(54) Title: ANTI-CD19 ANTIBODY HAVING ADCC AND CDC FUNCTIONS AND IMPROVED GLYCOSYLATION PRO-FILE

(57) Abstract: The present invention relates to an anti-CD19 antibody having a variant Fc region having some specific amino acid modifications relative to a wild-type Fc region which confer one or several useful effector functions. The present invention relates in particular to chimeric, humanized or full human anti-CD19 antibodies comprising such a variant Fc region. It relates advanta-geously to antibodies with an interesting and valuable glycosylation profile, especially a low fucose level and/or a high oligoman-nose level and low level of sialylated glycoform. The present invention also relates to the use of these antibodies in the treatment, prevention or management of disease or disorder, such as cancer, especially a B-cell malignancy, and auto-immune disease.

ANTI-CD19 ANTIBODY HAVING ADCC AND CDC FUNCTIONS
AND IMPROVED GLYCOSYLATION PROFILE

[0001]   FIELD OF THE INVENTION

[0002]   The present invention relates to an anti-CD19 antibody having a variant Fc region having some specific amino acid modifications relative to a wild-type Fc region which confer one or several useful effector functions. The present invention relates in particular to chimeric, humanized or full human anti-CD19 antibodies comprising such a variant Fc region. It relates advantageously to antibodies with an interesting and valuable glycosylation profile, especially a low fucose level and/or a high oligomannose level and/or higher level of sialylated glycoform. The present invention also relates to the use of these antibodies in the treatment, prevention or management of disease or disorder, such as cancer, especially a B-cell malignancy, and auto-immune disease.

[0003]   BACKGROUND OF THE INVENTION

[0004]   In autoimmune and/or inflammatory disorders, the immune system triggers an inflammatory response when there are no foreign substances to fight and the body's normally protective immune system cause damage to its own tissues by mistakenly attacking self. There are many different autoimmune disorders which affect the body in different ways. For example, the brain is affected in individuals with multiple sclerosis, the gut is affected in individuals with Crohn's disease, and the synovium, bone and cartilage of various joints are affected in individuals with rheumatoid arthritis.

[0005]   B-cell malignancies constitute an important group of cancer that includes B-cell non-Hodgkin's lymphoma (NHL), B-cell chronic lymphocytic leukaemia (B-CLL) and hairy cell leukaemia and B-cell acute lymphocytic leukaemia (B-ALL).

[0006]   Currently, cancer therapy may involve surgery, chemotherapy, hormonal therapy and/or radiation treatment to eradicate neoplastic cells in a patient. Recently, cancer therapy could also involve biological therapy or immunotherapy.

[0007]   There is a significant need for alternative cancer treatments, particularly for treatment of cancer that has proved refractory to standard cancer treatments, such as surgery, radiation therapy, chemotherapy, and hormonal therapy.

[0008]   A promising alternative is immunotherapy, in which cancer cells are specifically targeted by cancer antigen-specific antibodies.

[0009]   Major efforts have been directed at harnessing the specificity of the immune response, for example, hybridoma technology has enabled the development of tumor selective monoclonal antibodies and in the past few years, the Food and Drug Administration

has approved the first MAbs for cancer therapy: Rituxan® (anti-CD20) for non-Hodgkin's Lymphoma and Herceptin [anti-(c-erb-2/HER-2)] for metastatic breast cancer.

[0010]   Rituxan® (common name is rituximab) is a chimeric mouse-human monoclonal antibody to human CD20, a 35 kilodaltons, four transmembrane-spanning proteins found on the surface of the majority of B-cells in peripheral blood and lymphoid tissue.

[0011]   The antibody therapy (Rituxan®, U.S.Patent 5, 736, 137) was approved by the United States Food and Drug Administration (FDA) for the treatment of relapsed or refractory low grade or follicular, CD20-positive B-cell non-Hodgkin's lymphoma.

[0012]   In addition, lymphoma therapies employing radiolabeled anti-CD20 antibodies have been described in U.S. Patents 5,595,721, 5,843,398, 6,015,542, and 6,090,365.

[0013]   In oncology, Rituxan® /MabThera® is also indicated in the US for the treatment of relapsed or refractory, low-grade or follicular, CD20-positive, B-cell NHL as a single agent, for the treatment of NHL, for previously untreated follicular, CD20-positive, B-cell NHL in combination with cyclophosphamide, vincristine, prednisolone (CVP) chemotherapy, for the treatment of non-progressing (including stable disease), low-grade, CD20 positive, B-cell NHL as a single agent, after first-line CVP chemotherapy and for previously untreated diffuse large B-cell, CD20-positive, NHL in combination with standard chemotherapy (CHOP) or other anthracycline-based chemotherapy regimens.

[0014]   In oncology, Rituxan®/MabThera® is indicated in the EU for the treatment of patients with previously untreated or relapsed/refractory chronic lymphocytic leukaemia (CLL) in combination with chemotherapy, for the treatment of previously untreated patients with stage III-IV follicular lymphoma in combination with chemotherapy, as maintenance therapy for patients with relapsed/refractory follicular lymphoma responding to induction therapy with chemotherapy with or without Rituxan®/MabThera®, for the treatment of patients with CD20-positive diffuse large B-cell non-Hodgkin's lymphoma (NHL) in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, prednisolone) chemotherapy and as monotherapy for treatment of patients with stage III-IV follicular lymphoma who are chemoresistant or are in their second or subsequent relapse after chemotherapy.

[0015]   In addition, in rheumatology Rituxan®/MabThera® in combination with methotrexate is indicated for the treatment of adult patients with severe active rheumatoid arthritis who have had an inadequate response or intolerance to other disease-modifying anti-rheumatic drugs (DMARD) including one or more tumor necrosis factor (TNF) inhibitor therapies. MabThera is known as Rituxan in the United States, Japan and Canada.

[0016]   Alemtuzumab is another antibody targeting CD52 that is approved for use in relapsed chronic lymphocytic leukaemia (CLL) but is associated with significant toxicity

because of the ubiquitous expression of the target antigen on most normal immune cells including T-cells and natural killer (NK) cells.

[0017]   However, some resistance or relapses to rituximab treatment has appeared. Relapse may appear through a variety of mechanisms, some of which lead to loss CD20 expression and resistance to further rituximab treatment. Resistance may appear which may lead to loss or modulation of CD20 expression and is characterized by the lack of efficacy of repeated rituximab treatment or in some cases by the lack of efficacy of primary rituximab treatment. Resistance also occurs in lymphoma cases constitutively lacking CD20 expression, including some B-cel llymphomas such as plasmablastic lymphomas. Moreover, CD20+ patients may not respond to, or acquire resistance to Rituxan® therapy.

[0018]   Also, under conditions of high B-cell burden, exhaustion of the body's effector mechanisms, for example, NK-cell-mediated killing, may lead to substantial decreases in the immunotherapeutic efficacy of this MAb. Moreover, rituximab treatment of patients with chronic lymphocytic leukaemia and high levels of circulating B-cells can lead to removal of CD20 from the cells, thus allowing them to persist and resist clearance.

[0019]   On the basis of the success and limitations of rituximab and alemtuzumab, in particular resistance and relapse phenomena with rituximab therapy, identification of alternative antibodies targeting alternative antigens on B-cells is needed.

[0020]   CD19 is a 95-kDa glycoprotein member of the immunoglobulin (Ig) superfamily. CD19 is expressed on follicular dendritic cells and all B-cells from their early pre-B-cell stage until the time of plasma cell differentiation. CD19 surface expression is tightly regulated during B-cell development with higher levels seen in more mature cells and CD5+ (B-1) B-cells. CD19 is expressed later than CD20 through the plasmablast stage of B-cell differentiation. Consequently, CD19 expression is relatively high in many pre-B and immature B-lymphoblastic leukaemia and B-cell malignancies in which CD20 is poorly expressed. CD19+ plasmablasts may also play a role in the perpetuation of autoimmune diseases.

[0021]   CD19 is expressed on the surface of B-cells as a multiple molecular complex with CD21, CD81 and CD225. Together with this complex, CD19 is involved in co-signaling with the B-cell receptor and plays a role in the control of differentiation, activation and proliferation of B-lymphoid cells (Sato *et al.,* 1997).

[0022]   CD19 is present on the blasts of different types of human B-cell malignancies including pro- and pre-B-cell acute lymphoblastic leukaemia (ALL), common ALL (cALL) of children and young adults, NHL, B-CLL and hairy-cell leukaemia (HCL). It is not shed from malignant cells and is internalized after binding of some antibodies (Press *et al.,* 1989). Antigen density ranges from 10 000 to 30 000 molecules per cell on healthy peripheral B-

cells, and from 7 000 to 30 000 molecules per cell on malignant cells from a variety of lymphoid cancers (Olejniczak *et al.,* 1983).

[0023]   CD19 is expressed more broadly and earlier in B-cell development than CD20, which is targeted by the marketed anticancer MAb Rituxan® and so could have applications in a wider range of cancers including non-Hodgkin's lymphoma and acute lymphoblastic leukaemia as well as CLL.

[0024]   CD19 has been a focus of immunotherapy development for over 20 years, but initial clinical trials with monoclonal antibodies to CD19 did not result in durable effects despite demonstrating responses in some patients either as a single agent or in combination with other therapeutic agents (Hekman *et al.,* 1991; Vlasved *et al.,* 1995).

[0025]   Several CD19-specific antibodies have been evaluated for the treatment of B-lineage malignancies *in vitro,* in mouse models, and in clinical trials. These have included unmodified anti-CD19 antibodies, antibody-drug conjugates, and bispecific antibodies targeting CD19 and CD3 or CD16 to engage cytotoxic lymphocyte effector functions.

[0026]   The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations or alternative post-translational modifications that may be present in minor amounts, whether produced from hydridomas or recombinant DNA techniques.

[0027]   Antibodies are proteins, which exhibit binding specificity to a specific antigen. Native antibodies are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies between the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly intrachain disulfide bridges.

[0028]   Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain.

[0029]   The term 'variable' refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are responsible for the binding specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed through the variable domains of antibodies. It is concentrated in three segments called complementarity determining regions (CDRs) both in the light chain and the heavy chain variable domains.

[0030] The more highly conserved portions of the variable domains are called the framework regions (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a β-sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of the β-sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen binding site of antibodies (Kabat *et al.*, 1991).

[0031] The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions. Depending on the amino acid sequence of the constant region of their heavy chains, antibodies or immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgG, IgD, IgE and IgM, and several of these may be further divided into subclasses (isotypes), e.g. IgG1, IgG2, IgG3 and IgG4; IgA1 and IgA2. The heavy chain constant regions that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively. Of the various human immunoglobulin classes, only IgG1, IgG2, IgG3 and IgM are known to activate complement.

[0032] Immune effector functions which have been shown to contribute to antibody-mediated cytotoxicity include antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cell-mediated phagocytosis (ADCP), and complement-dependent cytotoxicity (CDC).

[0033] Cytotoxicity may also be mediated via antiproliferative effects. The mechanism of antibody modulation of tumor cell proliferation is poorly understood. However, advances in understanding the interactions of antibodies with Fcg receptors (FcgR) on immune effector cells have allowed the engineering of antibodies with significantly improved effector function.

[0034] The mechanism of action of MAbs is complex and appears to vary for different MAbs. There are multiple mechanisms by which MAbs cause target cell death. These include apoptosis, CDC, ADCC and inhibition of signal transduction.

[0035] The most studied is ADCC, which is mediated by natural killer (NK) cells. This involves binding of the Fab portion of an antibody to a specific epitope on a cancer cell and subsequent binding of the Fc portion of the antibody to the Fc receptor on the NK cells. This triggers release of perforin, and granzyme that leads to DNA degradation, induces apoptosis and results in cell death. Among the different receptors for the Fc portion of MAbs, the FcgRIIIa plays a major role in ADCC.

[0036] Previous research has shown that a polymorphism of the FcgRIIIa gene encodes for either a phenylalanine (F) or a valine (V) at amino acid 158. Expression of the valine isoform correlates with increased affinity and binding to MAbs (Rowland *et al.,* 1993; Sapra

et al., 2002; Molhoj *et al.,* 2007). Some clinical studies have supported this finding, with greater clinical response to rituximab in patients with non-Hodgkin's lymphoma who display the V/V polymorphism (Cartron et al., 2002, Bruenke *et al. 2005,* Hekmann *et al.,* 1991, Bargou *et al.,* 2008).

[0037]   WO1999051642 describes a variant human IgG Fc region comprising an amino acid substitution at positions 270 or 329, or at two or more of positions 270, 322, 329, and 331. These modifications aim at increasing the CDC and ADCC effector functions.

[0038]   WO2002080987 describes a method for treating a B-cell malignancy in a subject comprising administering an anti-CD19 immunotoxin, more particularly a humanized or human, monoclonal antibody. The B-cell malignancy may be one which comprises B-cells that do not express CD20.

[0039]   WO2007024249 is related to the modification of a human IgG Fc region in order to confer an increase effector cell function mediated by FcγR, especially ADCC. The Fc region comprises specific modifications at various amino acid positions.

[0040]   WO2008022152 is also related to antibodies that target CD19, wherein the antibodies comprise modifications to Fc receptors and alter the ability of the antibodies to mediate one or more effector functions, including ADCC, ADCP and CDC. ADCC assays are illustrated.

[0041]   Other patent applications related to anti-CD19 antibodies include WO2009052431, WO2009054863, WO2008031056, WO2007076950, WO2007082715, WO2004106381, WO1996036360, WO1991013974, US 7,462,352, US20070166306 and WO2005092925.

[0042]   US20090098124 also relates to engineering of antibodies with variant heavy chains containing the Fc region of IgG2, 3 or 4, having one or more amino acid modifications. A number of mutations and group of mutations within the Fc region are proposed. One of them is substitution at position 243 with leucine, at position 292 with proline, at position 300 with leucine, at position 305 with isoleucine, and at position 396 with leucine (MgFc88). Additional mutations may be introduced in the Fc regions to provide for altered C1q binding and/or CDC function. The amino acid positions to be modified are said to be generally selected from positions 270, 322, 326, 327, 329, 331, 333, and 334.

[0043]   J. B. Stavenhagen et al. (Cancer Res. 2007, 67 (18) : 8882-8890) disclose Fc optimization of therapeutic antibodies. The highest levels of ADCC were obtained with mutant 18 (F243L/R292P/Y300L/V305I/P396L). Anti-CD20 and anti-CD32B monoclonal antibodies having this mutated Fc are disclosed.

[0044]   Despite CD19 is a B-cell specific antigen expressed on chronic lymphocytic leukemia (CLL) cells, to date CD19 has not been effectively targeted with therapeutic

monoclonal antibodies. The authors describe XmAb5574, a novel engineered anti-CD19 monoclonal antibody with a modified Fc domain designed to enhance binding of FcγRIIIa. They demonstrate that this antibody mediates potent ADCC, modest direct cytotoxicity and ADCP, but no CDC (Awan *et al.*, 2010).

[0045] Moreover, glycoproteins mediate many essential functions in human beings including catalysis, signalling, cell-cell communication and molecular recognition and association. Many glycoproteins have been exploited for therapeutic purposes. The oligosaccharide component of protein can affect properties relevant to the efficacy of a therapeutic glycoprotein, including physical stability, resistance to protease attack, interactions with the immune system, pharmacokinetics, and specific biological activity. Such properties may depend not only on the presence or absence, but also on the specific structures, of oligosaccharides. For example, certain oligosaccharide structures mediate rapid clearance of the glycoprotein from the bloodstream through interactions, with specific carbohydrate binding proteins, while others can be bound by antibodies and trigger undesired immune reactions (Jenkins *et al.*, 1996).

[0046] Most of the existing therapeutic antibodies that have been licensed and developed as medical agents are of the human IgG1 isotype, the molecular weight of which is ~150 kDa. Human IgG1 is a glycoprotein bearing two *N*-linked biantennary complex-type oligosaccharides bound to the antibody constant region (Fc), in which the majority of the oligosaccharides are core fucosylated, and it exercises the effector functions of antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) through the interaction of the Fc with either leukocyte receptors (FcγRs) or complement.

[0047] Recently, therapeutic antibodies have been shown to improve overall survival as well as time to disease progression in a variety of human malignancies, such as breast, colon and haematological cancers, and genetic analysis of FcγR polymorphisms of cancer patients has demonstrated that ADCC is a major anti-neoplasm mechanism responsible for clinical efficacy. However, the ADCC of existing licensed therapeutic antibodies has been found to be strongly inhibited by serum due to non-specific IgG competing for binding of the therapeutics to FcγRIIIa on natural killer cells, which leads to the requirement of a significant amount of drug and very high costs associated with such therapies.

[0048] The enhanced ADCC of non-fucosylated forms of therapeutic antibodies through improved FcγRIIIa binding is shown to be inhibited by the fucosylated counterparts. In fact, non-fucosylated therapeutic antibodies, not including the fucosylated forms, exhibit the strongest and most saturable *in vitro* and *ex vivo* ADCC among such antibody variants with improved FcγRIIIa binding as those bearing naturally occurring oligosaccharide heterogeneities and artificial amino acid mutations, even in the presence of plasma IgG.

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[0049] Inhibiting glycosylation of a human IgG1, by culturing in the presence of tunicamycin, causes, for example, a 50-fold decrease in the affinity of this antibody for the FcγRI receptor present on monocytes and macrophages (Leatherbarrow *et al.*, 1990). Binding to the FcγRIII receptor is also affected by the loss of carbohydrates on IgG, since it has been described that a non-glycosylated IgG3 is incapable of inducing lysis of the ADCC type via the FcγRIII receptor of NK cells (Lund *et al.*, 1995). However, beyond the necessary presence of the glycan-containing residues, it is more precisely the heterogeneity of their structure which may result in differences in the ability to initiate effector functions.

[0050] Studies have been conducted to investigate the function of oligosaccharide residue on antibody biological activities. It has been shown that sialic acid of IgG has no effect on ADCC (Boyd *et al., 1995)*. Several reports have shown that Gal residues enhance ADCC (Kumpel *et al., 1994)*. Bisecting GlcNac, which is a beta,4-GlcNac residue transferred to a core beta-mannose (Man) residue, has been implicated in biological residue of therapeutic antibodies (Lifely *et al.,* 1995, Shield *et al.*, 2002) have revealed the effect of fucosylated oligosaccharide on antibody effector functions; the Fuc-deficient IgG1 have shown 50-fold increased binding to FcγRIII and enhanced ADCC.

[0051] Today, a wide range of recombinant proteins for therapeutic applications (i.e cancer, inflammatory diseases...) are composed of glycosylated monoclonal antibodies. For therapeutic and economical reasons, there is a large interest in obtaining higher specific antibody activity. One way to obtain large increases in potency, while maintaining a simple production process in cell line and potentially avoiding significant, undesirable side effects, is to enhance the natural, cell-mediated effector functions of MAbs. Consequently, engineering the oligosaccharides of IgGs may yield optimized ADCC which is considered to be a major function of some of the therapeutic antibodies, although antibodies have multiple therapeutic functions (e.g. antigen binding, induction of apoptosis, and CDC.

[0052] In general, chimeric and humanized antibodies are prepared using genetic recombination techniques and produced using CHO cells as the host cell. In order to modify the sugar chain structure of the antibodies, various methods have been attempted, say application of an inhibitor against an enzyme relating to the modification of a sugar chain, selection of a mutant, or introduction of a gene encoding an enzyme relating to the modification of a sugar chain.

[0053] GLYCART BIOTECHNOLOGY AG (Zurich, CH) has expressed N-acetyl-glucosaminyltransferase III (GnTIII) which catalyzes the addition of the bisecting GlcNac residue to the N-linked oligosaccharide, in a Chinese Hamster Ovary (CHO) cell line, and showed a greater ADCC of IgG1 antibody produced (WO 99/54342; WO 03/011878; WO 2005/044859).

[0054]   By removing or supplanting fucose from the Fc portion of the antibody, KYOWA HAKKO KOGYO (Tokyo, Japan) has enhanced Fc binding and improved ADCC, and thus the efficacy of the MAb (US 6,946,292).

[0055]   More recently, Laboratoire Français du Fractionnement et des Biotechnologies (LFB) (France) showed that the ratio Fuc/Gal in MAb oligosaccharide should be equal or lower than 0.6 to get antibodies with a high ADCC (FR 2 861 080).

[0056]   P. M. Cardarelli et al. (Cancer Immunol. Immunother. 2010, 59:257-265) produce an anti-CD19 antibody in Ms-704PF CHO cells deficient in the FUT8 gene which encodes alpha1,6-fucosyltransferase. Non-fucosylation of the antibody in this paper requires the engineering of an enzymes-deficient cell line. This paper does consider amino acid mutations.

[0057]   John Lund et al. (Journal of Immunology, 1996, vol. 157, no. 11, pp4963-4969) describe that aglycosylated human chimeric IgG3 retained a significant capacity to bind human C1q and trigger lysis mediated through guinea pig C.

[0058]   Effector functions such as CDC and ADCC are effector functions that may be important for the clinical efficacy of MAbs. All of these effector functions are mediated by the antibody Fc region and let authors to attempt amino acid modifications with more or less success. Glycosylation, especially fucosylation of the Fc region have a dramatic influence on the efficacy of an antibody. This let the authors to modify the conditions of production of the antibodies in the CHO cells in order to change the glycosylation profile in an attempt here again to improve some effector functions, with more or less success one again.

[0059]   A method of enhancing the ADCC of the chimeric MAb anti-CD19 MAb 4G7 was disclosed in US 2007/0166306. The MAb 4G7 was produced by using the human mammalian 293T-cell line in the presence of a beta (1,4)-N-acetylglucosaminyltransferase III (GnTIII) enzyme, under conditions effective to produce in the antibody, an Fc fragment characterized by Asn297-linked oligosaccharides containing (1) at least 60% N-acetylglucosamine biselecting oligosaccharides and (2) only 10% of non fucosylated N-acetylglucosamine biselecting oligosaccharides.

[0060]   H. M. Horton et al. (Cancer Res. 2008, 68 (19) : 8049-8057) describe an Fc-engineered anti-CD19 antibody, having S239D and I332E mutations. This Fc domain called herein Fc14 was compared to the Fc domain of the invention called chR005-Fc20 (F243L/R292P/Y300L/V305L/P396L). The Fc domain of the invention displays ADCC in the presence of whole blood effector cells whereas no significant ADCC activity was detected with Fc14 in the same conditions in the presence of whole blood containing circulating natural immunoglobulins.

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[0061] Mammalian cells are the preferred hosts for production of therapeutic glycoproteins, due to their capability to glycosylate proteins in the most compatible form for human applications (Jenkis *et al.,* 1996). Bacteria very rarely glycosylates proteins, and like other type of common hosts, such as yeasts, filamentous fungi, insect and plant cells yield glycosylation patterns associated with rapid clearance from the blood stream.

[0062] Among mammalian cells, Chinese hamster ovary (CHO) cells have been most commonly used during the last two decades. In addition to giving suitable glycosylation patterns, these cells allow consistent generation of genetically stable, highly productive clonal cell lines. They can be cultured to high densities in simple bioreactors using serum-free media, and permit the development of safe and reproducible bioprocesses. Other commonly used animal cells include baby hamster kidney (BHK) cells, NSO- and SP2/0-mouse myeloma cells. Production from transgenic animals has also been tested (Jenkins *et al.,* 1996).

[0063] Since the sugar chain structure plays a remarkably important role in the effector function of antibodies and differences are observed in the sugar chain structure of glycoproteins expressed by host cells, development of a host cell which can be used for the production of an antibody having higher effector function has been an objective.

[0064] In order to modify the sugar chain structure of the produced glycoprotein, various methods have been attempted, such as (1) application of an inhibitor against an enzyme relating to the modification of a sugar chain, (2) selection of a cell mutant, (3) introduction of a gene encoding an enzyme relating to the modification of a sugar chain, and the like. Specific examples are described below.

[0065] Examples of an inhibitor against an enzyme relating to the modification of a sugar chain includes tunicamycin which selectively inhibits formation of GlcNAc-P-P-Dol which is the first step of the formation of a core oligosaccharide which is a precursor of an N-glycoside-linked sugar chain, castanospermin and W-methyl-1-deoxynojirimycin which are inhibitors of glycosidase I, bromocondulitol which is an inhibitor of glycosidase II, 1-deoxynojirimycin and 1,4-dioxy-1,4-imino-D-mannitol which are inhibitors of mannosidase I, swainsonine which is an inhibitor of mannosidase II, swainsonine which is an inhibitor of mannosidase II and the like.

[0066] Examples of an inhibitor specific for a glycosyltransferase include deoxy derivatives of substrates against N-acetylglucosamine transferase V (GnTV) and the like. Also it is known that 1-deoxynojirimycin inhibits synthesis of a complex type sugar chain and increases the ration of high mannose type and hybrid type sugar chains (Glycobiology series 2 - Destiny of Sugar Chain in Cell, edited by Katsutaka Nagai, Senichiro Hakomori and Akira Kobata, 1993).

[0067]   Cell mutants regarding the activity of an enzyme relating to the modification of a sugar chain are mainly selected and obtained as a lectin-resistant cell line. For example, CHO cell mutants having various sugar chain structures have been obtained as a lectin-resistant cell line using a lectin such as WGA (wheat-germ agglutinin derived from *T.vulgaris*), ConA (cocanavalin A derived from *C. ensiformis*), RIC (a toxin derived from *R. communis*), L-PHA (leucoagglutinin derived from P. *vulgaris*), LCA (lentil agglutinin derived from *L. culinaris*), PSA (pea lectin derived from *P. sativum*) or the like (Genet *et al.* 1986).

[0068]   As an example of the modification of the sugar chain structure of a product obtained by introducing the gene of an enzyme relating to the modification of a sugar chain, into a host cell, it has been reported that a protein in which a number of sialic acid is added to the non-reducing end of the sugar chain can be produced by introducing rat β-galactosidase-a-5,6-sialyltransferase into CHO cell. Different types of glycoprotein-modifying glycosyl transferase may be also expressed in the host system such as GnT III, or, alternatively, b (1,4)-N-acetylglucosaminyltransferase V (GnT V), β(1,4)-galactosyl transferase (GalT) and mannosidase II (Man II).

[0069]   WO20070166306 is related to the use of avian embryonic derived stem cell lines, named EBx®, for the production of proteins and more specifically glycoproteins such as antibodies that are less fucosylated than with usual CHO cells.

[0070]   Ramesh Jassal et al. generate sialylation of anti NIP IgG3 antibody with FA243 mutation or by using a rat a2,6-sialyltransferase transfected CHO-K1 cell line. The FA243 IgG3 having both a2,6 and a2,3 silaylation restored target cell lysis by complement.

[0071]   John Lund et al. (Journal of Immunology, 1996, vol. 157, no. 11, pp4963-4969) describe that aglycosylated human chimeric IgG3 retained a significant capacity to bind human C1q and trigger lysis mediated through guinea pig C.

[0072]   The present inventors have evaluated the glycosylation profile of various Fc chimeric variant antibodies of human IgG1 subclass directed against the CD19 antigen produced by the Chinese hamster ovary cells CHO/DG44 cell, (purchased by ECACC) unmodified and untreated with glycosylation inhibitors. By analyzing and comparing structures of the sugar chains of the chR005-1 Fc0 and the optimized chR005-1 Fc20 variant antibodies produced, the present invention provides that a wild-type CHO host cell without any engineering expresses an interesting and valuable glycosylation profile, especially a low fucose level and/or a high oligomannose level and/or higher level of sialylated glycoforms.

[0073]   **SUMMARY OF THE INVENTION**

[0074] The present invention provides chimeric, humanized and human anti-CD19 antibodies, anti-CD19 antibody fusion proteins, and fragments thereof that bind a human B-cell marker.

[0075] Current approaches to optimize therapeutic antibody functionality (e.g. ADCC, CDC) have focused on amino acid modification or modification of the glycosylation state of the native Fc region. In contrast the present invention is based on a simultaneous combined approach concerning amino acid modification and glycosylation modification.

[0076] The present invention relates to the field of glycosylation engineering of proteins. More particular, the present invention is directed to the glycosylation engineering of proteins by using a wild-type mammalian CHO cell line to provide variant proteins following amino acid mutation with improved therapeutic properties such as efficient ADCC whereas the parent molecules (murine or wild-type chimeric antibodies) do not detectably exhibit this function.

[0077] The molecules of the invention still conserved apoptotic effector function conferred by the parental murine antibody.

[0078] More particularly, the present innovation relates to modifications of antibody functionality in immunoglobulin with Fc regions from IgG1 isotype to have capability to trigger CDC effector function.

[0079] More particularly the molecules of the invention exhibit both complement activation and efficient induction of ADCC.

[0080] The molecules of the invention are particularly useful for the treatment of B-cell disorders, such as but not limited to, B-cell malignancies, for the treatment and prevention of autoimmune disease, and for the treatment and prevention of graft-versus host diseases (GVHD), humoral rejection, and post-transplantation lymphoproliferative disorder in human transplant recipients, for patients refractory to treatment with exiting therapeutic antibody such as but not limited as anti-CD20 antibody or for combination treatment with exiting therapeutic antibody such as but not limited as anti-CD20 antibodies.

[0081] The present work aims at generating anti-CD19 antibodies having a modified Fc region that overcomes these drawbacks. The inventors have demonstrated that amino acid modifications in the Fc region of these anti-CD19 antibodies may have consequences not only on the effector functions, including ADCC and/or CDC, but also directly on the glycosylation profile of the antibody. They have in particular demonstrated that some modifications in the Fc region may lead to an anti-CD19 antibody having an interesting and valuable glycosylation profile, especially a low fucose level and/or a high oligomannose level and/or higher level of sialylated glycoforms when the antibody is produced or expressed in

mammal cells, including wild-type mammal cells, in particular rodent cells, especially CHO cells, such as the wild-type dhfr$^{-/-}$ CHO cells (purchased by ATCC collection).

[0082]   A first objective of the invention is to provide for a modified Fc region conferring an interesting and valuable glycosylation profile, especially a low fucose level and/or a high oligomannoses level and/or higher level of sialylated glycoforms to an anti-CD19 antibody, including an antibody produced or expressed in mammal cells, especially rodent cells, such as CHO cells, preferably wild-type cells, such as wild-type CHO cells. By definition, wild-type signifies that the cells have not been modified or engineered in order to modify the glycosylation machinery of the native cells.

[0083]   A second objective of the invention is to provide for a modified Fc region conferring ADCC function to an anti-CD19 antibody containing this Fc region.

[0084]   A third objective of the invention is to provide for a modified Fc region conferring CDC function to an anti-CD19 antibody containing this Fc region.

[0085]   A fourth objective of the invention is to provide for a modified Fc region conferring ADCC and CDC functions to an anti-CD19 antibody containing this Fc region.

[0086]   A fifth objective of the invention is to provide for a modified Fc region conferring ADCC and/or CDC function to an anti-CD19 antibody containing this Fc region, and in addition an interesting and valuable glycosylation profile, especially a low fucose level and/or a high oligomannoses level and/or higher level of sialylated glycoforms when produced or expressed in mammal cells, in particular rodent cells, such as CHO cells, including of the wild type.

[0087] DETAILED DESCRIPTION OF THE INVENTION

[0088]   A first object of the present invention is an anti-CD19 antibody modified to comprise a variant human IgG1 Fc region, wherein this variant region comprises an amino acid substitution at each of the amino acid positions 243, 292, 300, 305, 326, 396 or 243, 292, 300, 305, 326, 333, 396 of the human IgG1 Fc region. The amino acid residues in the Fc region are numbered according to the numbering system of Kabat®.

[0089]   The recombinant anti-CD19 antibodies according to the invention have an interesting glycosylation profile after production in a wild-type cell, especially rodent cell, such as in particular a wild-type CHO.

[0090]   In particular, from the transfected cells, e.g. wild-type CHO, the invention allows to express a large proportion of recombinant antibodies or fragment thereof, carrying a common N-linked oligosaccharide structure of a biantennary-type that comprises long chains with terminal GlcNac that are galactosylated and non-fucosylated.

[0091] One or more glycoforms are present in a recombinant anti-CD19 antibody population. A glycoform is an isoform of a protein that differs only with respect to the number or type of attached glycan.

[0092] In a very surprising and valuable embodiment, the antibodies have a low fucose level. This means that among a recombinant anti-CD19 antibody population produced in these cells, e.g. wild-type CHO, in this embodiment, the proportion of non-fucosylated antibodies represent approximately at least 40%, preferably approximately at least 60%, more preferably approximately at least 80% of the antibodies or higher, e.g. at least 85%. More particularly, the non-fucosylation concerns the Fc.

[0093] As CHO cells, mentioned may be done of CHO dhfr-/-, CHO/DG44 and CHO Easy C.

[0094] A specific object of the invention is an anti-CD19 antibody modified to comprise a variant human IgG1 Fc region, wherein this variant region comprises an amino acid substitution at each of the amino acid positions 243, 292, 300, 305, 326, 396 or 243, 292, 300, 305, 326, 333, 396 of the human IgG1 Fc region, and wherein the antibody has been produced in wild-type rodent cells, preferably wild-type CHO cells. According to a preferred feature, this antibody has a low level of fucose.

[0095] Another object of the invention is an anti-CD19 antibody modified to comprise a variant human IgG1 Fc region, wherein this variant region comprises an amino acid substitution at each of the amino acid positions 243, 292, 300, 305, 326, 396 or 243, 292, 300, 305, 326, 333, 396 of the human IgG1 Fc region, having a low fucose level.

[0096] In accordance with the invention, low level of fucose means either (1) a reduced amount of fucose in one antibody, especially in its Fc or (2) a high number of antibodies in a pool that have reduced amount of fucose and/or no fucose, especially in their Fc.

[0097] In the present description, one speaks about an Fc or the two Fc of an antibody according to the invention. Even if not mentioned every time, there is for every embodiment a preferred case where the antibody has two Fc and each one of the Fc has a similar structure (the same mutations) and a similar glycosylation profile.

[0098]

In an embodiment, the antibody of the invention has an Fc, preferably two Fc bearing no $(GlcNAc)_2(\textbf{Fuc})_1$ + $(Man)_3(GlcNAc)_2$ glycan.

[0099] In an embodiment, the antibody of the invention has an Fc, preferably two Fc bearing no $(Gal)_1(GlcNAc)_2(\textbf{Fuc})_1$ + $(Man)_3(GlcNAc)_2$ glycan.

[00100] In an embodiment, the antibody of the invention has an Fc, preferably two Fc bearing no $(GlcNAc)_2(\textbf{Fuc})_1$ + $(Man)_3(GlcNAc)_2$ glycan and no $(Gal)_1(GlcNAc)_2(\textbf{Fuc})_1$ + $(Man)_3(GlcNAc)_2$ glycan.

[00101]   In an embodiment, the antibody of the invention comprises an Fc, preferably two Fc bearing a $(Man)_5(GlcNAc)_2$ glycan. In an embodiment, the antibody of the invention comprises an Fc, preferably two Fc bearing a $(Man)_5(GlcNAc)_2$ glycan and no $(GlcNAc)_2$**(Fuc)**$_1$ + $(Man)_3(GlcNAc)_2$ glycan and/or, preferably and, no $(Gal)_1(GlcNAc)_2$**(Fuc)**$_1$ + $(Man)_3(GlcNAc)_2$ glycan.

[00102]   In an embodiment, the antibody comprises an Fc, preferably two Fc bearing one or two of the following glycans:

- $(Gal)_1(GlcNAc)_2$ **(Fuc)**$_1$ **(NeuAc)**$_1$ + $(Man)_3(GlcNAc)_2$
- $(Gal)_2(GlcNAc)_2$ **(Fuc)**$_1$ **(NeuAc)**$_1$ + $(Man)_3(GlcNAc)_2$

[00103]   In an embodiment, the antibody of the invention comprises an Fc, preferably two Fc bearing a $(Man)_5(GlcNAc)_2$ glycan and no $(GlcNAc)_2$**(Fuc)**$_1$ + $(Man)_3(GlcNAc)_2$ glycan and/or, preferably and, no $(Gal)_1(GlcNAc)_2$**(Fuc)**$_1$ + $(Man)_3(GlcNAc)_2$ glycan, and one or two of the following glycans:

- $(Gal)_1(GlcNAc)_2$ **(Fuc)**$_1$ **(NeuAc)**$_1$ + $(Man)_3(GlcNAc)_2$
- $(Gal)_2(GlcNAc)_2$ **(Fuc)**$_1$ **(NeuAc)**$_1$ + $(Man)_3(GlcNAc)_2$

[00104]   Another object of the invention is an anti-CD19 antibody an anti-CD19 antibody modified to comprise a variant human IgG1 Fc region, wherein this variant region comprises an amino acid substitution at each of the amino acid positions 243, 292, 300, 305, 326, 396 or 243, 292, 300, 305, 326, 333, 396 of the human IgG1 Fc region, and wherein the antibody has an Fc, preferably two Fc bearing no $(GlcNAc)_2$**(Fuc)**$_1$ + $(Man)_3(GlcNAc)_2$ glycan and/or, preferably and, no $(Gal)_1(GlcNAc)_2$**(Fuc)**$_1$ + $(Man)_3(GlcNAc)_2$ glycan. In an embodiment, the antibody of the invention comprises an Fc, preferably two Fc bearing a $(Man)_5(GlcNAc)_2$ glycan. In an embodiment, the antibody comprises an Fc, preferably two Fc bearing one or two of the following glycans:

- $(Gal)_1(GlcNAc)_2$ **(Fuc)**$_1$ **(NeuAc)**$_1$ + $(Man)_3(GlcNAc)_2$
- $(Gal)_2(GlcNAc)_2$ **(Fuc)**$_1$ **(NeuAc)**$_1$ + $(Man)_3(GlcNAc)_2$

[00105]   In still a very surprising and valuable embodiment, the antibodies have a high oligomannose level. This means that among a recombinant anti-CD19 antibody population produced in these cells, e.g. wild-type CHO the proportion of antibodies featured by a higher level of oligomannoses represent approximately at least 20%, preferably approximately at least 30%, more preferably approximately at least 40%, still more preferably approximately at least 50% of the antibodies or higher.

[00106]   In still a very surprising and valuable embodiment, among a recombinant antibody population produced in these cells, e.g. wild-type CHO, the proportion of antibodies featured by a higher level of sialylated glycoforms represent approximately at least 1.5%, preferably

approximately at least 2.5 %, more preferably approximately at least 5% of the antibodies or higher.

[00107] In a preferred embodiment, the antibodies of the invention combine three of these features, especially low fucose level and high oligomannose level and/or higher level of sialylated glycoforms.

[00108] In particular, the transfected cells of the invention, e.g. wild-type CHO cells allow to express a large proportion of antibodies or fragment thereof, carrying a common N-linked oligosaccharide structure of a biantennary-type that comprises long chains with terminal GlcNac that are galactosylated and non-fucosylated and which confer strong ADCC activity to antibodies.

[00109] In an embodiment, the anti-CD19 antibody having a specific glycosylation profile according to the invention, especially a low fucose level and/or a high oligomannose level and/or higher level of sialylated glycoforms, is produced or expressed, or is as produced or expressed, in mammal cells, preferably wild-type mammal cells, preferably of rodent origin, especially CHO cells.

[00110] The present invention has also as an object a pool of antibodies (or composition of antibodies) according to the invention, wherein it comprises anti-CD19 antibodies modified to comprise a variant human IgG1 Fc region, wherein this variant region comprises an amino acid substitution at each of the amino acid positions 243, 292, 300, 305, 326, 396 or 243, 292, 300, 305, 326, 333, 396 of the human IgG1 Fc region. According to a feature, the antibody pool has been produced in wild-type rodent cells, preferably wild-type CHO cells. According to another feature, the antibody pool has a low level of fucose. According to still another feature, the antibody pool has been produced in wild-type rodent cells, preferably wild-type CHO cells.

[00111] In a first embodiment, this pool comprises less or equal than 15 % of such anti-CD19 antibodies comprising an Fc, preferably two Fc bearing a $(GlcNAc)_2$**(Fuc)**$_1$ + $(Man)_3(GlcNAc)_2$ glycan and/or, preferably and, less or equal than 20 % of such antibodies comprising an Fc, preferably two Fc bearing a $(Gal)_1(GlcNAc)_2$**(Fuc)**$_1$ + $(Man)_3(GlcNAc)_2$ glycan.

Glycoform percentages are expressed in % in number.

[00112] In another embodiment, the pool of antibodies comprises at least 15, 20, 30, 40 or 50% of antibodies comprising an Fc, preferably two Fc bearing $(Man)_5(GlcNAc)_2$ glycans. In an embodiment, the value is at least 15 %.

[00113] In another embodiment, the pool comprises less or equal than 15 % of such anti-CD19 antibodies comprising an Fc, preferably two Fc bearing a $(GlcNAc)_2$**(Fuc)**$_1$ + $(Man)_3(GlcNAc)_2$ glycan and/or, preferably and, less or equal than 20 % of such antibodies

comprising an Fc, preferably two Fc bearing a $(Gal)_1(GlcNAc)_2(Fuc)_1$ + $(Man)_3(GlcNAc)_2$ glycan, and at least 15, 20, 30, 40 or 50% of antibodies comprising an Fc, preferably two Fc bearing $(Man)_5(GlcNAc)_2$ glycans. In a preferred embodiment the pool of antibodies comprises all these features. In an embodiment, the value for oligomannose is at least 15 %.

[00114]   In another embodiment, the pool of antibodies comprises

- less than 1.5 or 1 % of antibodies comprising an Fc, preferably two Fc bearing $(Gal)_1(GlcNAc)_2(Fuc)_1(NeuAc)_1$ + $(Man)_3(GlcNAc)_2$ (typical range 0.1-1.5 %)

- and/or, preferably, less than 2 or 1.5% of antibodies comprising an Fc, preferably two Fc bearing $(Gal)_2(GlcNAc)_2(Fuc)_1$ $(NeuAc)_1$ + $(Man)_3(GlcNAc)_2$ (typical range 0.1-2).

[00115]   In still another embodiment, the pool comprises less or equal than 15 % of such anti-CD19 antibodies comprising an Fc, preferably two Fc bearing a $(GlcNAc)_2(Fuc)_1$ + $(Man)_3(GlcNAc)_2$ glycan and/or, preferably and, less or equal than 20 % of such antibodies comprising an Fc, preferably two Fc bearing a $(Gal)_1(GlcNAc)_2$ $(Fuc)_1$ + $(Man)_3(GlcNAc)_2$ glycan, and at least 15, 20, 30, 40 or 50% of antibodies comprising an Fc, preferably two Fc bearing $(Man)_5(GlcNAc)_2$ glycans, and further

- less than 1.5 or 1% of antibodies comprising an Fc, preferably two Fc bearing $(Gal)_1(GlcNAc)_2(Fuc)_1(NeuAc)_1$ + $(Man)_3(GlcNAc)_2$

- and/or, preferably and, less than 2 or 1.5% of antibodies comprising an Fc, preferably two Fc bearing $(Gal)_2(GlcNAc)_2$ $(Fuc)_1(NeuAc)_1$ + $(Man)_3(GlcNAc)_2$. In a preferred embodiment the pool of antibodies comprises all these features. In an embodiment, the value for oligomannose is at least 15 %.

[00116]   Another object of the invention is a pool of antibodies (or composition of antibodies) according to the invention, wherein it comprises anti-CD19 antibodies modified to comprise a variant human IgG1 Fc region, wherein this variant region comprises an amino acid substitution at each of the amino acid positions 243, 292, 300, 305, 326, 396 or 243, 292, 300, 305, 326, 333, 396 of the human IgG1 Fc region, and wherein the pool comprises less or equal than 15 % of such anti-CD19 antibodies comprising an Fc, preferably two Fc bearing a $(GlcNAc)_2(Fuc)_1$ + $(Man)_3(GlcNAc)_2$ glycan and/or, preferably and, less or equal than 20 % of such antibodies comprising an Fc, preferably two Fc bearing a $(Gal)_1(GlcNAc)_2(Fuc)_1$ + $(Man)_3(GlcNAc)_2$ glycan.

[00117]   In another embodiment, the pool of antibodies comprises at least 20, 30, 40 or 50% of antibodies comprising an Fc, preferably two Fc bearing $(Man)_5(GlcNAc)_2$ glycans. In an embodiment, the value is at least 15 %.

[00118]   In another embodiment, the pool comprises less or equal than 15 % of such anti-CD19 antibodies comprising an Fc, preferably two Fc bearing a $(GlcNAc)_2(Fuc)_1$ + $(Man)_3(GlcNAc)_2$ glycan and/or, preferably and, less or equal than 20 % of such antibodies

comprising an Fc, preferably two Fc bearing a $(Gal)_1(GlcNAc)_2(\mathbf{Fuc})_1$ + $(Man)_3(GlcNAc)_2$ glycan, and at least 15, 20, 30, 40 or 50% of antibodies comprising an Fc, preferably two Fc bearing $(Man)_5(GlcNAc)_2$ glycans. In a preferred embodiment the pool of antibodies comprises all these features. In an embodiment, the value for oligomannose is at least 15%.

[00119]   In another embodiment, the pool of antibodies comprises

- less than 1.5 or 1% of antibodies comprising an Fc, preferably two Fc bearing $(Gal)_1(GlcNAc)_2(\mathbf{Fuc})_1(\mathbf{NeuAc})_1$ + $(Man)_3(GlcNAc)_2$

- and/or, preferably, less than 2 or 1.5% of antibodies comprising an Fc, preferably two Fc bearing $(Gal)_2(GlcNAc)_2(\mathbf{Fuc})_1$ $(\mathbf{NeuAc})_1$ + $(Man)_3(GlcNAc)_2$.

[00120]   In still another embodiment, the pool comprises less or equal than 15 % of such anti-CD19 antibodies comprising an Fc, preferably two Fc bearing a $(GlcNAc)_2(\mathbf{Fuc})_1$ + $(Man)_3(GlcNAc)_2$ glycan and/or, preferably and, less or equal than 20 % of such antibodies comprising an Fc, preferably two Fc bearing a $(Gal)_1(GlcNAc)_2$ $(\mathbf{Fuc})_1$ + $(Man)_3(GlcNAc)_2$ glycan, and at least 15, 20, 30, 40 or 50% of antibodies comprising an Fc, preferably two Fc bearing $(Man)_5(GlcNAc)_2$ glycans, and further

- less than 1.5 or 1% of antibodies comprising an Fc, preferably two Fc bearing $(Gal)_1(GlcNAc)_2(\mathbf{Fuc})_1(\mathbf{NeuAc})_1$ + $(Man)_3(GlcNAc)_2$

- and/or, preferably and, less than 2 or 1.5% of antibodies comprising an Fc, preferably two Fc bearing $(Gal)_2(GlcNAc)_2$ $(\mathbf{Fuc})_1(\mathbf{NeuAc})_1$ + $(Man)_3(GlcNAc)_2$. In a preferred embodiment the pool of antibodies comprises all these features. In an embodiment, the value for oligomannose is at least 15%.

[00121]   In an embodiment, the cells are rodent cells, in particular CHO cells.

[00122]   In an embodiment, the cells are wild-type rodent cells, especially wild-type CHO cells.

[00123]   In an embodiment, the anti-CD19 antibody is able to generate CDC or CDC + ADCC activity.

[00124]   In an embodiment, this anti-CD19 antibody is able to generate CDC or CDC + ADCC activity and to present a specific glycosylation profile according to the invention, especially a low fucose level and/or a high oligomannose level and/or higher level of sialylated glycoforms according to the invention.

[00125]   The antibody of the invention may comprise supplemental mutations in the Fc region.

[00126]   In an embodiment, the Fc region comprises further an amino acid substitution at the amino acid position 333.

[00127]   The human IgG Fc region may be a region of IgG sub-class. It may be an Fc region of IgG1, IgG2, IgG3 or IgG4. In a preferred embodiment, the Fc region is an IgG1 Fc region.

[00128]   The amino acids of the Fc region that are substituted in accordance with the invention may be substituted by any amino acid, the condition being that the whole set of substituted amino acids is able to generate this CDC or CDC + ADCC activity and/or to confer a specific glycosylation profile according to the invention, especially a low fucose level and/or a high oligomannose level and/or higher level of sialylated glycoforms according to the invention. Examples of possible substitutions are given thereafter.

[00129]   In an embodiment, Phe243 is substituted by Leu.

[00130]   In an embodiment, Arg292 is substituted by Pro.

[00131]   In an embodiment, Tyr300 is substituted by Leu.

[00132]   In an embodiment, Val305 is substituted by Leu.

[00133]   In an embodiment Lys326 is substituted by Ala.

[00134]   In an embodiment, Glu333 is substituted by Ala.

[00135]   In an embodiment, Pro396 is substituted by Leu.

[00136]   In an embodiment, the anti-CD19 antibody comprises an Fc region in which Phe243 is substituted by Leu, Arg292 is substituted by Pro, Tyr300 is substituted by Leu, Val305 is substituted by Leu, Lys326 is substituted by Ala and Pro396 is substituted by Leu.

[00137]   In an embodiment, this Fc region has the amino acid sequence depicted on SEQ ID NO: 1 (Fc34). A nucleic acid coding for this Fc region is depicted on SEQ ID NO: 2.

[00138]   In another embodiment, the anti-CD19 antibody comprises an Fc region in which Phe243 is substituted by Leu, Arg292 is substituted by Pro, Tyr300 is substituted by Leu, Val305 is substituted by Leu, Lys326 is substituted by Ala, Glu333 is substituted by Ala, and Pro396 is substituted by Leu.

[00139]   In another embodiment, this Fc region has the amino acid sequence depicted on SEQ ID NO: 3 (Fc24). A nucleic acid coding for this Fc region is depicted on SEQ ID NO: 4

[00140]   Modifications and changes may be made in the structure of a polypeptide of the present invention and still obtain a molecule having like characteristics. For example, certain amino acids can be substituted for other amino acids in a sequence without appreciable loss of activity. Because it is the interactive capacity and nature of a polypeptide that defines that polypeptide's biological functional activity, certain amino acid sequence substitutions can be made in a polypeptide sequence (or, of course, its underlying DNA coding sequence) and nevertheless obtain a polypeptide with like properties.

[00141]   In making such changes, the hydropathic index of amino acids can be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function

on a polypeptide is generally understood in the art (Kyte *et al.* 1982). It is known that certain amino acids can be substituted for other amino acids having a similar hydropathic index or score and still result in a polypeptide with similar biological activity. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics.

[00142] It is believed that the relative hydropathic character of the amino acid determines the secondary structure of the resultant polypeptide, which in turn defines the interaction of the polypeptide with other molecules, for example, enzymes, substrates, receptors, antibodies, antigens, and the like. It is known in the art that an amino acid may be substituted by another amino acid having a similar hydropathic index and still obtain a biologically functionally equivalent polypeptide. In such changes, the substitution of amino acids whose hydropathic indices are within ± 2 is preferred, those which are within ± 1 are particularly preferred, and those within +0.5 are even more particularly preferred.

[00143] Substitution of like amino acids can also be made on the basis of hydrophilicity, particularly where the biologically functionally equivalent peptide or polypeptide thereby created is intented for use in immunological embodiments. U.S. Patent 4,554,101, incorporated herein by reference, states that the greatest local average hydrophilicity of a polypeptide, as governed by the hydrophilicity of its adjacent amino acids, correlate with its immunogenicity and antigenicity, *i.e.* with a biological property of the polypeptide.

[00144] As detailed in U.S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 ± 1); glutamate (+3.0 ± 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); proline (-0.5 ± 1); threonine (-0.4); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). It is understood that an amino acid can be substituted for another having a similar hydrophility value and still obtain a biologically equivalent, and in particular, an immunologically equivalent, polypeptide. In such changes, the substitution of amino acids whose hydrophilicity values are within ± 2 is preferred, those which are within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

[00145] As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like.

Amino Acid	Index	Amino Acid	Index
isoleucine L	(+4,5)	tryptophan W	(-0,9)
valine V	(+4,2)	tyrosine Y	(-1,3)
leucine L	(+3,8)	proline P	(-1,6)
phenylalanine	(+2,8)	histidine H	(-3,2)

cysteine C	(+2,5)	glutamate E	(-3,5)
methionine M	(+1,9)	glutamine Q	(-3,5)
alanine A	(+1,8)	aspartate D	(-3,5)
glycine G	(-0,4)	asparagine N	(-3,5)
threonine T	(-0,7)	lysine K	(-3,9)
serine S	(-0,8)	arginine R	(-4,5)

[00146]   Amino acid substitution may be chosen or selected differently. Possible substitutions have been documented in WO99/51642, WO2007024249 and WO2007106707.

[00147]   In an embodiment, Phe243 is substituted by an amino acid chosen among Leu, Trp, Tyr, Arg and Gln. Preferably, Phe243 is substituted by Leu.

[00148]   In an embodiment, Arg292 is substituted by an amino acid chosen among Gly and Pro. Preferably, Arg292 is substituted by Pro.

[00149]   In an embodiment, Tyr300 is substituted by an amino acid chosen among Lys, Phe, Leu and Ile. Preferably, Tyr300 is substituted by Leu.

[00150]   In an embodiment, Val305 is substituted among Leu and Ile. Preferably, Val305 is substituted by Leu.

[00151]   In an embodiment, Lys326 is substituted by an amino acid chosen among Val, Glu, Ala, Gly, Asp, Met, Ser, Asn and Trp. Preferably, Lys326 is substituted by Ala.

[00152]   In an embodiment, Glu333 is substituted by an amino acid chosen among Val, Gly, Ala, Gln, Asp, Asn, Lys, Arg and Ser. Preferably, Glu333 is substituted by Ala.

[00153]   In an embodiment, Pro396 is substituted by Leu.

[00154]   In an embodiment, the present invention applies to any anti-CD19 antibody. That is to say, the anti-CD19 antibody comprises variable regions specific to CD19 antigen, or variable regions comprising CDRs specific to CD19 antigen, and the mutant Fc region according to the invention. Variable regions may be found in US20090098124, WO2002080987, WO2007024249, WO2008022152, WO2009052431, WO2009054863, WO2008031056, WO2007076950, WO2007082715, WO2004106381, WO1996036360, WO1991013974, US 7,462,352, US20070166306 and WO2005092925. The person skilled in the art may refer to these documents as source of variable regions or CDRs specific to CD19.

[00155]   In an embodiment, the antibody is specific for, or recognizes, a non-internalizing epitope on the CD19 antigen. The applicant has developed two murine anti-CD19 antibodies called mR005-1 and mR005-2, whose variable regions have been sequences and the CDRs identified. The present invention thus includes as preferred embodiments the use of the variable regions or the CDRs derived from mR005-1 and mR005-2.

[00156] In a preferred embodiment, the anti-CD19 antibody mR005-1 of the invention comprises the following CDRs:

	SEQ ID NO:	Sequence IMGT ®	SEQ ID NO:	Sequence Kabat ®	SEQ ID NO:	Sequence (Common numbering system)
VHmR005-1						
CDR1	5	GYAFSSYW	11	SYWVN	16	SSYW
CDR2	6	IYPGDGDT	12	QIYPGDGDTNYNGKFKG	6	IYPGDGDT
CDR3	7	ARSITTVVGCAMDY	13	SITTVVGCAMDY	13	SITTVVGCAMDY
VLmR005-1						
CDR1	8	DHINNW	14	KASDHINNWLA	8	DHINNW
CDR2	9	GAT	15	GATTLET	9	GAT
CDR3	10	QQSWNTPWT	10	QQSWNTPWT	10	QQSWNTPWT

[00157] In a preferred embodiment, the anti-CD19 antibody mR005-2 of the invention comprises the following CDRs:

	SEQ ID N°:	Sequence IMGT ®	SEQ ID NO:	Sequence Kabat ®	SEQ ID NO:	Sequence (Common numbering system)
VHmR005-2						
CDR1	17	GYTFTSYV	23	SYVMH	28	TSYV
CDR2	18	VNPYNDGT	24	YVNPYNDGTKYNEKFKG	18	VNPYNDGT
CDR3	19	ARGPYYGSSPFDY	25	GPYYGSSPFDY	25	GPYYGSSPFDY
VLmR005-2						
CDR1	20	QSLENSNGNTY	26	RSSQSLENSNGNTYLN	20	QSLENSNGNTY
CDR2	21	RVS	27	RVSNRFS	21	RVS
CDR3	22	LQVTHVPPT	22	LQVTHVPPT	22	LQVTHVPPT

[00158] By definition, these CDRs include variant CDRs, by deletion, substitution or addition of one or more amino acid(s), which variant keeps the specificity of the original CDR. The common numbering system provides for a CDR definition having the shortest amino acid sequences or the minimal CDR definition.

[00159] In an embodiment, the antibody comprises the CDRs of the antibodies mR005-1 or mR005-2 whose VH and VL amino acid sequences are depicted on the following table, or more preferably, the antibody comprises those sequences:

	Amino acid sequence VH	Amino acid sequence VL
mR005-1	SEQ ID NO: 29	SEQ ID NO: 31
mR005-2	SEQ ID NO: 33	SEQ ID NO: 35

[00160]   In an embodiment, the antibody comprises the CDRs whose sequence is depicted on SEQ ID NO: 16, 6, 13 and/or 8, 9, 10.

[00161]   In an embodiment, the antibody comprises the CDRs whose sequence is depicted on SEQ ID NO: 5, 6, 7 and/or 8, 9, 10.

[00162]   In an embodiment, the antibody comprises the CDRs whose sequence is depicted on SEQ ID NO: 11, 12, 13 and/or 14, 15, 10.

[00163]   In an embodiment, the antibody comprises the CDRs whose sequence is depicted on SEQ ID NO: 28, 18, 25 and/or 20, 21, 22.

[00164]   In an embodiment, the antibody comprises the CDRs whose sequence is depicted on SEQ ID NO: 17, 18, 19 and/or 20, 21, 22.

[00165]   In an embodiment, the antibody comprises the CDRs whose sequence is depicted on SEQ ID NO: 23, 24, 25 and/or 26, 27, 22.

[00166]   The invention further comprises each one of these embodiments wherein the antibody further comprises an Fc region having the amino acid sequence depicted on SEQ ID NO: 1 or 3.

[00167]   The antibody may be a monoclonal antibody, a chimeric antibody, a humanized antibody, a full human antibody, a bispecific antibody, an antibody drug conjugate or an antibody fragment. A "humanized antibody" or "chimeric humanized antibody" shall mean an antibody derived from a non human antibody, typically a murine antibody, that retains or substantially retains the antigen-binding properties of the parental antibody, but which is less immunogenic in humans.

[00168]   In an embodiment, the antibody triggers programmed cell death or apoptosis.

[00169]   In an embodiment, the antibody is for use for CD19 positive B-cell disorders.

[00170]   In an embodiment, the antibody is for use on refractory or relapse anti-CD20 antibody treated patients.

[00171]   In an embodiment, the antibodies of the invention have an Fc region having a specific glycosylation profile according to the invention, especially a low fucose level and/or a high oligomannose level and/or higher level of sialylated glycoforms and have been produced in wild-type rodent mammalian cells, preferably wild-type CHO cells. By definition, in the present study these cells have not been modified or cultured in specific conditions to alter the glycosylation. For example, these cells have not been modified to be deficient in 1,6-fucosyltransferase. The invention is thus based on the production of such anti-CD19 antibodies having an interesting and valuable glycosylation profile, especially a low fucose level and/or a high oligomannose level and/or higher level of sialylated glycoforms owing the mutations in the Fc region.

[00172] The present invention provides for this anti-CD19 antibody which after transfection and expression in mammalian cells, especially rodent cells, such as CHO cells, including of the wild-type, have a very interesting glycosylation profile and in particular have an interesting and valuable glycosylation profile, especially a low fucose level and/or a high oligomannose level and/or higher level of sialylated glycoforms.

[00173] The present invention provides the person skilled in the art with a method to produce an anti-CD19 antibody in mammalian cells, without the need to engineer these cells, for example to render these cells deficient in 1,6-fucosyltransferase, or in the presence of agents which are able to impact on the glycosylation profile, e.g. fucose level or to use special cell lines with specific glycosylation function.

[00174] In an embodiment, the method comprises the combination of an Fc as disclosed herein with at least a variable region to give an anti-CD19 antibody.

[00175] In an embodiment, the antibody is produced on mammalian cells, in particular rodent cells, preferably CHO cells, preferably wild-type, such as the wild type DG44 CHO cells (purchased by ATCC).

[00176] The method for producing the anti-CD19 antibody is another object of the invention. The mammal cells, preferably rodent cells such as CHO cells, preferably wild-type cells are transfected with one or several expression vectors. Preferably, the cells are co-transfected with an expression vector for light chain and with an expression vector for heavy chain.

[00177] The expression vector for the heavy chain contains a nucleic acid sequence SEQ ID NO: 2, 4 which encodes the variant Fc region according to the invention.

[00178] Cell transfection is also objects of the invention. As transfection that may be performed, one may mention without limitation standard transfection procedures, well-known from the man skilled in the art may be carried out, such as calcium phosphate precipitation, DEAE- Dextran mediated transfection, electroporation, nucleofection (AMAXA Gmbh, GE), liposome-mediated transfection (using lipofectin® or lipofectamine® technology for example) or microinjection.

[00179] The expression vector for the heavy chain contains a nucleic acid sequence which encodes the variant Fc region according to the invention which comprises an amino acid substitution at each of the amino acid positions 243, 292, 300, 305, 326 and 396, optionally 333. In an embodiment, this nucleic acid sequence also comprises a nucleic acid sequence encoding an antibody variable region which is specific for, or specifically recognizes, a CD19 antigen. Preferably, the variable region recognizes a non internalizing CD19 epitope or determinant.

[00180] Preferably, the vector contains a nucleic acid sequence encoding the variable region of the parental murine R005-1 which recognizes a non internalizing CD19 epitope or determinant.

[00181] Preferably, the vector contains a nucleic acid sequence encoding the variable region of the parental murine R005-2 which recognizes a non internalizing CD19 epitope or determinant.

[00182] The expression vector comprises a nucleic acid sequence or nucleic acid sequences which code(s) for the variable region that is wished. Various embodiments of variable regions which can be expressed by the vector are presented below.

[00183] In a first embodiment, the variable region VH comprises the VH CDRs CDR1, CDR2, and CDR3 of R005-1.

[00184] In an embodiment, the variable region VH comprises R005-1 CDRs whose sequence is depicted on SEQ ID NO: 16, 6, 13. In another embodiment, the variable region VH comprises the CDRs whose sequence is depicted on SEQ ID NO: 5, 6, 7. In another embodiment, the variable region VH comprises the CDRs whose sequence is depicted on SEQ ID NO: 11, 12, 13.

[00185] In a second embodiment, the variable region VH comprises the VH CDRs CDR1, CDR2, and CDR3 of R005-2.

[00186] In an embodiment, the variable region VH comprises the CDRs whose sequence is depicted on SEQ ID NO: 28, 18, 25. In another embodiment, the variable region VH comprises the CDRs whose sequence is depicted on SEQ ID NO: 17, 18, 19. In another embodiment, the variable region VH comprises the CDRs whose sequence is depicted on SEQ ID NO: 23, 24, 25.

[00187] In an embodiment, the variable region VH (R005-1) has the amino acid sequence as depicted on SEQ ID NO: 29. In another embodiment, the vector comprises a nucleic acid sequence as depicted on SEQ ID NO: 30.

[00188] In another embodiment, the variable region VH R005-2 has the nucleic acid sequence as depicted on SEQ ID NO: 33. In another embodiment, the vector comprises the nucleic acid sequence as depicted on SEQ ID NO: 34.

[00189] In an embodiment, the vector comprises a nucleic acid sequence comprising a nucleic acid coding for the CDRs according to SEQ ID NO: 16, 6, 13, or 5, 6, 7, or 11, 12, 13 and a nucleic acid coding for an Fc region having the amino acid sequence depicted on SEQ ID NO: 1 or 3.

[00190] In an embodiment, the vector comprises a nucleic acid sequence as depicted on SEQ ID NO: 30 and a nucleic acid coding for an Fc region having the sequence depicted on SEQ ID NO: 1 or 3.

[00191]   In another embodiment, the vector comprises a nucleic acid sequence coding for an amino acid sequence as depicted on SEQ ID NO: 29 and a nucleic acid coding for an Fc region having the sequence depicted on SEQ ID NO: 1 or 3.

[00192]   In another embodiment, the vector comprises a nucleic acid sequence comprising the CDRs according to SEQ ID NO:18, 28, 25, or 17, 18, 19, or 23, 24, 25 and a nucleic acid coding for an Fc region having the sequence depicted on SEQ ID NO: 1 or 3.

[00193]   In an embodiment, the vector comprises a nucleic acid sequence as depicted on SEQ ID NO: 34 and a nucleic acid coding for an Fc region having the sequence depicted on SEQ ID NO:1 or 3.

[00194]   In another embodiment, the vector comprises a nucleic acid sequence coding for an amino acid sequence as depicted on SEQ ID NO: 33 and a nucleic acid coding for an Fc region having the sequence depicted on SEQ ID NO: 1 or 3.

[00195]   The expression vector for the light chain contains a nucleic acid sequence which encodes a human Kappa or Lambda region, preferably a human Kappa region, and an antibody variable region which is specific for, or specifically recognizes, a CD19 antigen. Preferably, the variable region recognizes a non internalizing CD19 epitope or determinant.

[00196]   The expression vector comprises a nucleic acid sequence or nucleic acid sequences which code(s) for the variable region that is wished. Various embodiments of variable regions which can be expressed by the vector are presented below.

[00197]   In a first embodiment the variable region VL comprises the VL CDRs CDR1, CDR2, and CDR3 of R005-1.

[00198]   In an embodiment, the variable region VL comprises the CDRs whose sequence is depicted on SEQ ID NO: 8, 9, 10. In another embodiment, the antibody comprises the CDRs whose sequence is depicted on SEQ ID NO: 14, 15, 10. In an embodiment, the variable region has the amino acid sequence as depicted on SEQ ID NO: 31. In another embodiment, the vector comprises the nucleic acid sequence as depicted on SEQ ID NO: 32.

[00199]   In a second embodiment, the variable region VL comprises the VL CDRs CDR1, CDR2, and CDR3 of R005-2.

[00200]   In an embodiment, the variable region VL comprises the CDRs whose sequence is depicted on SEQ ID NO: 20, 21, 22. In another embodiment, the antibody comprises the CDRs whose sequence is depicted on SEQ ID NO: 26, 27, 22. In an embodiment, the variable region has the amino acid sequence as depicted on SEQ ID NO: 35. In another embodiment, the vector comprises the nucleic acid sequence as depicted on SEQ ID NO: 36.

[00201] The expression vectors comprise these nucleic acid sequences and regulatory sequences allowing expression of the former. In an embodiment, a unique vector comprises both nucleic acid sequences and is able to encode the VH and the VL.

[00202] Preferably, the invention comprises the use of one single vector or a set of vectors comprise (1) a nucleic acid sequence encoding an antibody variable region comprising the VH CDRs as mentioned above and a variant Fc region comprising an amino acid substitution at each of the amino acid positions 243, 292, 300, 305, 326 and 396, optionally 333, and (2) a nucleic acid sequence encoding an antibody variable region comprising the VL CDRs as mentioned above and a human Kappa region.

[00203] These vectors are also objects of the invention, alone or as a set of vectors.

[00204] These vectors are also objects of the invention, alone or as a set of vectors. As vectors that may be used, one may mention without limitation: pcDNA3.3, pOptiVEC, pFUSE, pMCMVHE, pMONO, pSPORT1, pcDV1, pCDNA3, pCDNA1, pRc/CMV, pSEC.

[00205] Based on the specific amino acid substitutions according to the invention, the person skilled in the art is fully able to design the nucleic acid sequences with the codon mutations which allow the sequence to encode the variant Fc region according to the invention, taking into account the degeneracy of the genetic code.

[00206] In an embodiment, the sequence encodes a variant Fc region wherein Phe243 is substituted by an amino acid chosen among Leu, Trp, Tyr, Arg and Gln. Preferably, Phe243 is substituted by Leu.

[00207] In an embodiment, the nucleic acid sequence encodes a variant Fc region wherein Arg292 is substituted by an amino acid chosen among Gly and Pro. Preferably, Arg292 is substituted by Pro.

[00208] In an embodiment, the nucleic acid sequence encodes a variant Fc region wherein Tyr300 is substituted by an amino acid chosen among Lys, Phe, Leu and Ile. Preferably, Tyr300 is substituted by Leu.

[00209] In an embodiment, the nucleic acid sequence encodes a variant Fc region among Ile and Leu. Preferably, Val305 is substituted by Leu.

[00210] In an embodiment, the nucleic acid sequence encodes a variant Fc region wherein Lys326 is substituted by an amino acid chosen among Val, Glu, Ala, Gly, Asp, Met, Ser, Asn and Trp. Preferably, Lys326 is substituted by Ala.

[00211] In an embodiment, the nucleic acid sequence encodes a variant Fc region wherein Glu333 is substituted by an amino acid chosen among Val, Gly, Ala, Gln, Asp, Asn, Lys, Arg and Ser. Preferably, Glu333 is substituted by Ala.

[00212] In an embodiment, the nucleic acid sequence encodes a variant Fc region wherein Pro396 is substituted by Leu.

[00213] In an embodiment, the nucleic acid sequence encodes a variant Fc region wherein Phe243 is substituted by Leu, Arg292 is substituted by Pro, Tyr300 is substituted by Leu, Val305 is substituted by Leu, Lys326 is substituted by Ala and Pro396 is substituted by Leu.

[00214] In an embodiment, the nucleic acid sequence encodes a variant Fc region wherein Phe243 is substituted by Leu, Arg292 is substituted by Pro, Tyr300 is substituted by Leu, Val305 is substituted by Leu, Lys326 is substituted by Ala, Glu333 is substituted by Ala, and Pro396 is substituted by Leu.

[00215] In an embodiment, the nucleic acid sequence comprises the CDRs or variable regions according to the invention and a nucleic acid coding for an Fc region having the sequence depicted on SEQ ID NO: 1 or 3.

[00216] In an embodiment, the nucleic acid sequence comprises the VH CDRs as mentioned above and a nucleic acid coding for an Fc region having the sequence depicted on SEQ ID NO: 1 or 3.

[00217] In an embodiment, the nucleic acid comprises a nucleic acid sequence as depicted on SEQ ID NO: 30 and a nucleic acid coding for an Fc region having the sequence depicted on SEQ ID NO:1 or 3.

[00218] In another embodiment, the nucleic acid comprises a nucleic acid sequence as depicted on SEQ ID NO: 34 and a nucleic acid coding for an Fc region having the sequence depicted on SEQ ID NO:1 or 3.

[00219] In an embodiment, the nucleic acid comprises a nucleic acid sequence as depicted on SEQ ID NO: 32, and a nucleic acid coding for a human Kappa or Lambda region.

[00220] In another embodiment, the nucleic acid comprises a nucleic acid sequence as depicted on SEQ ID NO: 36, and a nucleic acid coding for a human Kappa or Lambda region.

[00221] Another object of the invention is the use of these expression vectors or a set of expression vectors containing nucleic acid sequence(s) according to the invention and regulatory sequences allowing thefor the expression of the VH and VL regions of the anti-CD19 antibody in mammalian cells, in particular CHO cells, preferably of the wild type.

[00222] Another object of the invention is a host cell containing a vector or a set of vectors of the invention. The host cell may be a mammal cell, preferably a rodent cell, more preferably CHO cell. Still more preferably, the host cell may be a wild-type mammal cell, preferably a wild-type rodent cell, most preferably a wild-type CHO cell.

[00223]   The person skilled in the art fully owns the methods to generate the antibodies according to the invention using such a vector or vectors and cells such as CHO cells.

[00224]   Another object of the invention is a molecule or a polypeptide or an antibody which comprises the CDRs derived from R005-1 or R005-2 and has the property of binding specifically to the CD19 antigen. The antibody may be a monoclonal antibody, a chimeric antibody, a humanized antibody, a full human antibody or an antibody drug conjugate. It may be also an antibody fragment.

[00225]   The antibody fragment may be a molecule comprising or consisting essentially of one or two VH and VL regions, wherein the VH comprises the CDRs of SEQ ID NO: 16, 6, 13, or 5, 6, 7, or 11, 12, 13 and the VL comprises the CDRs of SEQ ID NO: 8, 9, 10, or 14, 15, 10. This molecule or antibody may be used for targeting the CD19 antigen in the treatment of disease. The remaining of the description fully applies to these molecules and antibodies, for their use in therapy.

[00226]   The antibody fragment may be a molecule comprising or consisting essentially of one or two VH and VL regions, wherein the VH comprises the CDRs of SEQ ID NO: 28, 18, 25, or 17, 18, 19 or 23, 24, 25 and the VL comprises the CDRs of SEQ ID NO: 20, 21, 22, or 26, 27, 22. This molecule or antibody may be used for targeting the CD19 antigen in the treatment of disease. The remaining of the description fully applies to these molecules and antibodies, for their use in therapy.

[00227]   Another object of the invention is thus a nucleic acid sequence which encodes a polypeptide containing one, two, preferably all the CDRs according to SEQ ID NO: 16, 6, 13, or 5, 6, 7, or 11, 12, 13.

[00228]   In an embodiment of the sequence or vector, the variable region VH or VL comprise the nucleic acid sequence as depicted on SEQ ID NO: 30 or 32 or a nucleic acid sequence coding for an amino acid sequence as depicted on SEQ ID NO: 29 or 31.

[00229]   In an embodiment, the nucleic acid sequence for the VH also encodes an Fc region, in particular a mutated Fc region according to the invention on SEQ ID NO: 1 or 3.

[00230]   Another object of the invention is thus a nucleic acid sequence which encodes a polypeptide or amino acid sequence containing one, two, preferably all the CDRs according to SEQ ID NO: 28, 18, 25, or 17, 18, 19, or 23, 24, 25.

[00231]   In an embodiment of the sequence or the vector, the variable region VH or VL comprises the nucleic acid sequence as depicted on SEQ ID NO: 34 or 36 or a nucleic acid sequence coding for an amino acid sequence as depicted on SEQ ID NO: 33 or 35.

[00232]   In an embodiment, this nucleic acid sequence also encodes a Kappa region, in particular a human Kappa region.

[00233] Another object of the invention is an expression vector containing such a nucleic acid sequence and regulatory sequences to allow expression of the polypeptide encoded by the nucleic acid sequence. Still another object is a host cell transfected with such a vector.

[00234] Another object of the invention is a composition containing an anti-CD19 antibody according to the invention and a vehicle.

[00235] In an embodiment, the invention concerns a pharmaceutical composition containing an anti-CD19 antibody according to the invention and a physiologically acceptable vehicle or excipient.

[00236] In an embodiment, said composition comprises at least one other antibody. This supplemental or other antibody may be an anti-CD19 antibody or an antibody directed against another tumoral antigen. This other tumoral antigen may be CD20, CD52, CD22, EGF receptor, VEGF receptor, mimics ganglioside, GD3, CEA, HER-2.

[00237] In an embodiment the composition comprises an anti-CD20 antibody.

[00238] In an embodiment the composition comprises an anti-CD52 antibody.

[00239] This other tumoral antigen may be in particular a murine monoclonal antibody, a chimeric antibody, a humanized antibody, a full human antibody.

[00240] In a particular embodiment, the pharmaceutical composition comprises an anti-CD19 antibody according to the invention and an anti-CD20 antibody. Among the anti-CD20 antibodies, one may mention in particular rituximab.

[00241] Another object of the invention is such a pharmaceutical composition, comprising at least two antibodies, including an anti-CD19 antibody according to the invention, and a physiologically acceptable vehicle or excipient, for a simultaneous, separated or delayed administration. In this case each one of the active principles is separately conditioned in a pharmaceutically acceptable vehicle.

[00242] Another object of the invention is a pharmaceutical composition comprising an anti-CD19 antibody according to the invention, as an anti-tumoral medicament.

[00243] Still another object of the invention is a pharmaceutical composition comprising an anti-CD19 antibody according to the invention and at least one other antibody directed against another tumoral antigen, such as CD20, as an anti-tumoral medicament.

[00244] Still another object of the invention is a pharmaceutical composition comprising an anti-CD19 antibody according to the invention and at least one chemotherapy drug. This drug may be endoxan, cyclophosphamide, doxorubicine, vincas alcaloides, steroides, platines (cisplatine, carboplatine, oxaliplatine), aracytine, bleomycine, etoposide, bendamustine … In an embodiment, the additional drug is doxorubicin.

[00245] In an embodiment, the composition comprises both active principles in the same composition, with a pharmaceutically acceptable vehicle.

[00246] Another object of the invention is such a pharmaceutical composition, comprising an anti-CD19 antibody according to the invention and an additional drug, and a physiologically acceptable vehicle or excipient, for a simultaneous, separated or delayed administration. In this case each one of the active principles is separately conditioned in a pharmaceutically acceptable vehicle.

[00247] In an embodiment, these compositions are for use in the treatment of B-cells lymphomas. In an embodiment, these compositions are for use in the treatment B-cell non-Hodgkin's lymphoma (NHL), B-cell chronic lymphocytic leukaemia (B-CLL), and hairy cell leukaemia and B-cell acute lymphocytic leukaemia (B-ALL).

[00248] Still another object of the invention is a method for the treatment of a patient in need thereof, comprising administering an antibody or a pharmaceutical composition according to the invention. The method aims at treating or preventing cancer or an inflammation or an autoimmune disease.

[00249] In some embodiments, the antibodies of the invention may be administered in combination with a therapeutically or prophylactically effective amount of one or more anti-cancer agents, therapeutic antibodies or other agents known to those skilled in the art for the treatment and / or prevention of cancer, inflammation and / or autoimmune diseases.

[00250] In an embodiment, the method aims at treating or preventing B-cell disorders, such as but not limited to, B-cell malignancies. B-cell malignancies include NHL, B-CLL, hairy cell leukaemia and B-ALL.

[00251] In an embodiment, the method is applied to refractory or relapsed patients treated with anti-CD20 antibody.

[00252] In an embodiment, the method aims at treating or preventing autoimmune disease, such as multiple sclerosis, rheumatoid arthritis and SLE.

[00253] . In an embodiment, the method is applied to refractory or relapsed patients treated with anti-CD20 antibody.

[00254] In an embodiment, the method aims at treating or preventing graft-versus host diseases (GVHD), humoral rejection, or post-transplantation lymphoproliferative disorder in human transplant recipients. In an embodiment, the method is applied to refractory or relapsed patients treated with anti-CD20 antibody.

[00255] In an embodiment, the method comprises the administration to the patient of an anti-CD19 antibody according to the invention and an antibody directed against another tumoral antigen. This other tumoral antigen may be CD20, CD52, CD22, EGF receptor, VEGF receptor, mimics ganglioside GD3, CEA, HER-2, This other tumoral antigen may be in particular a murine monoclonal antibody, a chimeric antibody, a humanized antibody, a full human antibody, a drug-conjugate antibody. In an embodiment the composition comprises

an anti-CD20 antibody. In another embodiment the composition comprises an anti-CD52 antibody.

[00256] In another embodiment, the method comprises the administration to the patient of an anti-CD19 antibody according to the invention, wherein the patient is, has been or will be treated with a chemotherapy drug. This drug may be for example endoxan, cyclophosphamide, doxorubicine, vincas alcaloides, steroides, platines (cisplatine, carboplatine, oxaliplatine), aracytine, bleomycine, etoposide, bendamustine .... In an embodiment, the additional drug is doxorubicin. Preferably, the patient is treated with both the antibody according to the invention and the additional drug. The antibody and the drug may be administered in the same composition or in separate compositions.

[00257] **BRIEF DESCRIPTION OF THE DRAWINGS**

[00258] **Figure 1:** CD19 epitope mapping. Cell lysat was used as CD19 antigen source by testing different combination MAbs used as tracer (biotinylated MAb) or as catcher MAb (purified MAb) respectively.

[00259] **Figure 2:** Higher level of apoptosis with the murine MAb anti-CD19 R005-1 in Burkitt's lymphoma cell line. The Burkitt's lymphoma cell line Raji was incubated with different MAb concentration for 5 hours. B-cells were double-stained with annexin V-FITC and propidium iodide (PI), and analyzed by flow cytometry. Mean +/- SD are shown from two independent experiments.

[00260] **Figure 3:** The murine MAb R005-1 is one of the best inducer of apoptosis in primary B-CLL cells. Primary B-CLL cells were isolated after ficoll centrifugation. 4×10^6 cells were incubated with interest MAbs and controls for 24 hours at 37°C in complete media. Then cells were harvested and stained with Annexin-V FITC/Propidium Iodide. 20 000 cells were acquired on LSRII cytometer (BD bioscience, USA) and analysis was done on FlowJo software. Apoptotic cells were defined as annexin V^+/ PI⁻ cells. Data represents the mean +/- SD of five independent experiments.

[00261] **Figure 4:** The nucleotide and amino acids sequences of the murine MAbs R005-1 and R005-2 MAbs, (A): V_H (B): VL. Amino acids are shown as one-letter codes.

	Amino acid sequence VH	Nucleic acid sequence VH	Amino acid sequence VL	Nucleic acid sequence VL
mR005-1	SEQ ID NO: 29	SEQ ID NO: 30	SEQ ID NO: 31	SEQ ID NO: 32
mR005-2	SEQ ID NO: 33	SEQ ID NO: 34	SEQ ID NO: 35	SEQ ID NO: 36

[00262] **Figure 5:** Comparative staining between the native chimeric R005-1 Fc0 and the murine parental mR005-1 MAb on PBMNC cells. Grey lines designated cytofluorometric

histograms of negative isotype murine IgG1 or human IgG1 control MAb and black lines showed cytofluorometric histograms obtained peripheral blood lymphocytes or monocytes at 5 µg/ml for 1.10^6 cells/ml. Representative experiments on three independent experiments.

[00263] **Figure 6:** Cross blocking experiment between the native chimeric R005-1 Fc0 and the murine parental mR005-1 MAb. Cells were pretreated or not with the native chR005-1 Fc0 or with hIgG1 control MAb (5 µg/ml) before the staining with 10 µl/well of FITC conjugated mR005-1 on Raji Burkitt's lymphoma cells. Grey lines designated cytofluorometric histograms of negative isotype mouse IgG1 control MAb and black lines showed cytofluorometric histograms obtained with the FITC conjugated mR005-1. Data represent one representative experiment on three independent experiments.

[00264] **Figure 7:** The cell labelling with the parental mR005-1 or chR005-1 Fc0 MAb triggered a down modulation of CD19 expression level. B-CLL cells were incubated with biotinylated MAbs at 4°C for 30 min. At T0, T3h, and T24h, cells were stained with streptavidin-Alexa 488 nm (final dilution 1/1000) 30 min at 4°C and fixed in formaldehyde 1%. 20 000 cells were then acquired on LSRII cytometer (BD bioscience, USA) and FlowJo analysis were done. Data represent one representative experiment of nine independent experiments.

[00265] **Figure 8:** The chR005-1 Fc0 MAb display a modest ADCC activity on Burkitt's lymphoma cells. Calcein-AM loaded Raji cells (1×10^5 cells/ml) were incubated with interest MAbs for 20 min at 4°C. Effector cells (PBMNC) were added at the ratio E/T 50:1 for 4 hours at 37°C under shaking condition. After centrifugation, supernatants were harvested and calcein-AM fluorescence was measured on fluorometer. ADCC lysis level was calculated following the formula: (experimental release − (target + effector spontaneous release) / (maximal release − target spontaneous release)*100. Maximal release value was obtained by treating target cells with Triton X-100. Data represent mean +/- SD of three independent experiments.

[00266] **Figure 9:** The chR005-1 Fc0 mab display a modest ADCC activity on primary B-CLL cells. Primary B-CLL cells were isolated after ficoll centrifugation. 1×10^5 cells/ml calcein-AM loaded cells were incubated with interest MAbs for 20 min at 4°C. Effector cells (PBMNC) were added at the ratio E/T 50:1 for 4 hours at 37°C under shaking condition. After centrifugation, supernatants were harvested and calcein-AM fluorescence was measured on fluorometer. ADCC lysis level was calculated following the formula: (experimental release − (target + effector spontaneous release)) / (maximal release − target spontaneous release)*100. Maximal release value was obtained by treating target cells with Triton X-100. Data represent mean +/- SD of five independent experiments.

[00267]   **Figure 10:** The chR005-1 Fc0 did not trigger CDC activity on Burkitt's lymphoma cells. Raji cells (2.10^6 cells/ml) were incubated with interest MAbs for 20 min at 4°C. Then 5 µl of natural human complement was added for 4 hours at 37°C under shaking condition. After incubation, supernatants were harvested and lactate deshydrogenase (LDH) was measured on fluorometer. CDC lysis level was calculated following the formula: (experimental release − target spontaneous release) / (maximal release − target spontaneous release)*100, where target without natural complement represented spontaneous release. Maximal release value was obtained by treating target cells with Triton X-100. Data represent mean +/- SD of three independent experiments.

[00268]   **Figure 11:** The amino acids and nucleic acid sequences of chimeric Fc24 (SEQ ID NO: 3 and 4) or Fc34 (SEQ ID NO: 1 and 2) variant MAb. Amino acids are shown as one-letter codes. According to the literature, the amino acid numbering of Fc region is based to the Kabat® data base, (CH1: aa n°118 to 215; Hinge: aa n°216 to 230; CH2: aa n°231 to 340; CH3: aa n°341 to 447).

Variations between the various Fc used in the invention with respect to native Fc (Fc0):

Name of the mutant	Mutations with respect to Fc0
Fc34	F243L/R292P/Y300L/V305L/K326A/P396L
Fc24	F243L/R292P/Y300L/V305L/ K326A/E333A/P396L
Fc39	F243L/R292P/Y300L/V305I/ K326A/E333A/P396L
Fc18	F243L
Fc28	Y300L/V305L
Fc19	F243L/R292P/P396L
Fc29	F243L/R292P/V305L/P396L
Fc30	F243L/R292P/Y300L/P396L
Fc20	F243L/R292P/Y300L/V305L/P396L
Fc23	F243L/R292P/Y300L/V305L/E333A/P396L
Fc6	E333A
Fc7	K326A/E333A
Fc9	K326A

[00269]   **Figure 12:** Enhanced ADCC activity with the chR005-1 Fc24 or chR005-1 Fc34 MAb on Burkitt's lymphoma cells in the presence of PBMNC as effector cells. Calcein-AM loaded Raji cells (1×10^5 cells/ml) were incubated with interest MAbs for 20 min at 4°C. Effector cells (PBMNC) were added at the ratio E/T 50:1 for 4 hours at 37°C under shaking condition. After centrifugation, supernatants were harvested and calcein-AM fluorescence was measured on fluorometer. ADCC lysis level was calculated following the formula:

(experimental release – (target + effector spontaneous realease)) / (maximal release – target spontaneous release)*100. Maximal release value was obtained by treating target cells with Triton X-100. Data represent mean +/- SD of four independent experiments.

[00270] **Figure 13**: The chR005-1 Fc24 mediated strong ADCC activity on primary B-CLL cells. Primary B-CLL cells were isolated after ficoll centrifugation. (1×10^5 cells/ml) calcein-AM loaded Raji cells were incubated with interest MAbs for 20 min at 4°C. Effector cells (PBMNC) were added at the ratio E/T 50:1 for 4 hours at 37°C under shaking condition. After centrifugation, supernatants were harvested and calcein-AM fluorescence was measured on fluorometer. ADCC lysis level was calculated following the formula: (experimental release – (target + effector spontaneous release)) / (maximal release – target spontaneous release)*100. Maximal release value was obtained by treating target cells with Triton X-100. Data represent mean +/- SD of five independent experiments.

[00271] **Figure 14:** The chR005-1 Fc24 or the chR005-1 Fc34 MAb induced CDC activity on Burkitt's lymphoma cell. Raji cells (2.10^6 cells/ml) were incubated with interest MAbs for 20 min at 4°C. Then 5 µl of natural human complement was added for 4 hours at 37°C under shaking condition. After incubation, supernatants were harvested and lactate deshydrogenase (LDH) was measured on fluorometer. CDC lysis level was calculated following the formula: (experimental release - spontaneous release) / (maximal release – spontaneous release)*100, where target without natural complement represented spontaneous release. Maximal release value was obtained by treating target cells with Triton X-100. Data represent mean +/- SD of three independent experiments.

[00272] **Figure 15:** The chR005-2 Fc24 also induced CDC activity on Burkitt's lymphoma cell. Raji cells (2.10^6 cells/ml) were incubated with interest MAbs for 20 min at 4°C. Then 5 µl of natural human complement was added for 4 hours at 37°C under shaking condition. After incubation, supernatants were harvested and lactate deshydrogenase (LDH) was measured on fluorometer. CDC lysis level was calculated following the formula: (experimental release - spontaneous release) / (maximal release – spontaneous release)*100, where target without natural complement represented spontaneous release. Maximal release value was obtained by treating target cells with Triton X-100. Data represent mean +/- SD of one independent experiment.

[00273] **Figure 16:** The chR005-1 Fc24 MAb mediated strong CDC activity on primary B-CLL cells. B-CLL cells ($2-10^6$ cells/ml) were incubated with interest MAbs for 20 min at 4°C. Then 5 µl of natural human complement was added for 4 hours at 37°C under shaking condition. After incubation, supernatants were harvested and lactate deshydrogenase (LDH) was measured on fluorometer. CDC lysis level was calculated following the formula: (experimental release – spontaneous release) / (maximal release – spontaneous release)*100, where target without natural complement represented spontaneous release.

Maximal release value was obtained by treating target cells with Triton X-100. Data represent mean +/- SD of one independent experiment.

[00274]   **Figure 17:** Recombinant human C1q binding on chR005-1 wild type or mutant MAb. The binding of human recombinant C1q was assessed by an ELISA binding assay. The murine parental MAb mR005-1 was used as negative control and Rituximab as positive control. Data represent mean +/- SD of two independent experiments.

[00275]   **Figure 18:** Fc24 or Fc34 engineering of anti-CD19 chR005-1 did not influence PCD in Burkitt's lymphoma cell line. The Burkitt's lymphoma cell line Raji was incubated with different MAb concentration for 5 hours. B-cells were double-stained with annexin V-FITC and propidium iodide (PI), and analyzed by flow cytometry. Data represent mean +/- SD of two independent experiments.

[00276]   **Figure 19:** The chR005-1 Fc24 or Fc34 engineering of anti-CD19 chR005-1 did not influence PCD in primary B-CLL cells. Cells were incubated with different MAb concentration for 5 hours. B-cells were double-stained with annexin V-FITC and propidium iodide (PI), and analyzed by flow cytometry. Data represent mean +/- SD of four independent experiments.

[00277]   **Figure 20:** The mR005-1 MAb combined with chimeric anti-CD20 (Rituximab) synergized their apoptotic effect in Burkitt's lymphoma cells. Daudi cells (4×10^4 cells) were incubated with interest MAbs in combination or not, and controls for 5 hours at 37°C in complete media. B-cells were double-stained with annexin V-FITC and propidium iodide (PI), and analyzed by flow cytometry. Data represent mean +/- SD of three independent experiments.

[00278]   **Figure 21:** The chR005-1 Fc24 or the chR005-1 Fc34 MAb combined with chimeric anti-CD20 (Rituxan®) synergized their apoptotic effect in Burkitt's lymphoma cells. Daudi cells (4×10^4 cells) were incubated with interest MAbs in combination or not, and controls for 5 hours at 37°C in complete media. B-cells were double-stained with annexin V-FITC and propidium iodide (PI), and analyzed by flow cytometry. Data represent mean +/- SD of two independent experiments.

[00279]   **Figure 22:** The parental mR005-1 combined with chimeric anti-CD20 (Rituximab®) synergized their apoptotic effect in primary B-CLL cells. Primary B-CLL cells were isolated after ficoll centrifugation. B-CLL (4×10^4 cells) were incubated with interest MAbs in combination or not, and controls for 24 hours at 37°C in complete media. B-cells were double-stained with annexin V-FITC and propidium iodide (PI), and analyzed by flow cytometry. Data represent mean +/- SD of seven independent experiments.

[00280]   **Figure 23:** The chR005-1 Fc24 or the chR005-1 Fc34 combined with chimeric anti-CD20 (Rituxan®) synergized their apoptotic effect in primary B-CLL cells. Primary B-CLL

were isolated after ficoll centrifugation. B-CLL (4×10^4 cells) were incubated with interest MAbs in combination or not, and controls for 24 hours at 37°C in complete media. B-cells were double-stained with annexin V-FITC and propidium iodide (PI), and analyzed by flow cytometry. Data represent mean +/- SD of four independent experiments.

[00281]   **Figure 24:** The murine MAb R005-1 triggered apoptosis in primary B-CLL cells Rituxan® refractory. Primary B-CLL cells were isolated after ficoll centrifugation. 4×10^6 cells were incubated with interest MAbs and controls for 24 hours at 37°C in complete media. Then cells were harvested and stained with Annexin-V FITC/Propidium Iodide. Apoptotic cells were defined as annexin V^+/ PI^- cells. Data represent mean +/- SD of two independent experiments.

[00282]   **Figure 25:** The MAb chR005-1 Fc24 or the MAb chR005-1 Fc34 triggered apoptosis in primary B-CLL cells Rituxan® refractory. Primary B-CLL were isolated after ficoll centrifugation. B-CLL (4×10^4 cells) were incubated with interest MAbs in combination or not, and controls for 24 hours at 37°C in complete media. B-cells were double-stained with annexin V-FITC and propidium iodide (PI), and analyzed by flow cytometry. Data represent one representative experiment.

[00283]   **Figure 26:** Similar level of ADCC was observed whatever FcγRIIIA allotypes F/F or V/V. Lymphocytes staining was performed with the MAb unconjugated 3G8 or MEM-154. For each FcγRIIIA - 158 polymophism (VV or FF). Data represent mean +/- SD of two independent experiments.

[00284]   **Figure 27:** General characteristics of iDD biotech MAb Heavy or Light chain expression vector. The empty CHO cells were co-transfected with the pcDNA3.3-TOPO expression vector for light chain (Invitrogen) and with the pcDNA3.3 expression vector for heavy chain (Invitrogen) following transient transfection procedure established in our laboratory.

[00285]   **Figure 28:** Glycosylation profile of the MAb chR005-1 Fc0. Antibody oligosaccharides released by PNGase F digestion were analyzed using a MALDI-TOF Voyager DE PRO spectrometer. The m/z value corresponds to the sodium-associated oligosaccharide ion. The sugar composition of each peak shown is detailed in Figure 32. The schematic oligosaccharide structure of each major peak is illustrated on the right side of the charts: GlcNAc (closed circles), mannose (open squares), galactose (open diamonds) and fucose (open triangles).

[00286]   **Figure 29:** Glycosylation profile of the MAb chR005-1 Fc24. Antibody Fc oligosaccharides released by PNGase F digestion were analyzed using a MALDI-TOF MS spectrometer Reflex III. The m/z value corresponds to the sodium-associated oligosaccharide ion. The sugar composition of each peak shown is detailed in Figure 32. The

schematic oligosaccharide structure of each major peak is illustrated on the right side of the charts: GlcNAc (closed circles), mannose (open squares), galactose (open diamonds) and fucose (open triangles).

[00287] **Figure 30:** Glycosylation profile of the MAb chR005-1 Fc24. Antibody Fc oligosaccharides released by PNGase F digestion were analyzed using a MALDI-TOF MS spectrometer Reflex III. The m/z value corresponds to the sodium-associated oligosaccharide ion. The sugar composition of each peak shown is detailed in Figure 32. The schematic oligosaccharide structure of each major peak is illustrated on the right side of the charts: GlcNAc (closed circles), mannose (open squares), galactose (open diamonds) and fucose (open triangles).

[00288] **Figure 31:** Assignment of carbohydrate structures by comparison with glycan standards following capillary electrophoresis with laser-induced fluorescence detection relative intensities of N-glycan types between the parental chR005-1 Fc0 and the optimized chR005-1 Fc24 or Fc34 MAbs expressed in wild type CHO cells.

[00289] **Figure 32:** Oligosaccharide analysis of variants MAbs anti-CD19. Comparison of relative intensities of N-glycan types between the parental chR005-1 Fc0 and the optimized chR005-1 Fc24 or Fc34 MAbs expressed in wild type CHO cells.

[00290] **Figure 33:** Glycan profiles according Fc variants. IgG N-linked glycans of chR005-1 Fc variant antibodies produced from the wild type CHO Easy C cells. Molecular mass are permethylated glycans, detected as [M+Na]+ by Maldi mass spectrometry (A-B). The MAb panel was produced from CHO Easy C cells. Different glycosylation profiles among the Fc variant antibody panel were observed. One representative experiment.

[00291] **Figure 34:** Differential MAb activity according Fc variant.

A- Calcein-AM loaded Raji cells (1×10^5 cells/mL) were incubated with interest MAbs for 20 min at 4°C. Effector cells (PBMNC) were added at the ratio E/T 50:1 for 4 hours at 37°C under shaking condition. After centrifugation, supernatants were harvested and calcein-AM fluorescence was measured on fluorometer. ADCC lysis level was calculated following the formula: [(experimental release − (target + effector spontaneous release)) / (maximal release − target spontaneous release) * 100]. Maximal release value was obtained by treating target cells with Triton X-100. Data represent mean +/- SD of five independent experiments.

B- Raji cells ($2\text{-}10^6$ cells/ml) were incubated with interest MAbs for 20 min at 4°C. Then 5 µl/well of natural human complement was added for 4 hours at 37°C under shaking condition. After incubation, supernatants were harvested and lactate deshydrogenase (LDH) was measured on fluorometer. CDC lysis level was calculated following the formula: (experimental release − target spontaneous release) / (maximal release − target spontaneous release) * 100, where target without natural complement represented

spontaneous release. Maximal release value was obtained by treating target cells with Triton X-100. Data represent mean +/- SD of three independent experiments.

[00292]   **Figure 35:** Differential MAb activity according Fc variant.

A- Calcein-AM loaded Raji cells (1x10⁵ cells/mL) were incubated with interest MAbs for 20 min at 4℃. Effector cells (PBMNC) were added at the ratio E/T 50:1 for 4 hours at 37℃ under shaking condition. After centrifugation, supernatants were harvested and calcein-AM fluorescence was measured on fluorometer. ADCC lysis level was calculated following the formula: [(experimental release – (target + effector spontaneous release)) / (maximal release – target spontaneous release) * 100]. Maximal release value was obtained by treating target cells with Triton X-100. Data represent mean +/- SD of three independent experiments.

B- Calcein-AM loaded Raji cells (1x10⁵ cells/mL) were incubated with interest MAbs for 20 min at 4℃. 50 µl per well of effector cells from whole blood were added for 4 hours at 37℃ under shaking condition. After centrifugation, supernatants were harvested and calcein-AM fluorescence was measured on fluorometer. ADCC lLysis level was calculated following the formula: [(experimental release – (target + effector spontaneous release)) / (maximal release – target spontaneous release) * 100]. Maximal release value was obtained by treating target cells with Triton X-100. One representative experiment.

[00293]   **Figure 36:** Differential MAb activity according Fc variant. Calcein-AM loaded Raji cells (1x10⁵ cells/mL) were incubated with interest MAbs for 20 min at 4℃. 50 µl per well of effector cells from whole blood were added for 4 hours at 37℃ under shaking condition. After centrifugation, supernatants were harvested and calcein-AM fluorescence was measured on fluorometer. ADCC lysis level was calculated following the formula: [(experimental release – (target + effector spontaneous release)) / (maximal release – target spontaneous release) * 100]. Maximal release value was obtained by treating target cells with Triton X-100. One representative experiment.

[00294]   **Figure 37:** Inhibition of tumour growth in vivo by the chR005-1 MAb Fc24 or chR005-1 Mab Fc34. Mice injected with Raji cells subcutaneously were treated by intravenous MAb infusion once a week starting on day 21 at 100 mg / kg with or without leukocytes. The tumors were measured twice a week.

[00295]   **Figure 38:** Complement human serum binding on chR005-1 wild type or mutant MAb. The binding of human complement serum to MAbs was assessed by an ELISA binding assay. The 96-well plates (Nunc) were coated overnight at 4℃ with varying MAb concentrations. After washing, the plates were blocked with PBS - 5% BSA for 1h, and incubated for 1h with 2.5 µl of natural human complement (Sigma). Then, 100 µl of a 1/500 dilution of sheep anti-human C1q peroxidase-conjugated Ab (Abd Serotec) added and incubated for 1h. The plates were developed with 100 µl per well of TMB substrate (Uptima

Interchim). After H_2SO_4 addition, the OD was measured at 450nm / 630 nm using a MRX II microplate reader. Data represent mean +/- SD of two independent experiments.

[00296]   **Figure 39:** The growth of Rituxan refractory RL established tumor was inhibited with the Fc24 optimized chR005-1 MAb. Mice injected with refractory Rituxan RL cells subcutaneously were treated by intravenous MAb infusion once a week starting on day 20 at 100 mg / kg.

[00297]   **Figure 40:** The combination of the Fc24 optimized chR005-1 MAb and the drug Doxorubicine increased the level of growth inhibition Raji established tumor. Mice bearing established SC Burkitt's lymphoma tumors were treated weekly. The control group received vehicle (NaCl 0.9%) while the treated groups received one of the following: chR005-1 Fc24 MAb 100 mg/kg, doxorubicine 2 mg/kg, chR005-1 Fc24 MAb + doxorubicine.

[00298]   **MATERIALS AND METHODS**

[00299]   Cells: The CHO dhfr$^{-/-}$ cell line was obtained for research purpose from ATCC (American Type Culture Collection, USA). Burkitt's lymphoma Raji or Daudi cell lines as the T lymphoma Cem cells were obtained from ECACC (European Collection of Cell Culture, UK). Human PBMNCs were purified from leukapheresis of anonymous healthy volunteer donors (Blood Center, Lyon France) using Ficoll-Histopaque density gradient (Sigma, Saint Quentin Fallavier, France). All B-CLL patients enrolled in this study had been defined immunophenotypically as outlined by criteria from the National Cancer Institute Working Group in 1996 (Hallek *et al.,* 2008). Blood was obtained from patients after written informed consent in accordance with the Declaration of Helsinki.

[00300]   Reagents and antibodies: The IgG1 chimeric negative control was produced at iDD biotech (Dardilly, France). The murine parental MAb mR005-1 or mR005-2 were isolated from iDD biotech hybridoma library. The wild type chR005-1 Fc0 (also called native IgG1) and all the modified antibodies were generated and produced by iDD biotech (Dardilly, France). FITC labelled annexin-V and propidium iodide (PI) were purchased from BD Biosciences (Pont de Claix, France) and Sigma (Saint Quentin Fallavier, France), respectively. Goat Fab' 2 anti-human RPE IgG antibody and Goat anti-mouse FITC Ig antibody were respectively purchased by Sigma (Saint Quentin Fallavier, France) and MP Biomedical (Illkirch, France). Rituximab were produced by Genentech and purchased commercially. Others MAbs anti-CD19 4G7 (IgG1), Bu12 (IgG1), HD37 (IgG1), B4 (IgG1) were purchased by Santa Cruz Biotechnology (California, USA), Abd Serotec (Düsseldorf, Germany), Santa Cruz Biotechnology (California, USA), Biogenex (San Ramon, USA) respectively. The Camptothecin used as positive control for apoptosis assays was purchased by Sigma (Saint Quentin Fallavier, France).

[00301] Murine antibody generation: The Balb/c mice were immunised with CD19 expressing cells such as human chronic lymphoid leukaemia cells. Murine MAbs were generated using standard hybridoma techniques (Zola *et al.*, 1987) and screened initially for their ability to bind by flow cytometry only CD19 positive cell line. Purified MAbs were produced following ascitis purification then purified by using protein-A Sepharose (Pharmacia, Uppsala, Sweden).

[00302] Conversion of murine MAb to native chimeric MAb: cDNA corresponding to the variable region of the hybridoma was obtained using two approaches, the first approach consist to the utilisation in PCR of the degenerate N-term amino acid related primer set generate since the N-Terminal sequencing and the second approach consist to the utilisation in PCR of degenerate primer set generate by IMGT® primer database and specific primers previously described (Essono *et al.*, 2003; Wang *et al.*, 2000). The sequence of N-terminal variable region was determined by Edman degradation. Total RNA extraction was carried out using the Tri Reagent kit according to the protocol described by the supplier Sigma. The amplified VL and VH fragments were cloned into the TOPO-TA cloning vector (Invitrogen) for sequence analyses by the dideoxytermination method (Sanger *et al.*, 1977). Then antibody variants constructs were amplified by PCR and cloned into the vector pcDNA3.3.

[00303] Construction of antibody variants: Substitutions in the Fc domain were introduced using "megaprimer" method of site-directed mutagenesis (Sarkar *et al.*, 1990). Positions are numbered according to the Kabat® index (Identical V region amino acid sequences and segments of sequences in antibodies of different specificities). Relative contributions of VH and VL genes, minigenes, and complementarity-determining regions to binding of antibody-combining sites were analyzed (Kabat *et al.*, 1991). Heavy and light chain constructs were co-transfected into CHO DG44 (ATCC) suitable for MAb screening. Antibodies were purified using protein A affinity chromatography (GE Healthcare).

[00304] CD19 MAb ELISA competition : For competition binding ELISA experiments, cell lysate was used as CD19 antigen source by testing different combination MAbs used as tracer (biotinylated MAb) or as catcher MAb (purified MAb) respectively.

[00305] FACS analysis of MAb binding to human CD19: The binding to human CD19 of all MAb generated in the present study were measured with a FACScan (BD Biosciences, Pont de Claix, France) by using the goat anti-mouse FITC Ig from Sigma (Saint Quentin Fallavier, France) for the detection of murine MAbs and with the goat anti-human PE Ig from Sigma (Saint Quentin Fallavier, France) for the detection of chimeric MAbs. For competition binding experiments, cells were pre-incubated with an excess of either the parental MAb or a mouse IgG1 isotype control antibody.

[00306] Laser scanning confocal microscopy by using biotinylated antibodies: Cell fluorescence has been visualized using an Inverted Zeiss Axiovert 100M LSM 510 Meta confocal microscope following cell labelling with biotinylated MAbs.

[00307] Glycosylation analysis: Release of *N*-glycans & permethylation were carried out following standard procedures (Ciucanu *et al.,* 1984). Analysis of protein glycosylation was determined by mass spectrometry (Morelle *et al.,* 2007).

[00308] Antibody affinity: Determination of antibody KD values was performed as previously described (Benedict *et al.,* 1997) by using binding assay analyzed by flow cytometry to detect cell-bound antibody.

[00309] Assessment of apoptosis by flow cytometry: The apoptosis of cells after incubation with antibodies was measured using annexin V / PI staining followed by FACS analysis. The apoptotic cells were determined in the gate showing a positive staining for annexin V and negative staining for propidium iodide.

[00310] Antibody dependent cell cytoxicity Assay (ADCC): Primary B-CLL cells or B-cell lines (Raji) (Target cells) were loaded with 12.5 µM Calcein-AM dye (Sigma, France). 5 000 target cells per well were the pre-incubated with different concentration of interest MAbs and controls for 20 min at +4°C. Effector cells were then added to the target cells at the ratio E/T equal to 50:1. Specific ADCC lysis was calculated using the formula above: (experimental release – (spontaneous release Target + Effector)) / (maximal release – spontaneous release target) * 100, where target and effector cells without antibody represented spontaneous release. Maximal release value was obtained by treating target cells with Triton X-100.

[00311] Complement dependent cytoxicity Assay (CDC): Target cells (50 000 cells per well) either primary B-CLL cells or B-cell lines (Ramos, Raji, and Daudi cell lines) were incubated with various MAbs concentration. Then, human normal serum were added the culture and then cells were incubated 4 hours at +37°C under shaking condition. At the end of incubation, lactate deshydrogenase present in supernatant was measured with LDH assay kit (Promega, France). Fluorescence was recorded at the 590 nm excitation wavelength. Specific CDC lysis was calculated using the formula above: (experimental release – target spontaneous release) / (maximal release – target spontaneous release) * 100, where target and effector cells without antibody represented spontaneous release. Maximal release value was obtained by treating target cells with Triton X-100.

[00312] Complement binding assay: The binding of human C1q to MAbs was assessed by an ELISA binding assay. The 96-well plates (Nunc) were coated overnight at 4°C with varying MAb concentrations. After washing, the plates were blocked with PBS-5% BSA for 1 h, and incubated for 1h with 0.2 µg/ml of recombinant human C1q (Abd Serotec) or 2.5 µl of

natural human complement (Sigma). Then, 100 µl of a 1/500 dilution of sheep anti-human C1q peroxidase-conjugated Ab (Abd Serotec) added and incubated for 1h. The plates were developed with 100 µl per well of TMB substrate (Uptima Interchim). After H_2SO_4 addition, the OD was measured at 450nm / 630 nmusing a MRX II microplate reader.

[00313]   Detection VV or FF polymorphism by flow cytometry: The FcγRIIIA – 158 V/F polymorphism was based on the MEM-154 / 3G8 fluorescence ratio as described (Böttcher *et al.*, 2005). Human blood samples (50 µl) were incubated 15 min in the dark at RT with 10 µg/ml of unconjugated 3G8 (Becton Dickinson, USA), MEM-154 (Abcam, USA) or with the isotype control MAb. 2 ml of a red blood lysing buffer (BD biosciences, USA) diluted at 1/10 is added during 5 min. After washes, goat anti-mouse FITC Ig from Sigma (Saint Quentin Fallavier, France) is added (100 µl diluted to 1/800). Cells were incubated a further 30 min at RT then washed twice and assayed using a FACScan (BD Biosciences, Pont de Claix, France).

[00314]   **RESULTS**

[00315]   1. CD19 epitope mapping.

[00316]   By ELISA competition, we determined the CD19 epitope mapping by using a MAb panel anti-CD19 including the MAb mR005-1, mR005-2, 4G7, B4, Bu12, HD37. As shown in Figure 1, a strong competition was observed by using as tracer / catcher MAbs the combination 4G7 / mR005-1 and HD37 / mR005-1 revealing that these antibodies recognized the same epitope or an adjacent epitope. By contrast, no significant competition was observed with the mR005-2 or BU12 MAbs.

[00317]   2. The parental murine MAb mR005-1 triggers apoptosis in Burkitt's lymphoma cell line or in primary B-CLL cells.

[00318]   The murine MAb mR005-1 was highly effective at inducing PCD than the murine MAb mR005-2 in Burkitt's lymphoma Raji cell line (Figure 2). It is tempting to suggest that the superior apoptotic effect of the mR005-1 MAb could be related to the fine specificity of these molecules. Our results also demonstrated that the mR005-1 MAb was more efficient to trigger PCD than rituximab.

[00319]   3. The murine MAb biological activity to trigger apoptosis is linked to epitope on CD19.

[00320]   A panel of murine MAb against human CD19 was tested to evaluate the apoptotic potential of various clones. We used the annexin-V/PI approach to determine the level of apoptosis induced by these antibodies at 24 hours post incubation. We have found that mR005-1 is one of the best MAbs as inducer of apoptotic process in B-CLL cells isolated from patients (Figure 3). Similar data was observed by using the Burkitt's lymphoma cell line

(data not shown). Our observations presented here suggest that treatment of B-CLL tumor cells with derivated chimeric MAb related to the mR005-1 MAb, which are capable of inducing an apoptotic signal, may contribute to their direct killing and elimination.

[00321] 4. Chimerisation of the murine MAb R005-1 and of the murine MAb R005-2.

[00322] In comparison with the IMGT® database, the sequence of VL mR005-1 and the sequence of VH mR005-1 were validated recognizing at least 96% and at least 92% respectively (Figure 4). The sequences of VL mR005-2 and VH mR005-2 were also validated (99% and 98%, respectively). The authenticity of the VH and VL sequences obtained by cDNA cloning were also confirmed by N-terminal amino acid sequencing of the target mouse monoclonal antibody. Heavy and light chains were separated before amino acid sequencing by polyacrylamide gel electrophoresis under reducing conditions and the the 20 first amino were sequencing by Edman degradation. Then sequences were then cloned in the Light chain expression vector (VL mR005-1 or VL mR005-2) and in the Heavy MAb expression vectors (VH mR005-2 or VH mR005-2) encoding also for a native Fc region named Fc0. Transient transfection on CHO dhfr$^{-/-}$ cells by lipofection was performed.

[00323] 5. Authenticity and selection of VH and VL chR005-1 chains confirmed by flow cytometry analysis.

[00324] The native chimeric MAb chR005-1 Fc0 constructed from the VH1 and VL1 sequences was compared directly with the parental murine MAb mR005-1 for staining and specificity. As shown in Figure 5, comparable staining was observed on peripheral blood lymphocytes. Cell binding competition between the murine parental mR005-1 and the native chR005-1 Fc0 was also showed in Figure 6. The chR005-1 Fc0 completely blocked the murine parental mR005-1 MAb binding.

[00325] 6. The induction of CD19 internalization following MAb binding is not a general effect and could be related to the different MAb anti-CD19 used.

[00326] By an indirect staining in flow cytometry, the presence or not of the naked antibody at B-CLL cell surface following incubation at 4°C or at 37°C was determined (Figure 7). A significant shift of geometric mean fluorescence intensity was observed with rituximab at 37°C. for an extended time period beyond 3 or 24 hours. Similar effect was noticed with the parental murine MAb mR005-1, although a lower modulation of geometric mean fluorescence intensity was observed with the wild type chimeric MAb chR005-1 Fc0.

[00327] 7. Characterization of the native chR005-1 Fc0 cytotoxicity activity.

[00328] In many applications, chimeric antibodies have demonstrated improved effector function in complement-mediated tumor cells lysis and in antibody-dependent cellular cytotoxicity assays as compared to the parental murine monoclonal antibody (Liu *et al.,* 1987; Nishimura *et al.,* 1987; Hamada *et al.,* 1990). The native chimeric MAb chR005-1 Fc0

induced modest ADCC against Burkitt's lymphoma cell line (Figure 8) or against *ex vivo* B-CLL cells from patients (Figure 9). In a standard cytotoxicity assay complement dependent, only rituximab used as positive control killed the Raji cells, whereas the chR005-1 Fc0 failed to trigger cell cytotoxicity (Figure 10).

[00329] 8. Generation of variants for human IgG1 C$_H$2 domain.

[00330] As used herein, the term "heavy chain" is used to define the heavy chain of an IgG antibody. In an intact, native IgG, the heavy chain comprises the immunoglobulin domains VH, CH1, Hinge, CH2 and CH3. Throughout the present specification, the numbering of the residues in an IgG heavy chain is that of the EU index (Kabat *et al*, 1991), expressly incorporated herein by references. The "EU index as in Kabat" refers to the numbering of the human IgGl EU antibody.

[00331] We constructed several variants including single, double, three, four or five substitution variants to enhance ability to mediate effector function, (Figure 11).

Fc34   LPLLAL   F243L / R292P / Y300L / V305L / K326A /P396L

Fc24   LPLLAAL   F243L / R292P / Y300L / V305L / K326A /E333A / P396L

[00332] 9. The Fc24 or Fc34 variant chR005-1 efficiently triggered ADCC.

[00333] The MAb activity to mediate ADCC from the MAb variant panel was measured using Raji target cells firstly in whole blood based assays (Figure 12). The potency and efficacy of the chR005-1 Fc24 or Fc34 MAb was notably higher when compared to parental chR005-1 Fc0.

[00334] The activity of chR005-1 Fc24 was also assessed by using *ex vivo* B-CLL cells from patients (Figure 13). Similar to observations with ADCC on Raji cell line, the potency and efficacy of the chR005-1 Fc20 MAb was notably higher when compared to parental chR005-1 Fc0.

[00335] 10. Investigation on the MAb variant panel to CDC using B-cell lymphoma.

[00336] As shown in Figure 14, the six mutants Fc34 (F243L / R292P / Y300L / V305L / K326A /P396L) or the seven mutants Fc24 (F243L / R292P / Y300L / V305L / K326A /E333A / P396L) generated from the wild type chR005-1 Fc0 induced CDC using the Raji cell line.

[00337] As shown in Figure 15, the five mutants Fc24 (F243L / R292P / Y300L / V305L / K326A /E333A / P396L) generated from the wild type chR005-2 Fc0 also induced CDC using the Raji cell line.

[00338] The six mutants Fc34 (F243L / R292P / Y300L / V305L / K326A /P396L) or the five mutants Fc24 (F243L / R292P / Y300L / V305L / K326A /E333A / P396L) generated from the wild type chR005-1 Fc0 also induced CDC when targeting *ex vivo* B-CLL (Figure 16).

[00339] 11. The variants chR005-1 Fc24 or Fc34 bound the complement with a higher efficient for the chR005-1 Fc34.

[00340]   The human or the natural complement present in serum (Figure 17) bound the variants chR005-1 Fc24 or chR005-1 Fc34 revealing a higher efficacy with the chR005-1 Fc34 variant.

[00341]   12. The MAb variants triggered similar level of cell apoptosis.

[00342]   The biological activity on programmed cell death of chR005-1 Fc24 or chR005-1 Fc34 MAb was tested on Raji CD19 positive cell line (Figure 18) or on *ex vivo* B-CLL (Figure 19). The Fc MAb engineering did not influence CD19 triggered apoptosis.

[00343]   13. Monoclonal antibodies may be used in combination.

[00344]   The MAb activity to mediate PCD alone or in combination was compared using Daudi target cells. A higher level of apoptotic cells was observed in the presence of murine parental mR005-1 (Figure 20) or with the variant Fc24 or Fc34 chR005-1 MAb (Figure 21), combined with rituximab demonstrated the feasibility and the benefit of the MAb combination.

[00345]   The MAb activity to mediate PCD alone or in combination was compared using on *ex vivo* B-CLL. A higher level of apoptotic cells was observed in the presence of the murine parental mR005-1 (Figure 22) or with the variant Fc24 or Fc34 chR005-1 MAb (Figure 23), combined with rituximab demonstrated the feasibility and the benefit of the MAb combination.

[00346]   14. The MAb directed against CD19 can be used in patient with rituximab recurrent and refractory disease.

[00347]   *Ex vivo* B-CLL samples from patients with recurrent and refractory disease following rituximab treatment were treated *in vitro* with the murine parental mR005-1 (Figure 24) or with MAb chR005-1 Fc24 or chR005-1 Fc34 (Figure 25). The higher level of apoptosis observed with MAbs against CD19 compared with rituximab demonstrated the feasibility and the efficacy to use MAbs directed another antigen than CD20.

[00348]   15. No influence of Fc polymorphism.

[00349]   Similar level of ADCC was observed whatever FcγRIIIA allotypes F/F or V/V. Lymphocytes staining was performed with the MAb unconjugated 3G8 or MEM-154 (Figure 26).

[00350]   16. MAb expression.

The empty CHO dhfr-/- cells (purchased by the ATCC collection) were co-transfected with the pcDNA3.3 expression vector for light chain and with the pcDNA3.3 expression vector for heavy chain following transient transfection procedure established in our laboratory. General characteristics of this MAb expression vector are shown in Figure 27. The empty CHO cells were co-transfected with the pcDNA3.3- expression vector for light chain (Invitrogen) and with the pcDNA3.3 expression vector for heavy chain (Invitrogen) following transient transfection procedure established in our laboratory. General characteristics of this research MAb expression vector are shown in Figure 27. By using the pcDNA3.3 vector, expression of

these chimeric antibody chains in mammalian cells was controlled by the full-length human CMV immediate early promoter/enhancer. Secretion of H and L chains were enabled by the respective human IgH leader sequence. And in the 3' region, a Herpes Simplex Virus thymidine kinase polyA tail allows for efficient induction and stabilization of mRNA. The coding regions for light and heavy chains of MAb anti-CD19 are introduced into the expression vector pcDNA3.3-TOPO in the TOPO cloning site. The transformants are analyzed for correct orientation and reading frame, the expression vector may be transfected into CHO cell line

[00351]   17. Generation of low fucosylated anti-CD19 MAb chR005-1.

[00352]   The sugar core found in the Fc region of IgG is a bi-antennary complex [Asn297-GN-GN-M-(M-GN)2] where GN is N-acetylglucosamine, and M is mannose. Oligosaccharides can contain zero (G0), one (G1) or two (G2) galactose (G). Variations of IgG glycosylation patterns can include core fucosylation (F). As shown in Figure 28 and 31. the three major peaks in the chR005-1 Fc0 sample correspond to masses of fucosylated oligosaccharides with (GlcNAc)2 (Fuc)1 + (Man)3 (GlcNAc)2 (m/z 1836), (Gal)1 (GlcNAc)2 (Fuc)1 + (Man)3(GlcNAc)2 (m/z 2040) and (Gal)2 (GlcNAc)2 (Fuc)1 + (Man)3(GlcNAc)2 (m/z 2245). By contrast, as shown in Figure 29-30, the two peaks G0F and G1F were present at much lower levels in the chR005-1 Fc24 or chR005-1 Fc34 antibody (7.2% or 5.2 % and 16.8% or 16.1% respectively) compared to the native chR005-1 Fc0 (28% or 38,3% respectively). No significant impact was observed on the peak G2F. A higher level of oligomannoses between $(Man)_5(GlcNAc)_2$ (m/z 1579), $(Man)_6(GlcNAc)_2$ (m/z 1784) $(Man)_7(GlcNAc)_2$ (m/z 1988), (Man)8(GlcNAc)2 (m/z 2192) and (Man)9(GlcNAc)2 (m/z 2396) was observed (29.9 % or 27.9% versus 0%) as also a higher level of sialylated glycoforms (1.6%% or 2.4% versus 0.8%). Including $(Gal)1 (GlcNAc)_2 (Fuc)_1 (NeuAc)_1 + (Man)_3(GlcNAc)_2.$ (m/z 2401), $(Gal)2 (GlcNAc)_2 (Fuc)_1 (NeuAc)_1 + (Man)_3(GlcNAc)_2.$ (m/z 2605) and $(Gal)3 (GlcNAc)_2 (Fuc)_1 (NeuAc)_1 + (Man)_3(GlcNAc)_2.$ (m/z 2966) in the chR005-1 Fc24 or chR005-1 Fc34 antibody compared to the native chR005-1 Fc0.

[00353]   As shown in Figure 32, data demonstrated accumulation of non-fucosylated in the optimized antibody from MAb chR005-1 Fc24 or chR005-1 Fc34 instead of fucosylated structures in the native chimeric chR005-1 Fc0 antibody.

[00354]   18. Determination of the binding MAb affinity.

[00355]   The binding properties of MAbs mR005-1, chR005-1 Fc0, chR005-1 Fc24 and chR005-1 Fc34 were examined by flow cytometry analysis and binding equilibrium studies on CD19 positive Raji cell line. Therefore neither the chimerization nor MAb optimization resulted in significant changes in MAb affinity:

MAb	KD nM
mR005-1	2,82

chR005-1 Fc0	1,83
chR005-1 Fc24	1,26
chR005-1 Fc34	2,63

[00356] 19. Different glycan profile according Fc variant

[00357] Figure 33 shows Fc variant impact on glycans such as fucosylated oligosaccharides (m/z 1836, 2040, 2245), oligomannoses (m/z 1579, 1784, 1988, 2192, 2396) and/or sialylated glycoforms (2401), 2605, 2966) compared to the native chR005-1 Fc0.

[00358] 20. Differential MAb activity according Fc variant.

[00359] Figure 34 shows different Fc variant anti-CD19 antibodies and their ability to trigger CDC and / or ADCC with isolated PBMNC. The present invention provided that only the chR005-1 Fc24 or chR005-1 Fc34 variant triggered both ADCC and CDC MAb activities.

[00360] 21. Figure 35 shows different Fc variant anti-CD19 antibodies (Fc14, chR005-1, Fc34) and their ability to trigger CDC and / or ADCC in the presence of whole blood. Only the chR005-1 Fc34 triggered ADCC in the presence of natural circulating immunoglobulins.

[00361] 22. Figure 36 shows different Fc variant anti-CD19 antibodies (chR005-1 Fc24; Fc39) and their ability to trigger ADCC in the presence of whole blood at different levels according the optimized Fc.

[00362] 23.The invention includes, that the improved ADCC with the chR005-1 Fc34 was not limited in CD16A off rates.

[00363] Figure 37 shows *in vivo* comparative effect of the chR005-1 MAb Fc24 or Fc 34 optimized variant on tumour clearance in established mouse Raji lymphoma xenograft model with or without human leukocyte inoculation. The invention includes, that the improved ADCC with the chR005-1 Fc34 was not limited in CD16A off rates compared to the chR005-1 Fc24 containing further the E333A modification.

[00364] 24. Impact of sialylation on CDC ability.

[00365] Figure 38 shows that the modification at position 243 leading additional sialylation was not enough sufficient to restore recognition target cell lysis by complement. The present invention reveals that only the chR005-1 Fc24 or chR005-1 Fc34 MAbs which exhibits around the same chR005-1 Fc20 sialylated glycoform pattern but with a different protein sequence and a higher level of bound complement induced a target cell lysis by complement.

[00366] 25. Impact of the MAb anti-CD19 on Rituxan refractory follicular lymphoma.

[00367] Figure 39 shows that the growth of Rituxan refractory RL established tumour was inhibited with the Fc24 optimized chR005-1 MAb.

[00368] 26. Influence of MAb and drug combination

[00369] Figure 40 shows that the combination of the Fc24 optimized chR005-1 MAb and the drug Doxorubicine increased the level of growth inhibition Raji established tumour.

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CLAIMS

1.   An anti-CD19 antibody modified to comprise a variant human IgG Fc region, preferably IgG1 Fc region, wherein this variant region comprises an amino acid substitution at each of the amino acid positions 243, 292, 300, 305, 326 and 396 and optionally 333 of the human IgG Fc region, wherein the numbering of the amino acid residues in the Fc region is the one of the Kabat®.

2.   The antibody of claim 1, wherein said antibody has been produced in wild-type rodent cells, preferably wild-type CHO cells.

3.   The antibody of any one of the preceding claims, which has an Fc, preferably two Fc bearing no $(GlcNAc)_2$**(Fuc)**$_1$ + $(Man)_3(GlcNAc)_2$ glycan.

1.   The antibody of any one of the preceding claims, which has an Fc, preferably two Fc bearing no $(Gal)_1(GlcNAc)_2$**(Fuc)**$_1$ + $(Man)_3(GlcNAc)_2$ glycan.

2.   The antibody of any one of the preceding claims, comprising an Fc, preferably two Fc bearing a $(Man)_5(GlcNAc)_2$ glycan.

3.   The antibody of any one of the preceding claims, comprising an Fc, preferably two Fc bearing one or two of the following glycans:

- $(Gal)_1(GlcNAc)_2$ **(Fuc)**$_1$ **(NeuAc)**$_1$ + $(Man)_3(GlcNAc)_2$

- $(Gal)_2(GlcNAc)_2$ **(Fuc)**$_1$ **(NeuAc)**$_1$ + $(Man)_3(GlcNAc)_2$

4.   The antibody of any one of the preceding claims, which is ADCC+ and CDC+.

5.   The antibody of any one of the preceding claims, wherein the antibody recognizes a non-internalizing epitope on the CD19 antigen, preferably the antibody comprises the CDRs of the antibodies mR005-1 or mR005-2 whose VH and VL amino acid sequences are depicted on the following table, or more preferably, the antibody comprises those sequences:

	Amino acid sequence VH	Amino acid sequence VL
R005-1	SEQ ID NO: 29	SEQ ID NO: 31
R005-2	SEQ ID NO: 33	SEQ ID NO: 35

6.   The antibody of any one of the preceding claims, which comprises an Fc region in which Phe243 is substituted by Leu, Arg292 is substituted by Pro, Tyr300 is substituted by Leu, Val305 is substituted by Leu, Lys326 is substituted by Ala and Pro396 is substituted by Leu, and possibly Glu333 is substituted by Ala.

7.   The antibody of any one of the preceding claims, wherein said antibody is a chimeric antibody, a humanized antibody, a full human antibody, a bispecific antibody, an antibody drug conjugate or an antibody fragment.

8.   The antibody of any one of the preceding claims, that triggers programmed cell death.

9.   The antibody of any one of the preceding claims, for use in a method of treating a cancer.

10.   The antibody of any one of the preceding claims, for use in a method of treating a cancer on refractory or relapse anti-CD20 antibody treated patients.

11.   A pool of antibodies according to any one of the preceding claims, wherein it comprises less or equal than 15 % of such antibodies comprising an Fc, preferably two Fc bearing a $(GlcNAc)_2$**(Fuc)**$_1$ + $(Man)_3(GlcNAc)_2$ glycan and/or less or equal than 20 % of such antibodies comprising an Fc, preferably two Fc bearing a $(Gal)_1(GlcNAc)_2$ **(Fuc)**$_1$ + $(Man)_3(GlcNAc)_2$ glycan.

12.   The pool of antibodies of claim 15, wherein it comprises at least 15, 20, 30, 40 or 50% of antibodies comprising an Fc, preferably two Fc bearing $(Man)_5(GlcNAc)_2$ glycans.

13.   The pool of antibodies of claim 15 or 16, wherein it comprises

- less than 1.5 or 1% of antibodies comprising an Fc, preferably two Fc bearing $(Gal)_1(GlcNAc)_2$**(Fuc)**$_1$**(NeuAc)**$_1$ + $(Man)_3(GlcNAc)_2$

- and/or less than 2 or 1.5% of antibodies comprising an Fc, preferably two Fc bearing $(Gal)_2(GlcNAc)_2$**(Fuc)**$_1$**(NeuAc)**$_1$ + $(Man)_3(GlcNAc)_2$.

14.   A pharmaceutical composition comprising an antibody of any one of the preceding claims, and a physiologically acceptable vehicle or excipient, and possibly an antibody directed against a tumoral antigen that is different from CD19, preferably an antibody directed against CD20.

15.   The composition of claim 16, for use in a method of treating a cancer or an autoimmune disease.

16.   The composition of claim 10, for use as an anti-tumoral medicament or as an anti-tumoral treatment for use in treating a cancer on refractory or relapse anti-CD20 antibody treated patients.

17.   The composition of claim 14, 15 or 16, comprising another anti-cancerous agent.

18.   A nucleic acid sequence encoding a variant Fc region in which Phe243 is substituted by Leu, Arg292 is substituted by Pro, Tyr300 is substituted by Leu, Val305 is substituted by Leu, Lys326 is substituted by Ala and Pro396 is substituted by Leu, and possibly Glu333 is substituted by Ala.

19.   An expression vector comprising a nucleic acid sequence encoding a polypeptide containing the VH CDRs of the antibodies mR005-1 or mR005-2 whose VH nucleic acid sequence are depicted on SEQ ID NO: 30 or SEQ ID NO: 34, or more preferably, the nucleic acid sequence comprises SEQ ID NO: 30 or SEQ ID NO: 34, and a human Fc region comprising an amino acid substitution at each of the amino acid positions 243, 292, 300, 305, 326 and 396, and optionally 333 depicted on SEQ ID NO: 1, 3.

20.   An expression vector comprising a nucleic acid sequence encoding a polypeptide containing the VL CDRs of the antibodies mR005-1 or mR005-2 whose VL nucleic acid sequence are depicted on SEQ ID NO: 32 or SEQ ID NO: 36, or more preferably, the nucleic acid sequence comprises SEQ ID NO: 32 or SEQ ID NO: 36, and a human Kappa region.

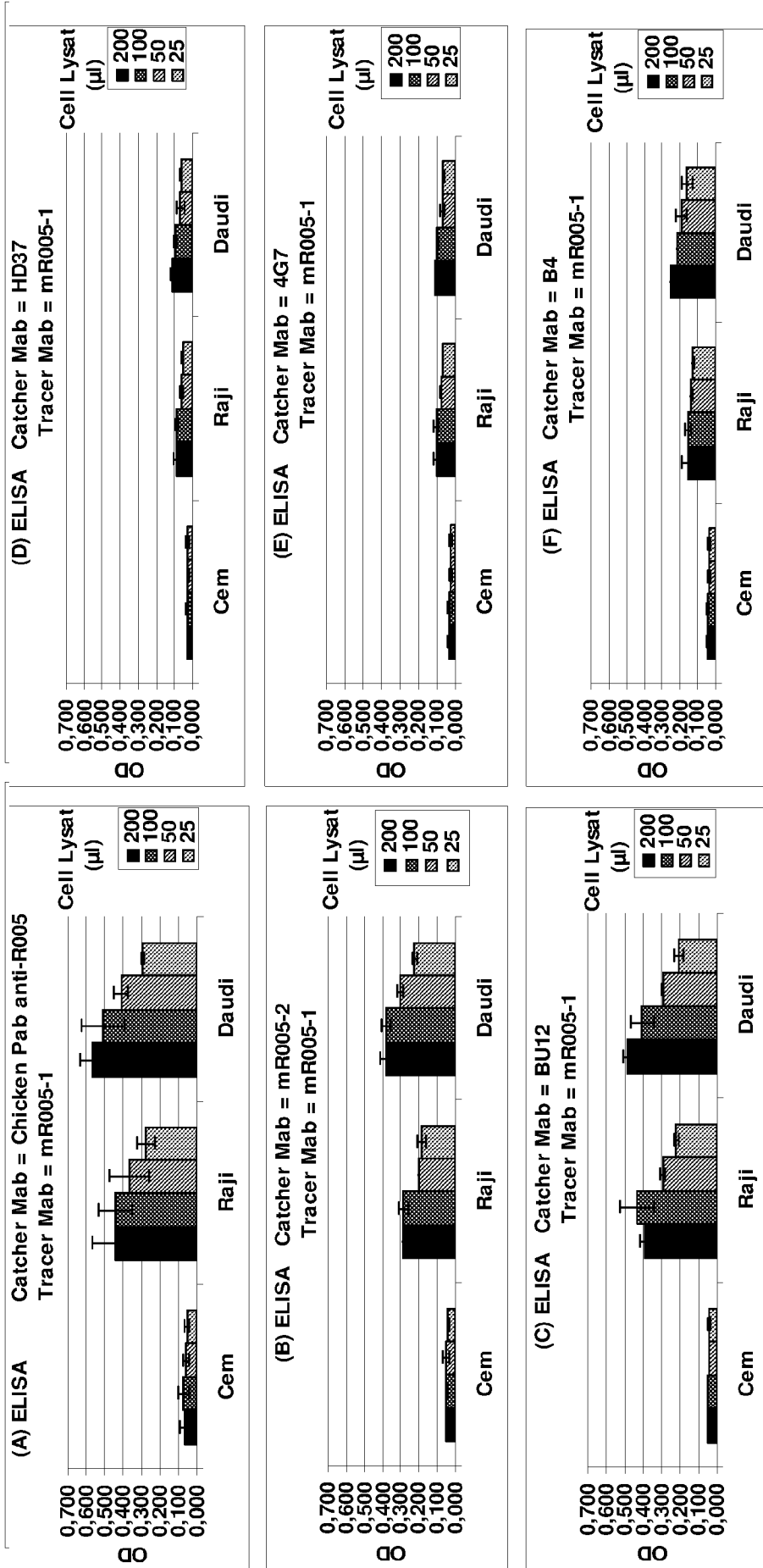21.   A host cell containing a vector of claim 13 and/or 14, the host cell being preferably a wild-type CHO cell.
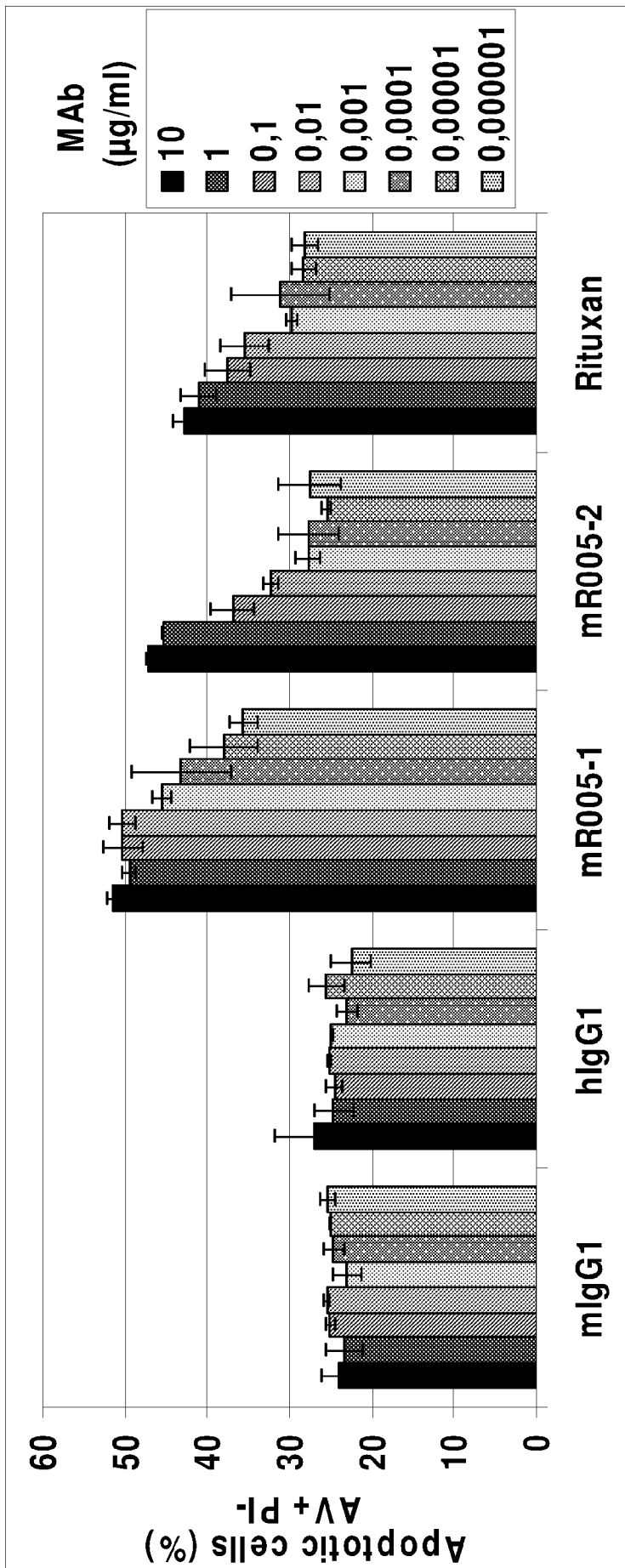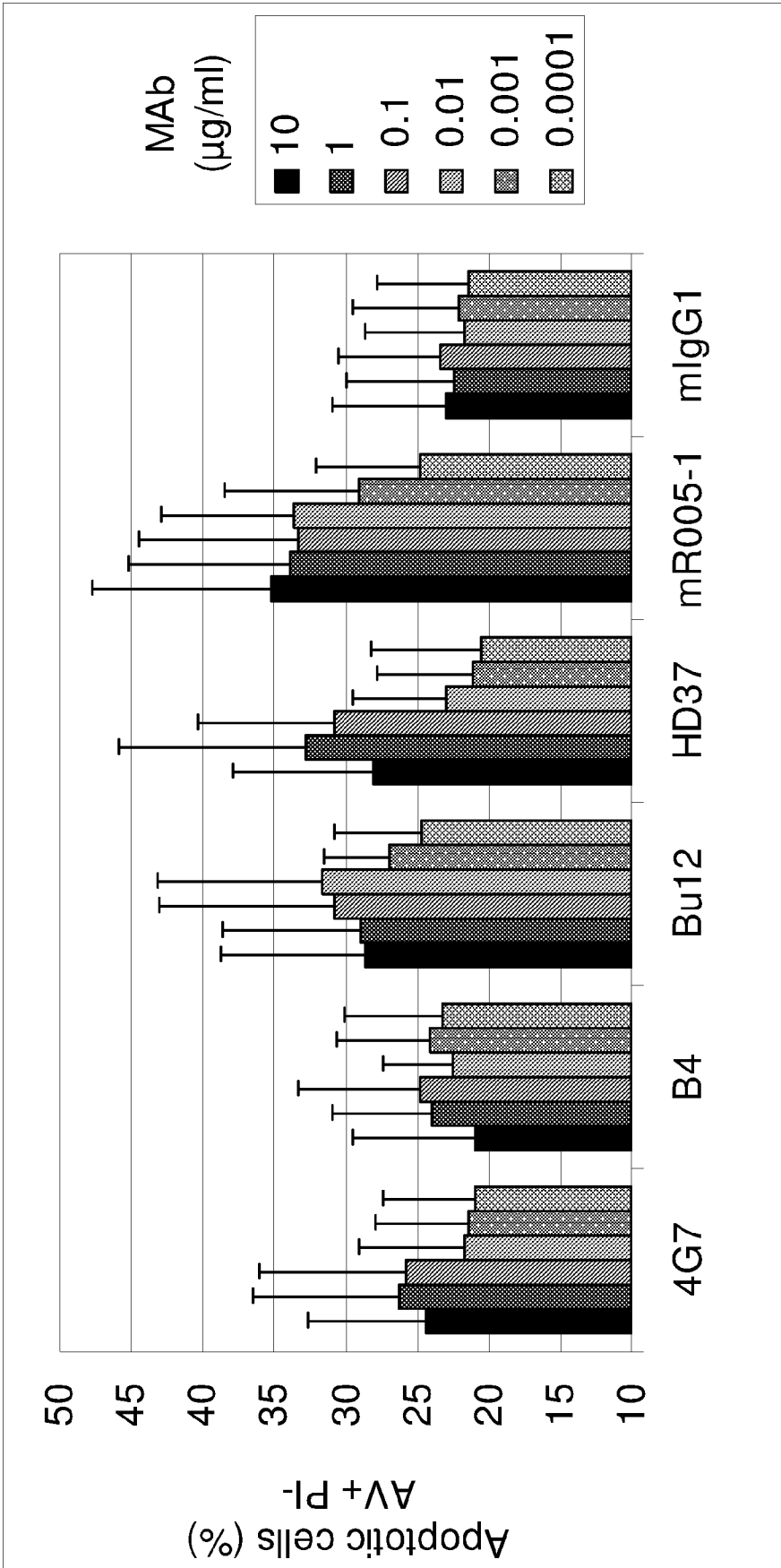
Figure 1

Figure 2

Figure 3

VH-R005-1

```
                  <------------------------------ H-FR1 - IMGT ------------------------------
       Q     V     Q     L     Q     Q     S     G     A     E     L     V     R     P     G     S     S     V     K
      CAG   GTC   CAG   CTG   CAG   CAG   TCT   GGG   GCT   GAA   CTG   GTG   AGG   CCT   GGG   TCC   TCA   GTG   AAG

                                       <----- H-CDR1 - IMGT ----->
       I     S     C     K     A     S     G     Y     A     F     S     S     Y     W     V     N     W     M     K
      ATT   TCC   TGT   AAG   GCT   TCT   GGC   TAT   GCA   TTC   AGT   AGC   TAC   TGG   GTG   AAC   TGG   ATG   AAG

           <---- H-FR2 - IMGT ---->                                <----- H-CDR2 - IMGT
       Q     R     P     G     Q     G     L     E     W     I     G     Q     I     Y     P     G     D     G     D
      CAG   AGG   CCT   GGA   CAG   GGA   CTT   GAG   TGG   ATT   GGA   CAG   ATT   TAC   CCT   GGA   GAT   GGT   GAT

                                           <------------------------------ H-FR3 - IMGT
       T     N     Y     N     G     K     F     K     G     R     A     T     V     T     A     D     K     S     S
      ACT   AAT   TAC   AAT   GGA   AAG   TTC   AAG   GGA   CGA   GCC   ACA   GTG   ACT   GCA   GAC   AAA   TCC   TCC

       S     T     S     Y     M     Q     F     S     S     L     T     S     E     D     S     A     V     Y     F
      AGC   ACA   TCC   TAC   ATG   CAG   TTC   AGC   AGC   CTA   ACA   TCT   GAG   GAC   TCT   GCG   GTC   TAT   TTC

                               <--------------- H-CDR3 - IMGT --------------->
       C     A     R     S     I     T     T     V     V     G     C     A     M     D     Y     W     G     Q     G
      TGT   GCA   AGA   TCT   ATT   ACT   ACG   GTG   GTA   GGC   TGT   GCT   ATG   GAC   TAC   TGG   GGT   CAA   GGA

       T     S     V     T     V     S     S
      ACC   TCG   GTC   ACC   GTC   TCC   TCA
```

VL-R005-1

```
<------------------------------------------ L-FR1 - IMGT--------------------------
 D   I   Q   M   T   Q   S   S   A   Y   L   S   V   S   L   G   G   R   V
GAC ATT CAG ATG ACC CAG TCT TCT GCC TAC CTG TCT GTA TCT CTA GGA GGC AGG GTC

-------------------->   ____ L-CDR1 - IMGT _____              <---------------
 T   I   T   C   K   A   S   D   H   I   N   N   W   L   A   Y   Q   H
ACC ATT ACT TGC AAG GCA AGT GAC CAC ATT AAT AAT TGG TTA GCC TGG TAT CAA CAT

---- L-FR2 - IMGT --------------------->   ____ L-CDR2- IMGT< ---------------
 K   P   G   N   A   P   R   L   L   I   S   G   A   T   L   E   T   G
AAA CCA GGA AAT GCT CCT AGG CTC TTA ATA TCT GGT GCA ACT ACT TTG GAA ACT GGG

-------------------------------- L-FR3 - IMGT ------------------------------
 V   P   S   R   F   S   G   S   G   K   D   Y   T   L   S   I   T
GTT CCT TCA AGA TTC AGT GGC AGT GGA TCT GGA AAG GAT TAC ACT CTC AGC ATT ACC

--------------------->   ____ L-CDR3 - IMGT _____
 S   L   Q   T   E   D   V   A   T   Y   Y   C   Q   Q   S   W   N   T   P
AGT CTT CAG ACT GAA GAT GTT GCT ACT TAT TAC TGT CAA CAG TCT TGG AAT ACT CCG

 W   T   F   G   G   G   T   K
TGG ACG TTC GGT GGA GGC ACC AAG
```

VH-R005-2

```
              H-FR1 - IMGT
E   V   Q   L   Q   Q   S   G   P   E   L   V   K   P   G   A   S   V   K
GAG GTT CAG CTG CAA CAG TCT GGA CCT GAG CTG GTA AAG CCT GGG GCT TCA GTG AAG

                      H-CDR1 - IMGT
M   S   C   K   A   S   G   Y   T   F   T   S   Y   V   M   H   W   V   K
ATG TCC TGC AAG GCT TCT GGA TAC ACA TTC ACT AGC TAT GTT ATG CAC TGG GTG AAG

H-FR2 - IMGT                              H-CDR2- IMGT
Q   K   P   G   Q   G   L   E   W   I   G   Y   N   P   Y   N   D   G
CAG AAG CCT GGG CAG GGC CTT GAG TGG ATT GGA TAT AAT CCT TAC AAT GAT GGT

                                  H-FR3 - IMGT
T   K   Y   N   E   K   F   K   G   K   A   T   L   T   S   D   K   S   S
ACT AAG TAC AAT GAG AAG TTC AAA GGC AAG GCC ACA CTG ACT TCA GAC AAA TCC TCC

S   T   A   Y   M   E   L   S   S   L   T   S   E   D   S   A   V   Y   Y
AGC ACA GCC TAC ATG GAG CTC AGC AGC CTG ACC TCT GAG GAC TCT GCG GTC TAT TAC

            H-CDR3 - IMGT
C   A   R   G   P   Y   Y   Y   G   S   P   F   D   Y   W   G   Q   G
TGT GCA AGA GGG CCT TAT TAC TAC GGT AGT CCC TTT GAC TAC TGG GGC CAA GGC

Q   V   T   V   S   S
CAG GTC ACC GTC TCC TCA
```

VL-R005-2

```
                      <------------------------------- L-FR1 - IMGT -------------------------------
                       D   A   V   M   T   Q   T   P   L   S   L   P   V   S   L   G   D   Q   A
                      GAC GCT GTG ATG ACC CAA ACT CCA CTC TCC CTG CCT GTC AGT CTT GGA GAT CAA GCC

                      ------------------------->              <------------------- L-CDR1 - IMGT
                       S   I   S   C   R   S   S              Q   S   L   E   N   S   N   G   N   T   Y   L
                      TCC ATC TCT TGC AGG TCT AGT            CAG AGC CTT GAA AAC AGT AAT GGA AAC ACC TAT TTG

                      ------------------- L-FR2 - IMGT ------------------->
                       N   W   Y   L   Q   K   P   G   Q   S   P   Q   L   L   I   Y   R   V   S
                      AAC TGG TAC CTC CAG AAA CCA GGC CAG TCT CCA CAG CTC CTG ATC TAC AGG GTT TCC

                      L-CDR2- IMGT _                          <------------------- L-FR3 - IMGT
                       N   R   F   S   G   V   L   D   R   F   S   G   S   G   T   D   F
                      AAC CGA TTT TCT GGG GTC CTA GAC AGG TTC AGT GGT AGT GGA TCA GGG ACA GAT TTC

                      ------------------- L-FR3 - IMGT ------------------------>
                       T   L   K   I   S   R   V   E   A   E   D   L   G   V   Y   F   C   L   Q
                      ACA CTG AAA ATC AGC AGA GTG GAG GCT GAG GAT TTG GGA GTT TAT TTC TGC CTC CAA

                      L-CDR3 - IMGT
                       V   T   H   V   P   P   T   F   G   A   G   T   K
                      GTT ACA CAT GTC CCT CCC ACG TTC GGT GCT GGG ACC AAG
```

Figure 4

MAb   (A) PBL   (B) Monocytes

mR005-1

chR005-1 Fc0

Rituxan

anti-CD3

anti-CD14

Figure 5

FITC conjugated mR005-1 MAb

alone

+ hIgG1
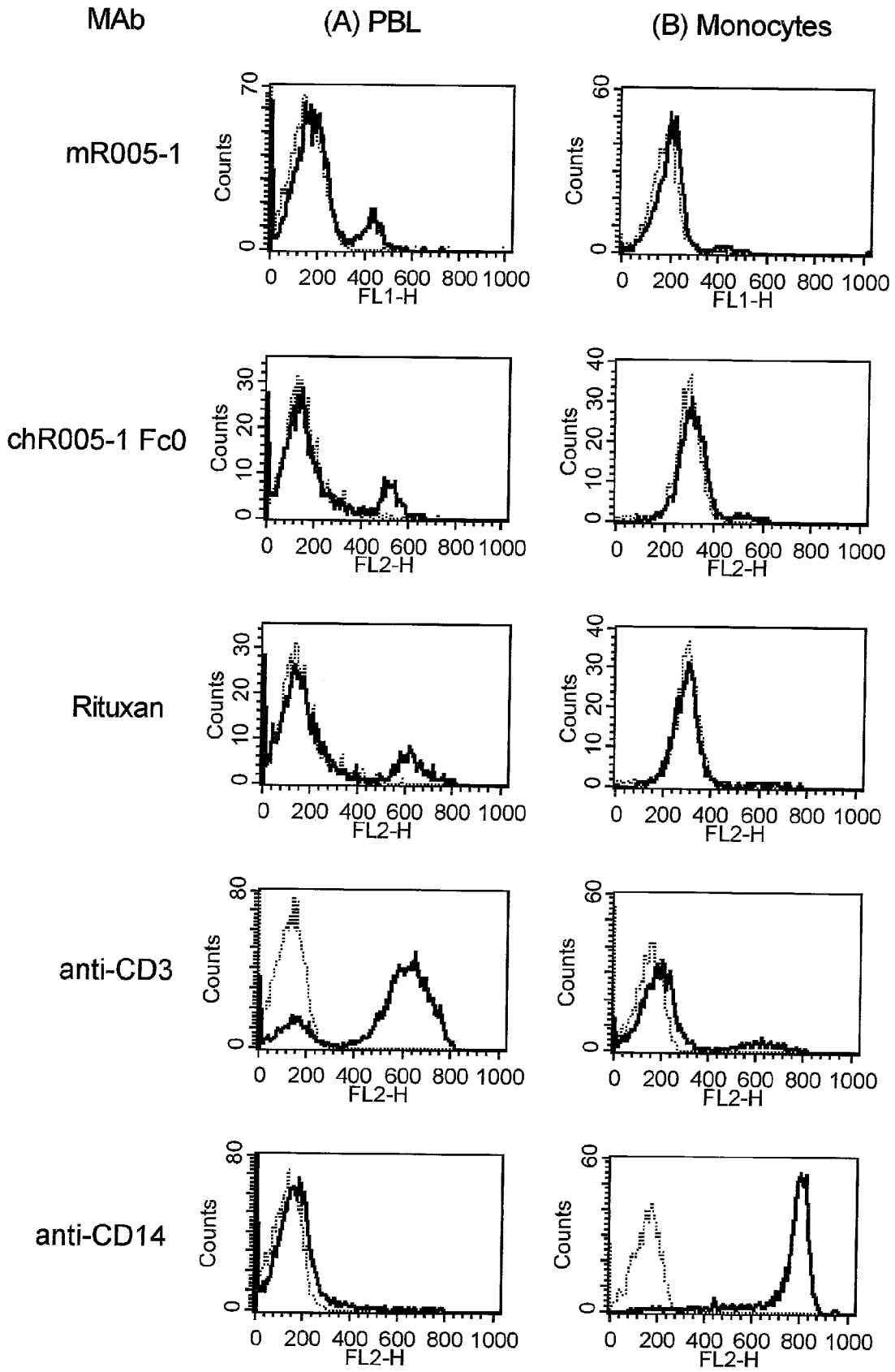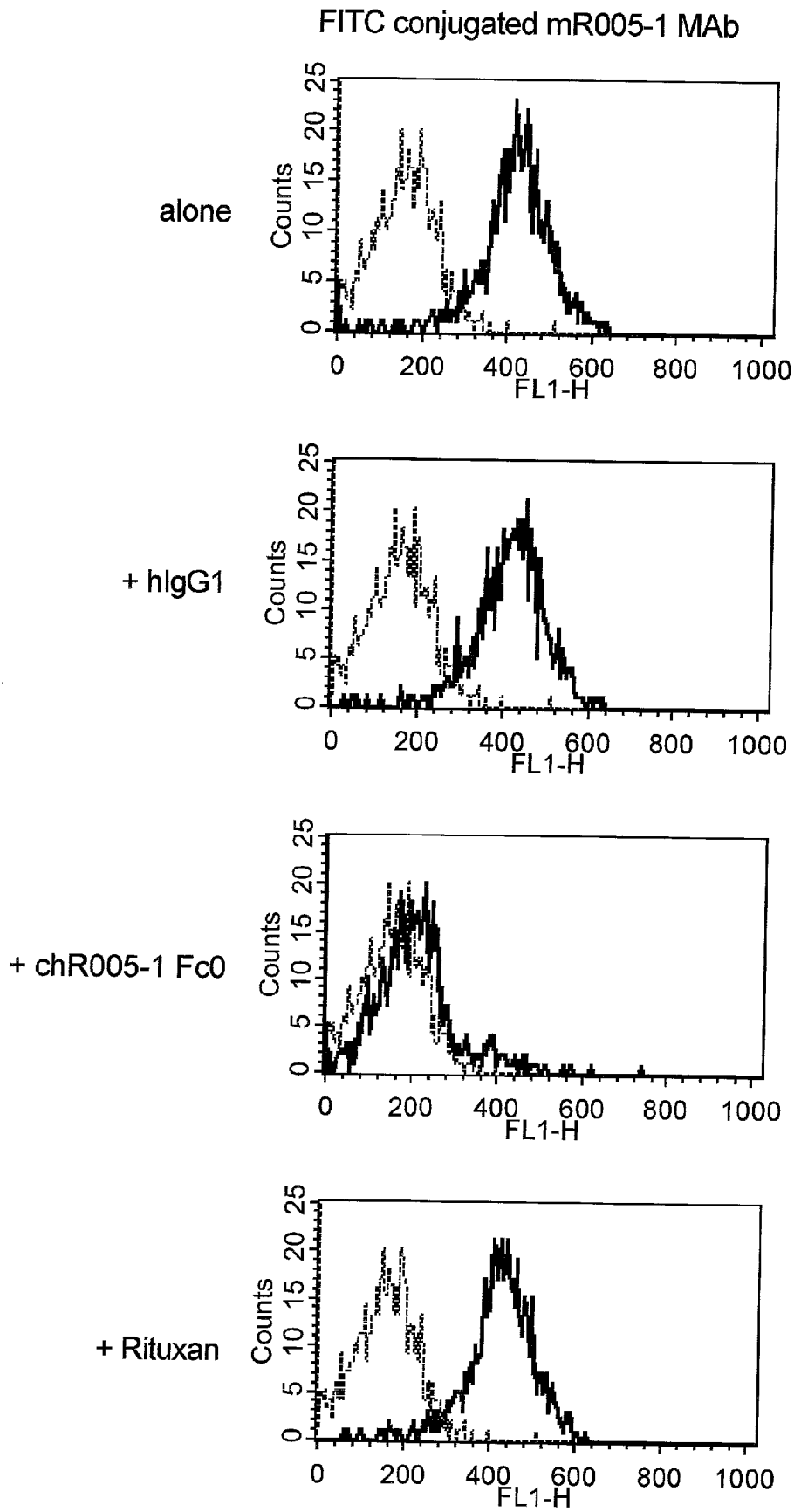
+ chR005-1 Fc0

+ Rituxan

Figure 6

Rituxan
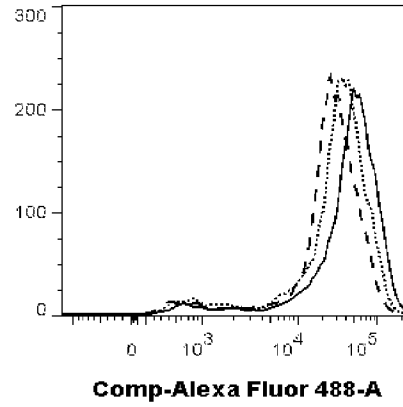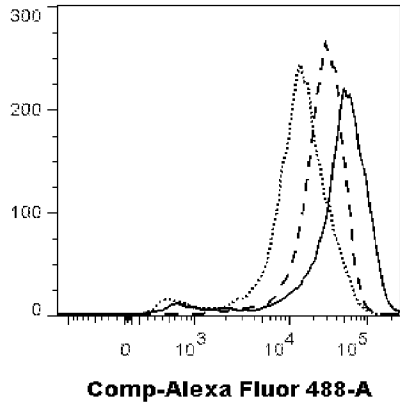
chR005-1 Fc0
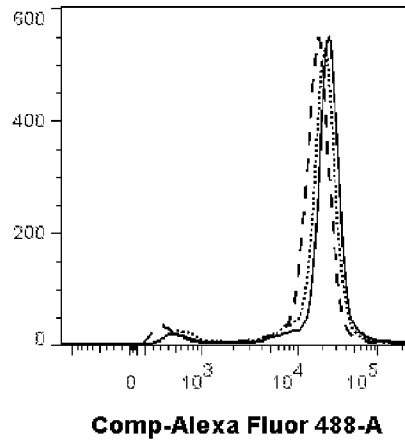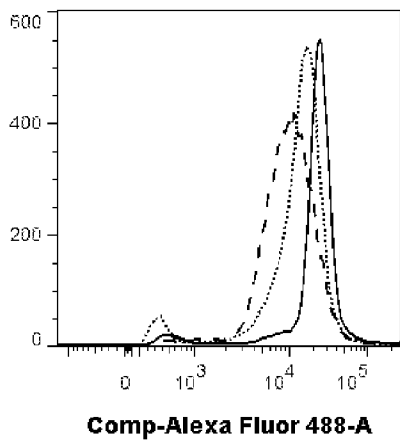
mR005-1
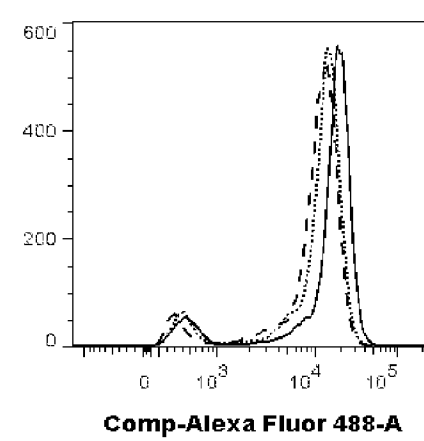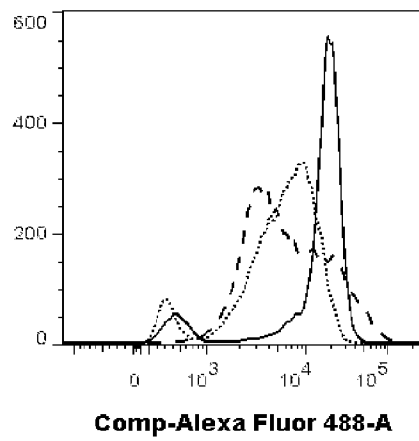
_____ T0   ......T3h   ---- T24h

Figure 7

Figure 8

Figure 9

Figure 10

Fc24

```
A    S    T    K    G    P    S    V    F    P    L    A    P    S    S    K    T    S
gcc  tcc  acc  aag  ggc  cca  tcg  gtc  ttc  ccc  ctg  gca  ccc  tcc  aag  agc  acc  tct
```

CH1

```
G    G    T    A    A    L    G    C    L    V    K    D    Y    F    P    E    P    V    T
ggg  ggc  aca  gcg  gcc  ctg  ggc  tgc  ctg  gtc  aag  gac  tac  ttc  ccc  gaa  ccg  gtg  acg
```

```
V    S    W    N    S    G    A    L    T    S    G    V    H    T    F    P    A    V    L
gtg  tcg  tgg  aac  tca  ggc  gcc  ctg  acc  agc  ggc  gtg  cac  acc  ttc  ccg  gct  gtc  cta
```

```
Q    S    S    G    L    Y    S    L    S    S    V    V    T    V    P    S    S    S    L
cag  tcc  tca  gga  ctc  tac  tcc  ctc  agc  agc  gtc  gtg  acc  gtg  ccc  tcc  agc  agc  ttg
```

```
G    T    Q    T    Y    I    C    N    V    N    H    K    P    S    N    T    K    V    D
ggc  acc  cag  acc  tac  atc  tgc  aac  gtg  aat  cac  aag  ccc  agc  aac  acc  aag  gtg  gac
```

Hinge

```
K    K    V    E    P    K    S    C    D    K    T    H    T    C    P    P    C    P    A
aag  aaa  gtt  gag  ccc  aaa  tct  tgt  gac  aaa  act  cac  aca  tgc  cca  ccg  tgc  cca  gca
```

```
P    E    L    L    G    G    P    S    V    F    L    L    P    P    K    P    K    D    T
cct  gaa  ctc  ctg  ggg  gga  ccg  tca  gtc  ttc  ctc  ttc  ccc  cca  aaa  ccc  aag  gac  acc
```

```
L    M    I    S    R    T    P    E    V    T    C    V    V    V    D    V    S    H    E
ctc  atg  atc  tcc  aga  acc  cct  gag  gtc  acg  tgc  gtg  gtg  gtg  gac  gtg  agc  cac  gag
```

```
     L    M    I    S    R    T    P    E    V    T    C    V    V    V    D    V    S    H    E
    ctc  atg  atc  tcc  cgg  acc  cct  gag  gtc  aca  tgc  gtg  gtg  gtg  gac  gtg  agc  cac  gaa
```

CH2

```
     D    P    E    V    K    F    N    W    Y    V    D    G    V    E    V    H    N    A    K
    gac  cct  gag  gtc  aag  ttc  aac  tgg  tac  gtg  gac  ggc  gtg  gag  gtg  cat  aat  gcc  aag

     T    K    P    R    E    E    Q    Y    N    S    T    L    R    V    V    S    L    T
    aca  aag  ccg  cgg  gag  gag  cag  tac  aac  agc  acg  ctc  cgt  gtg  gtc  agc  ctc  acc

     V    L    H    Q    D    W    L    N    G    K    E    Y    K    C    K    V    S    N    A
    gtc  ctg  cac  cag  gac  tgg  ctg  aat  ggc  aag  gag  tac  aag  tgc  aag  gtc  tcc  aac  gca

     A    L    P    A    P    I    A    K    T    I    S    K    A    K    G    Q    P    R    E
    gcc  ctc  cca  gcc  ccc  atc  gcg  aaa  acc  atc  tcc  aaa  gcc  aaa  ggg  cag  ccc  cga  gaa
```

CH3

```
     P    Q    V    Y    T    L    P    P    S    R    D    E    L    T    K    N    Q    V    S
    ccc  cag  gtg  tac  acc  ctg  ccc  cca  tcc  cgg  gat  gag  ctg  acc  aag  aac  cag  gtc  agc

     L    T    C    L    V    K    G    F    Y    P    S    D    I    A    V    E    W    E    S
    ctg  acc  tgc  ctg  gtc  aaa  ggc  ttc  tat  ccc  agc  gac  atc  gcc  gtg  gag  tgg  gag  agc

     N    G    Q    P    E    N    N    Y    K    T    T    P    P    V    L    D    S    D    G
    aat  ggg  cag  ccg  gag  aac  aac  tac  aag  acc  acg  cct  ccc  gtg  ctg  gac  tcc  gac  ggc

     S    F    F    L    Y    S    K    L    T    V    D    K    S    R    W    Q    Q    G    N
    tcc  ttc  ttc  ctc  tac  agc  aag  ctc  acc  gtg  gac  aag  agc  agg  tgg  cag  cag  ggg  aac
```

```
V    F    S    C    S    V    M    H    E    A    L    H    N    H    Y    T    Q    K    S
gtc  ttc  tca  tgc  tcc  gtg  atg  cat  gag  gct  ctg  cac  aac  cac  tac  acg  cag  aag  agc

L    S    P    G    K    *
ctc  tcc  ctg  tct  ccg  ggt  aaa  tga
```

Fc34

```
A    S    T    K    G    P    S    V    F    P    L    A    P    S    S    K    S    T    S
gcc  tcc  acc  aag  ggc  cca  tcg  gtc  ttc  ccc  ctg  gca  ccc  tcc  tcc  aag  agc  acc  tct
```

CH1

```
G    G    T    A    A    L    G    C    L    V    K    D    Y    F    P    E    P    V    T
ggg  ggc  aca  gcg  gcc  ctg  ggc  tgc  ctg  gtc  aag  gac  tac  ttc  ccc  gaa  ccg  gtg  acg
```

```
V    S    W    N    S    G    A    L    T    S    G    V    H    T    F    P    A    V    L
gtg  tcg  tgg  aac  tca  ggc  gcc  ctg  acc  agc  ggc  gtg  cac  acc  ttc  ccg  gct  gtc  cta
```

```
Q    S    S    G    L    Y    S    L    S    S    V    V    T    V    P    S    S    S    L
cag  tcc  tca  gga  ctc  tac  tcc  ctc  agc  agc  gtc  gtg  acc  gtg  ccc  tcc  agc  agc  ttg
```

```
G    T    Q    T    Y    I    C    N    V    N    H    K    P    S    N    T    K    V    D
ggc  acc  cag  acc  tac  atc  tgc  aac  gtg  aat  cac  aag  ccc  agc  aac  acc  aag  gtg  gac
```

Hinge

```
K    K    V    E    P    K    S    C    D    K    T    H    T    C    P    P    C    P    A
aag  aaa  gtt  gag  ccc  aaa  tct  tgt  gac  aaa  act  cac  aca  tgc  cca  ccg  tgc  cca  gca
```

```
P    E    L    L    G    G    P    S    V    F    L    L    P    P    K    P    K    D    T
cct  gaa  ctc  ctg  ggg  gga  ccg  tca  gtc  ttc  ctc  ttc  ccc  cca  aaa  ccc  aag  gac  acc
```

```
L    M    I    S    R    T    P    E    V    T    C    V    V    V    D    V    S    H    E
ctc  atg  atc  tcc  cgg  acc  cct  gag  gtc  aca  tgc  gtg  gtg  gtg  gac  gtg  agc  cac  gaa
```

```
D   P   E   V   K   F   N   W   Y   V   D   G   V   E   V   H   N   A   K
gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc aag
                                    CH2

T   K   P   P   E   E   Q   Y   N   S   T   L   R   V   V   S   L   T
aca aag ccg ccg gag gag cag tac aac agc acg ctc cgt gtg gtc agc ctc acc

V   L   H   Q   D   W   L   N   G   K   E   Y   K   C   K   V   S   N   A
gtc ctg cac cag gac tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc aac gca

A   L   P   A   P   I   E   K   T   I   S   K   A   K   G   Q   P   R   E
gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa

P   Q   V   Y   T   L   P   P   S   R   D   E   L   T   K   N   Q   V   S
cca cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc
                                    CH3

L   T   C   L   V   K   G   F   Y   P   S   D   I   A   V   E   W   E   S
ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc

N   G   Q   P   E   N   N   Y   K   T   T   P   P   V   L   D   S   D   G
aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac tcc gac ggc

S   F   F   L   Y   S   K   L   T   V   D   K   S   R   W   Q   Q   G   N
tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag agc agg tgg cag cag ggg aac
```

```
V   F   S   C   S   V   M   H   E   A   L   H   N   H   Y   T   Q   K   S
gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc

L   S   L   S   P   G   K   *
ctc tcc ctg tct ccg ggt aaa tga
```

Figure 11
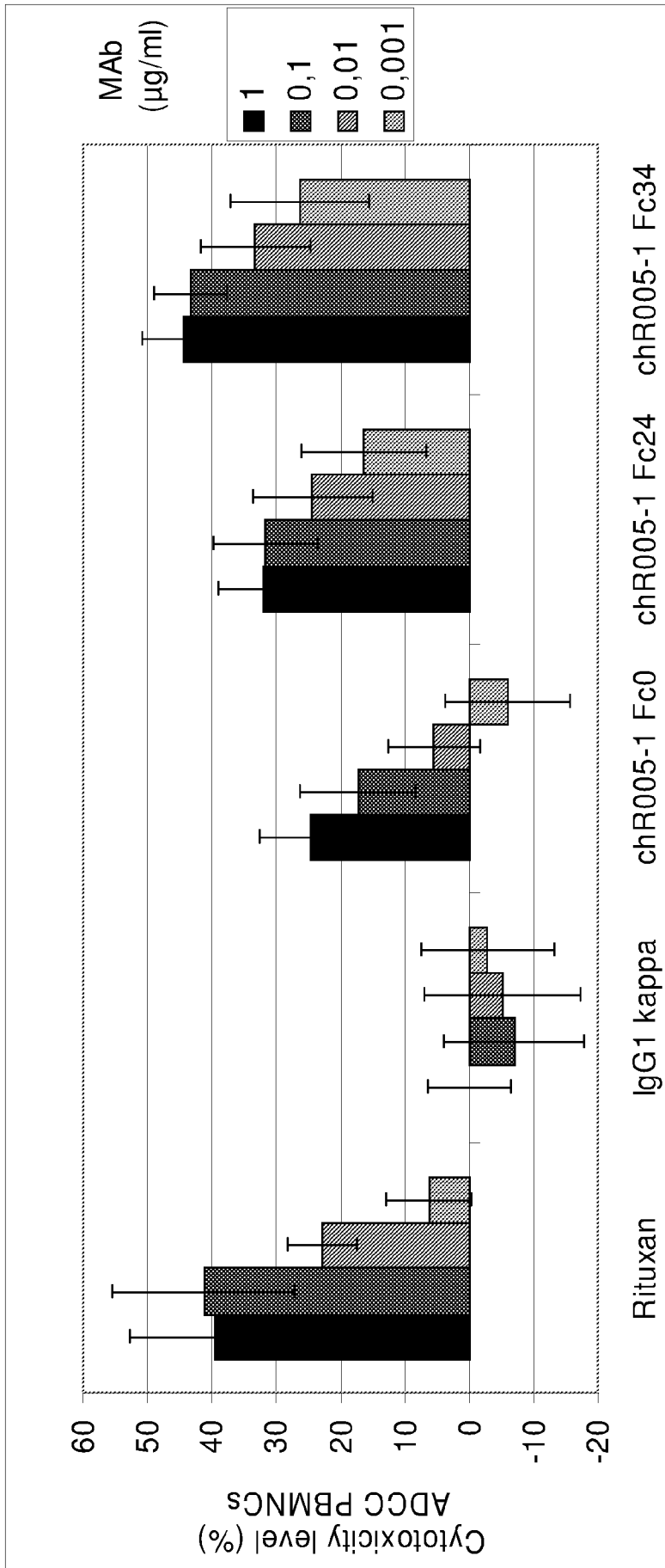
Figure 12
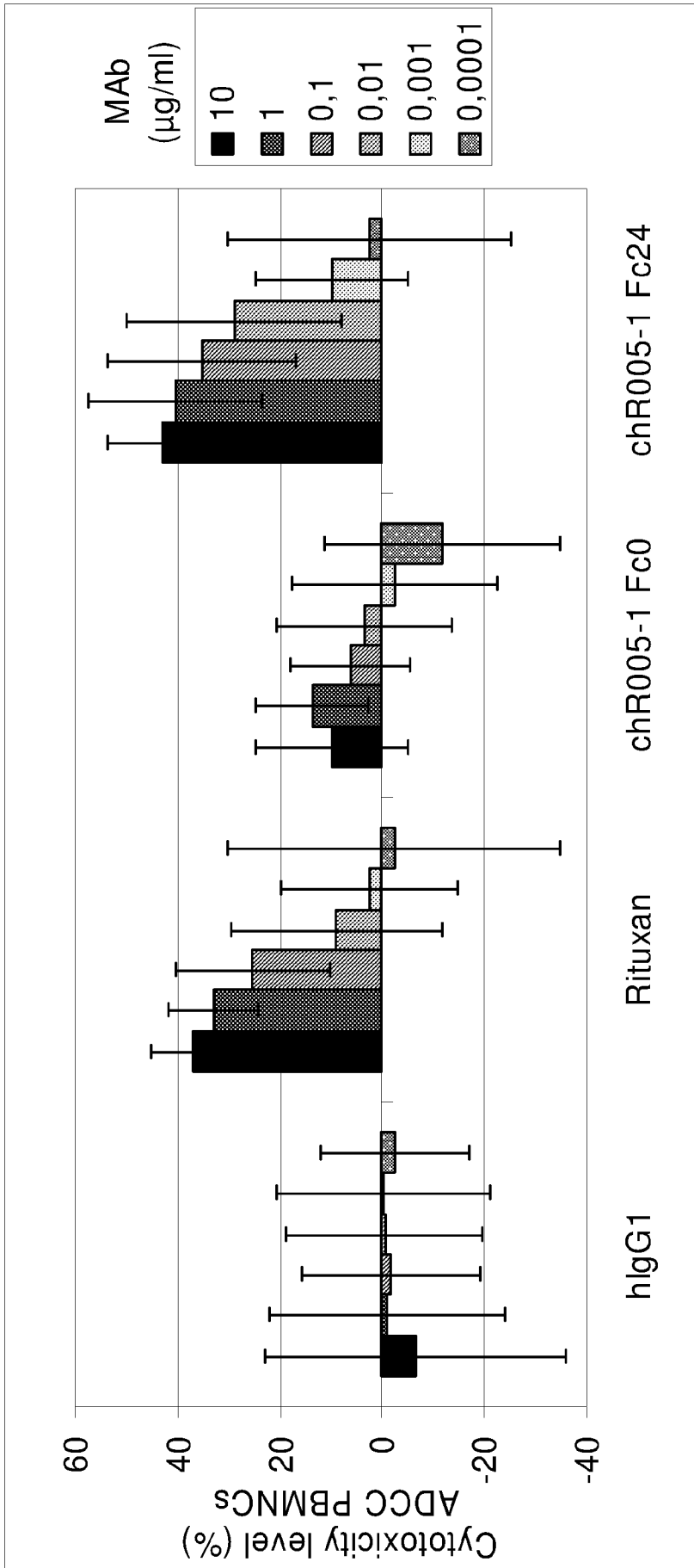
Figure 13
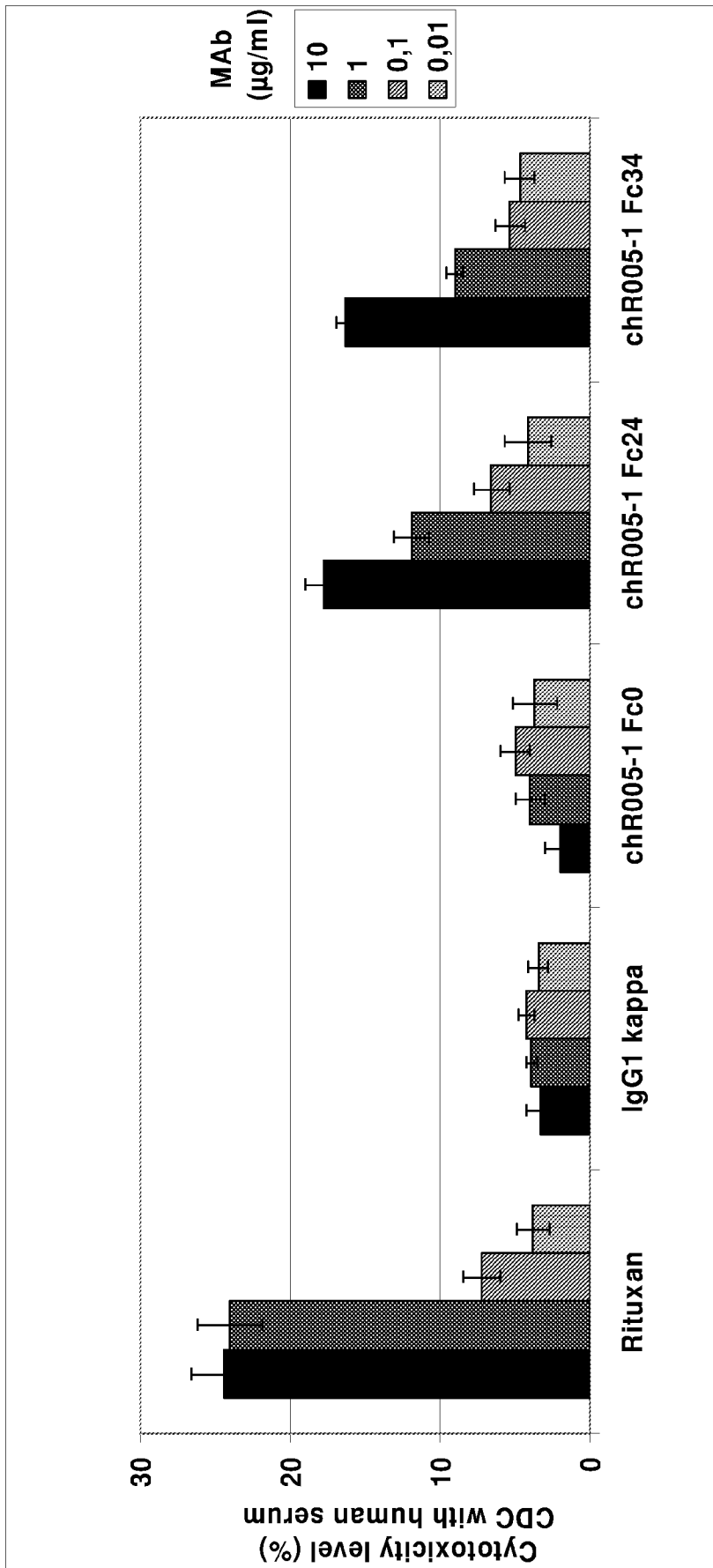
Figure 14
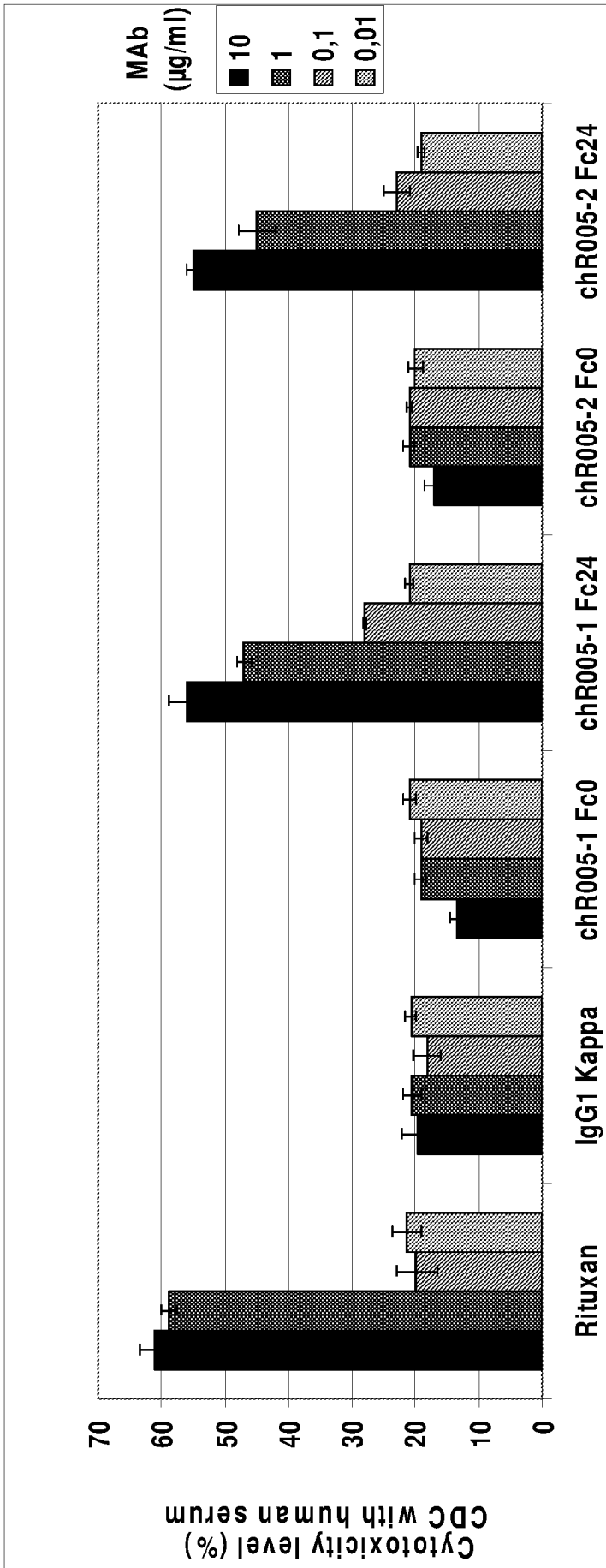
Figure 15
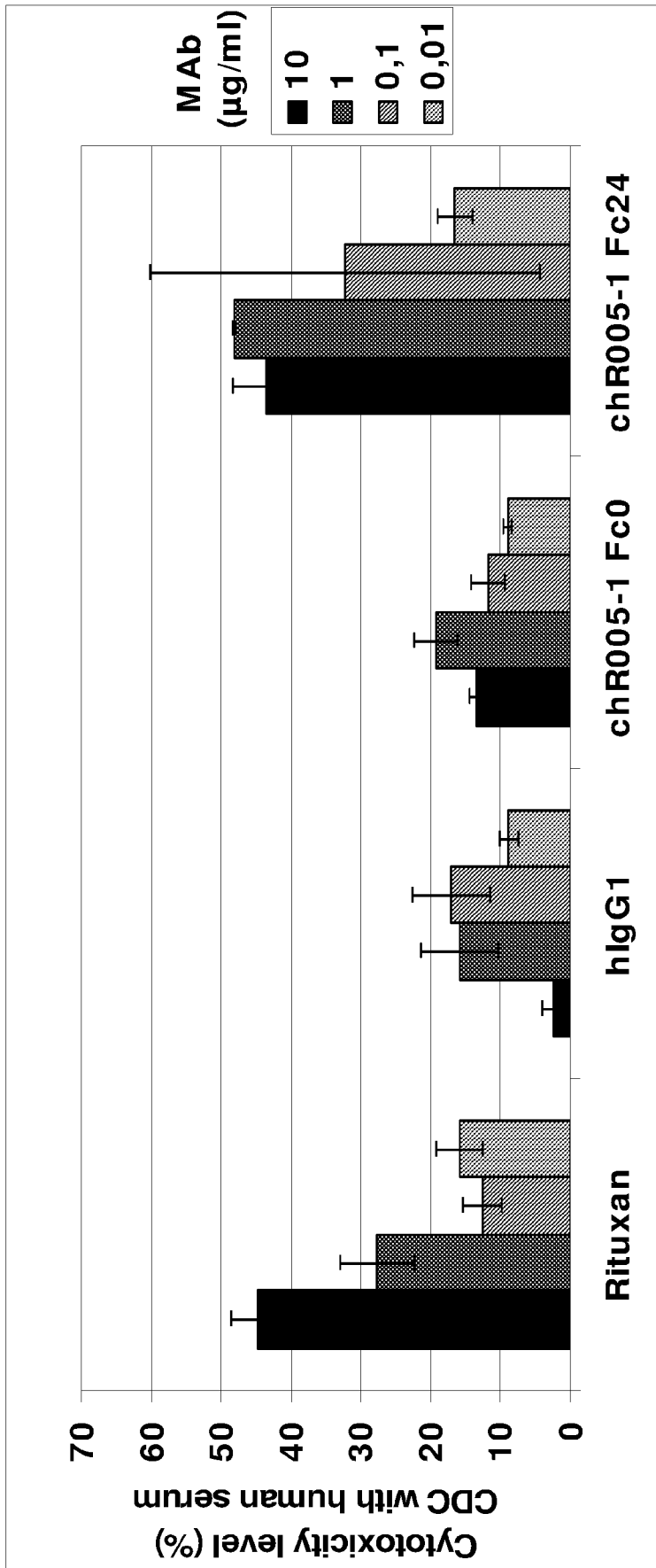
Figure 16
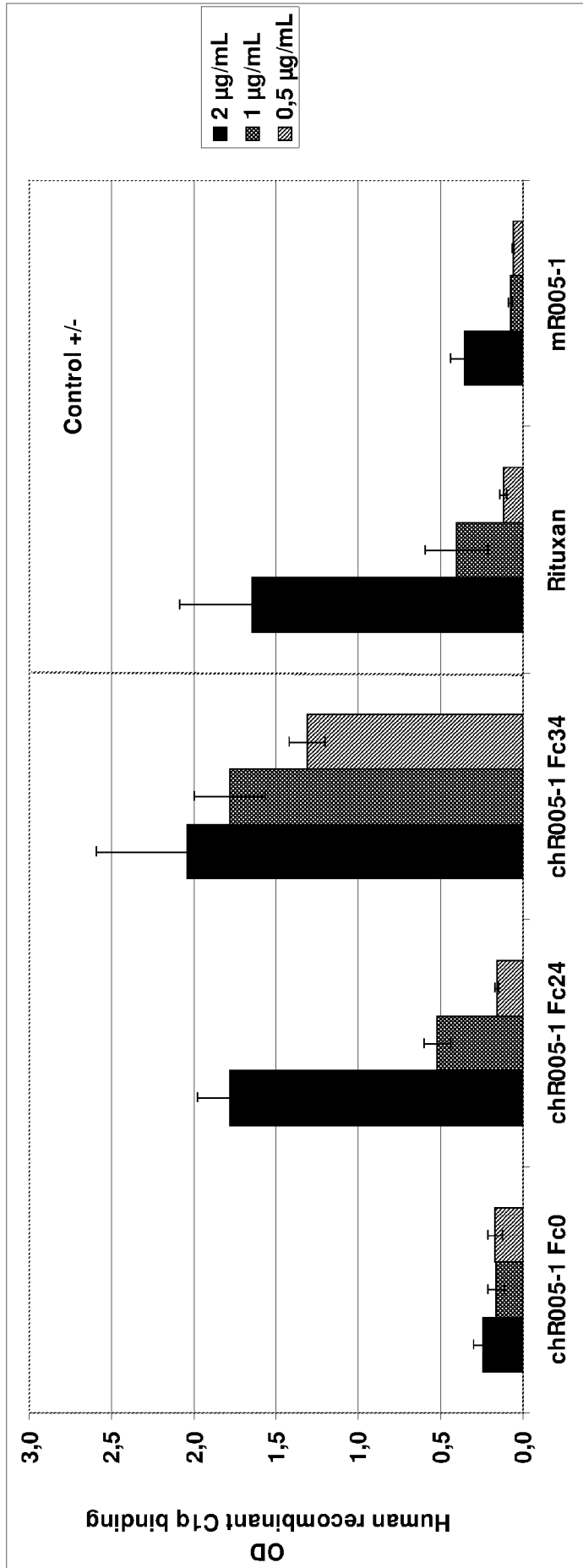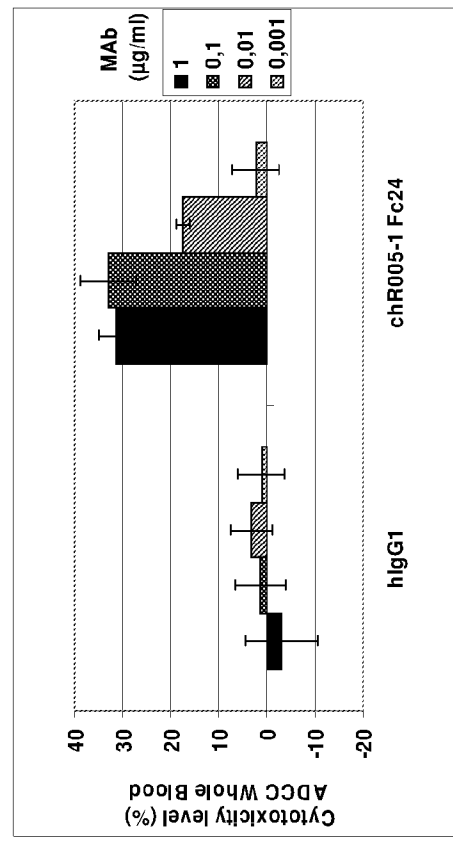
Figure 17
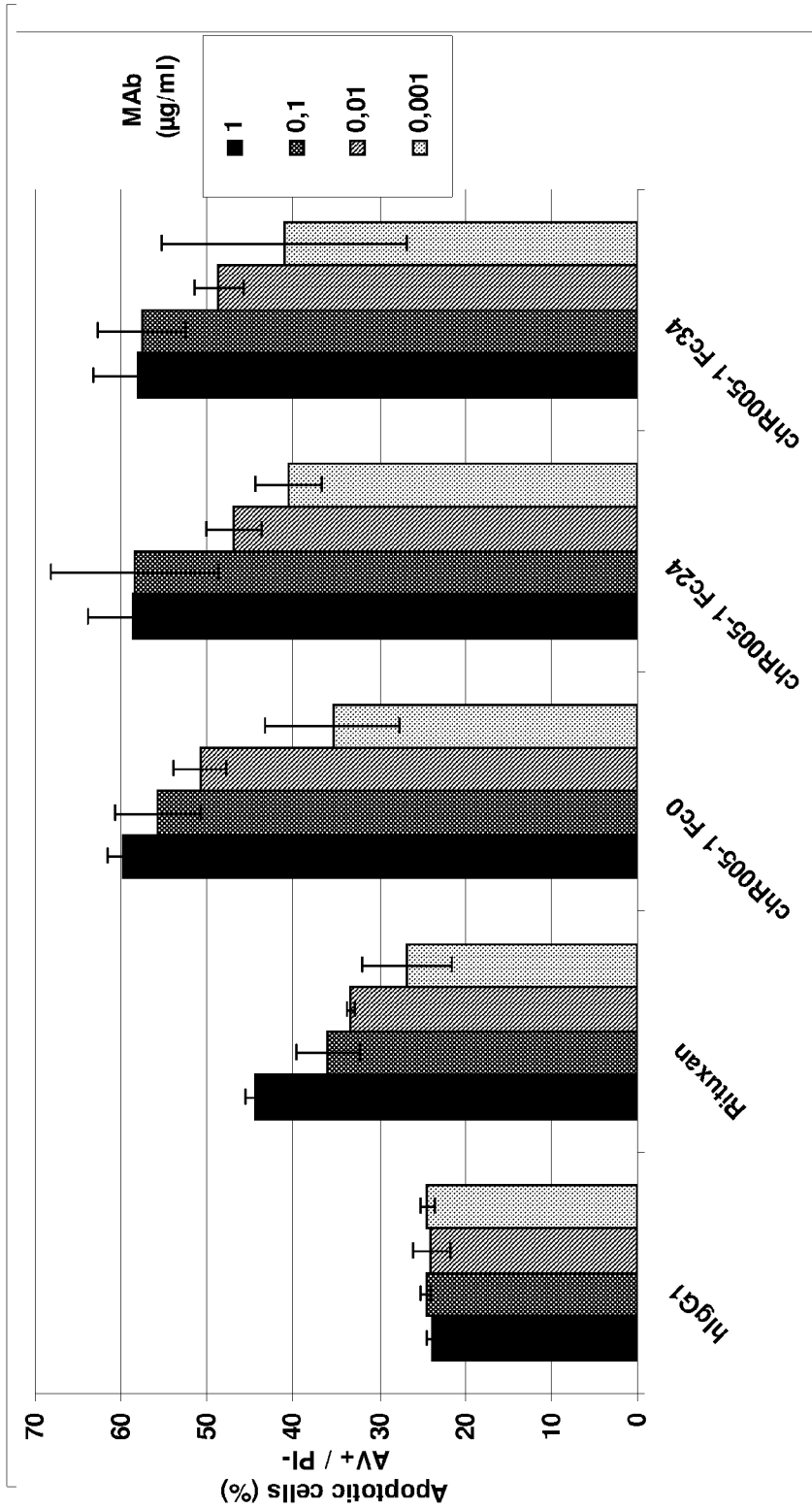
Figure 18
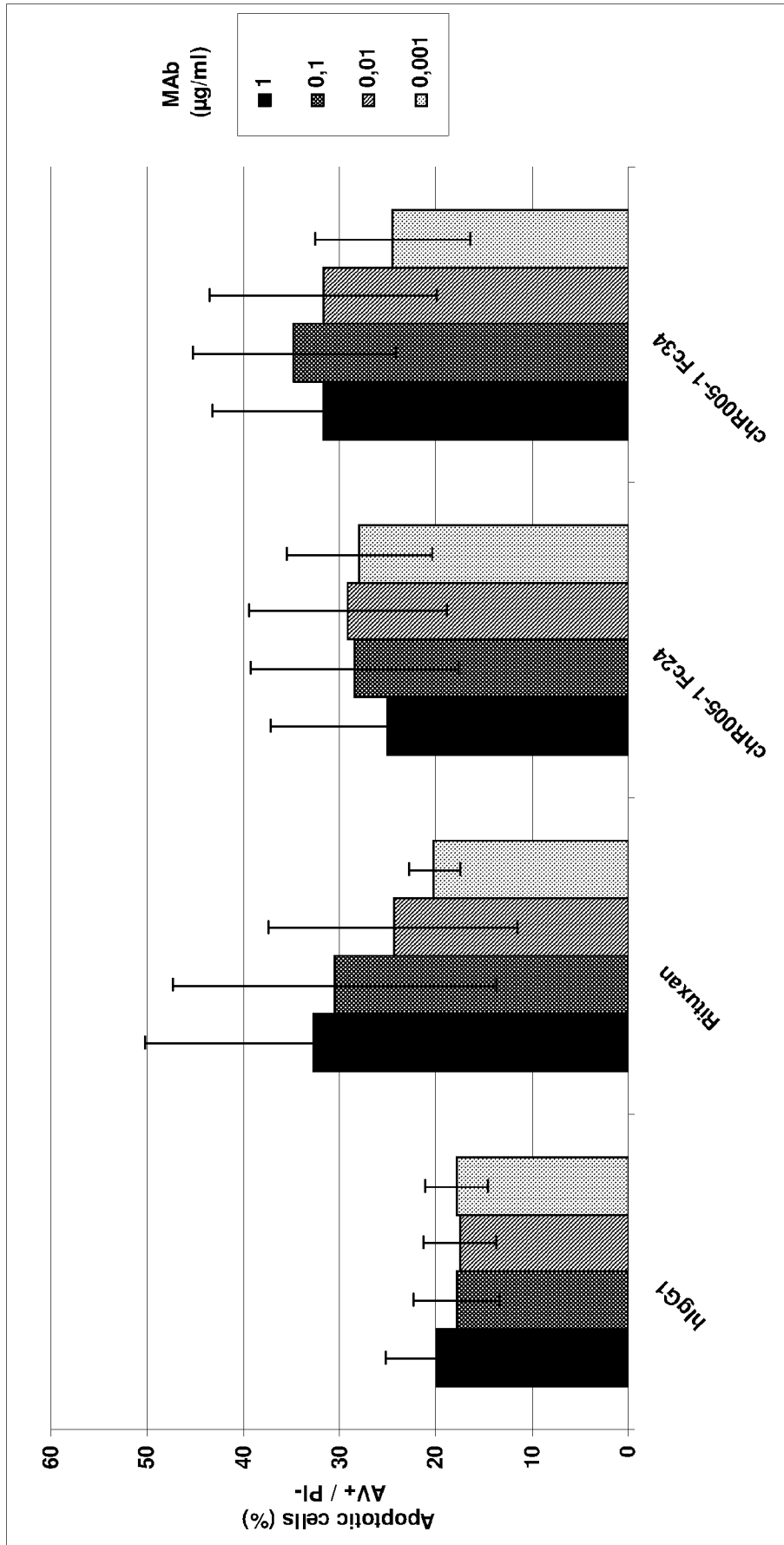
Figure 19
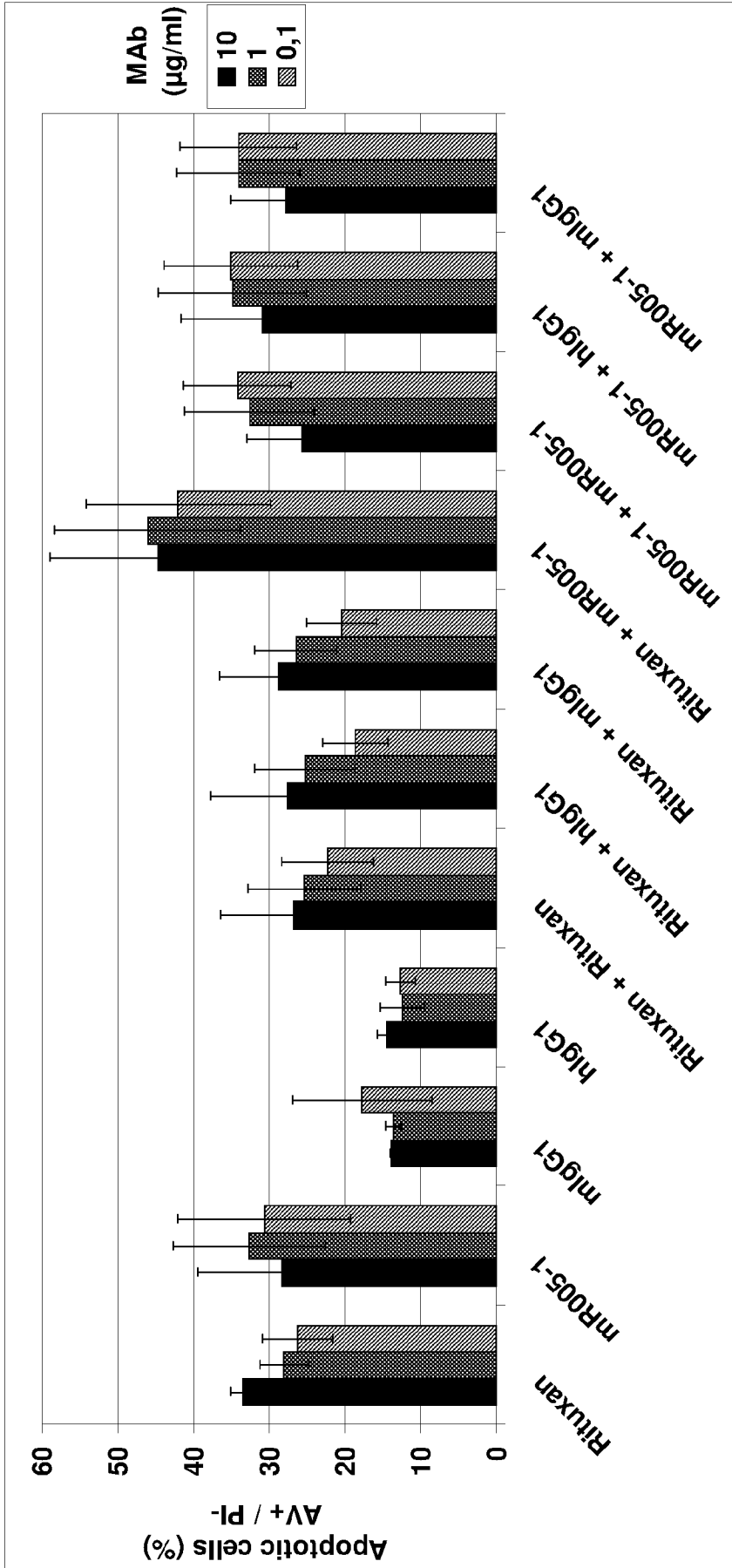
Figure 20
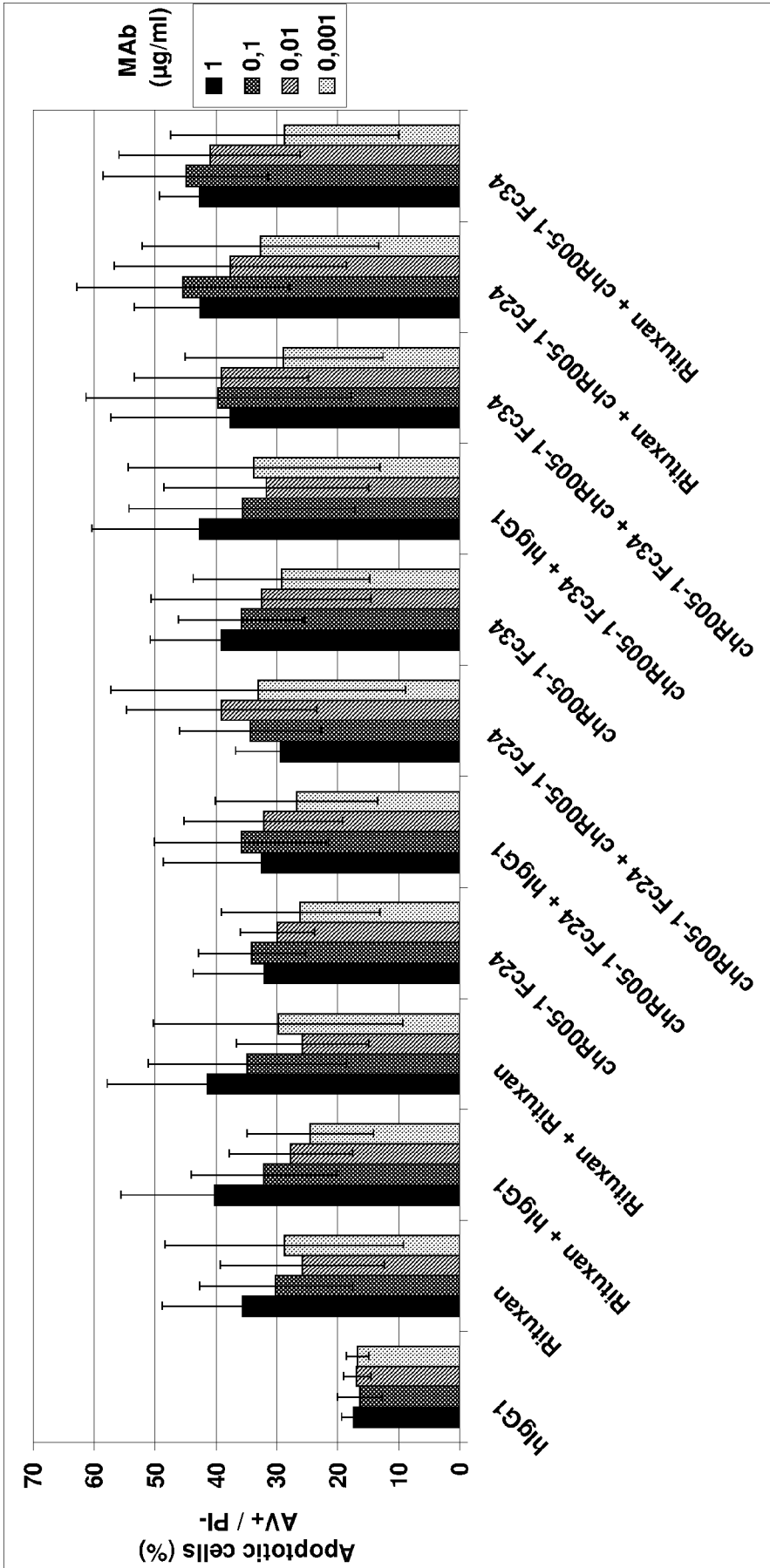
Figure 21
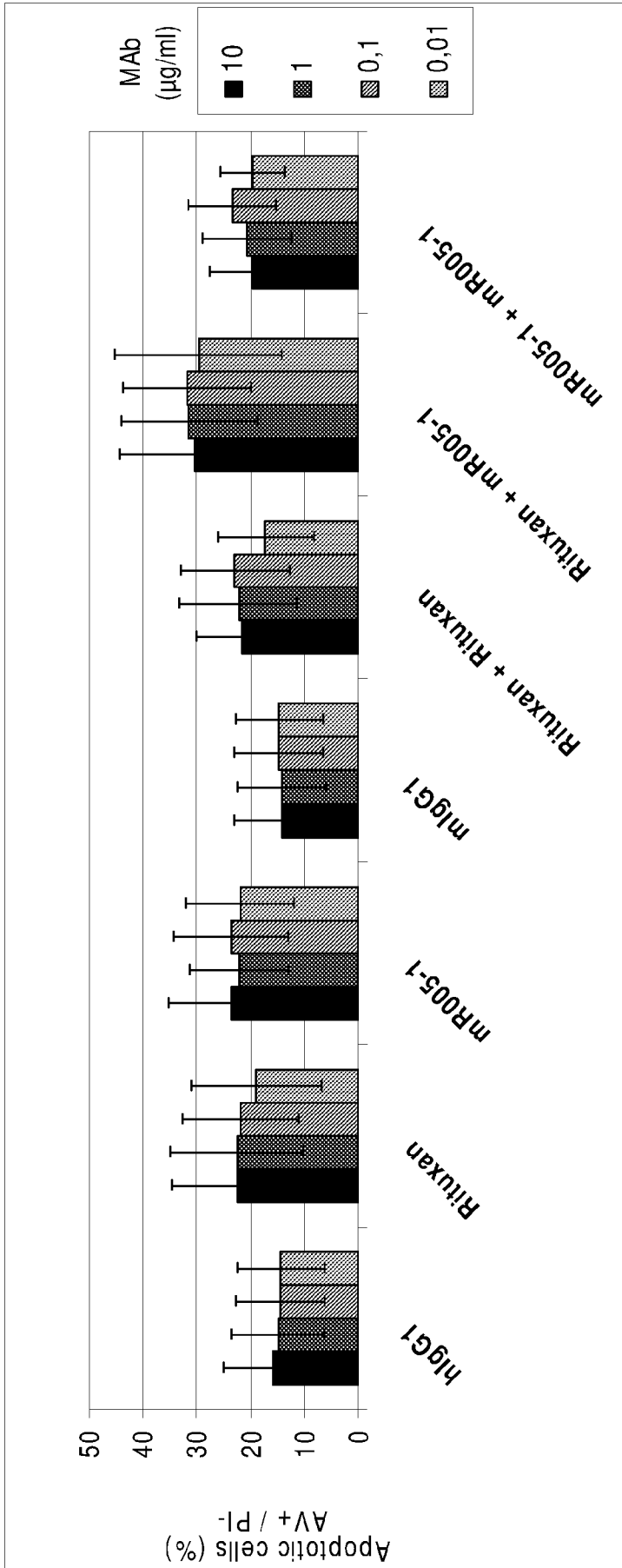
Figure 22
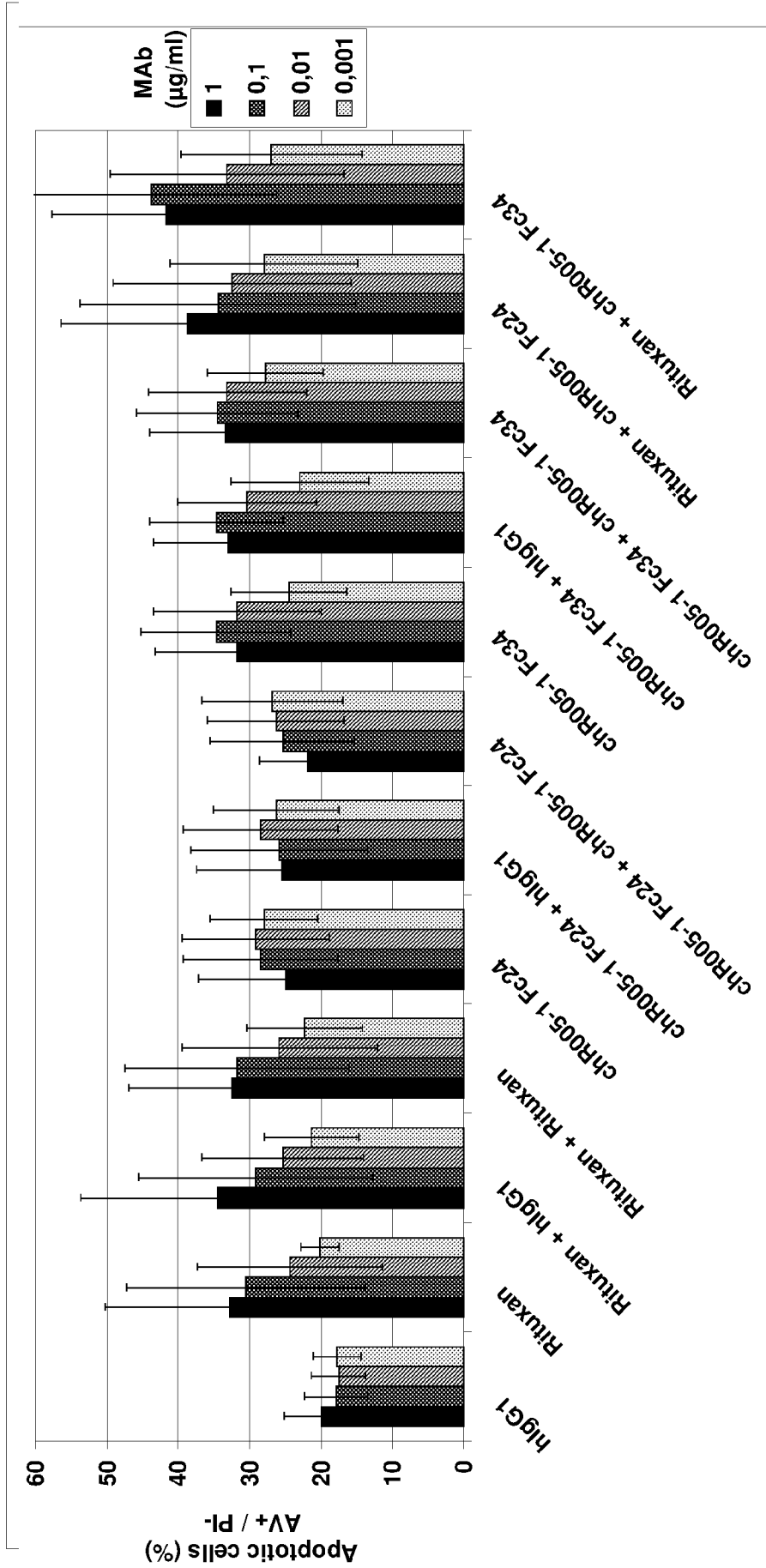
Figure 23
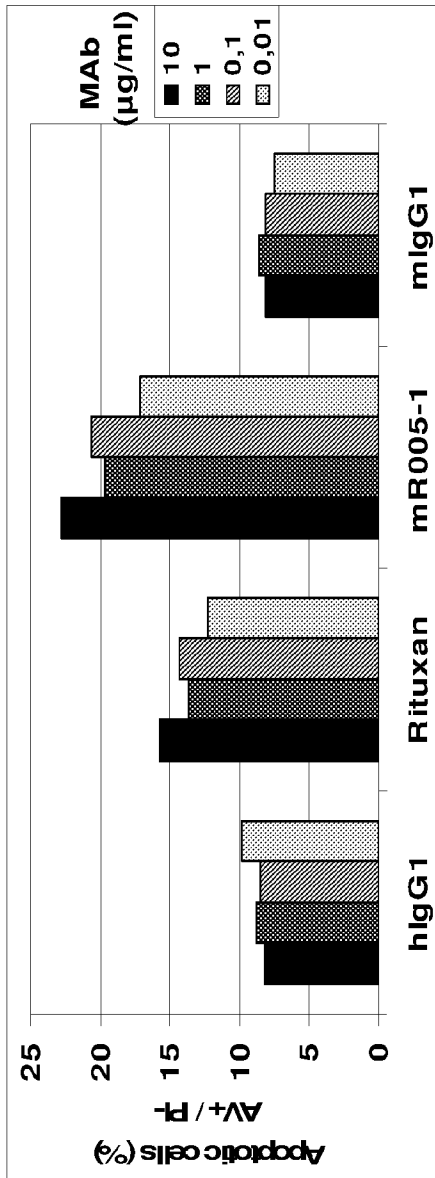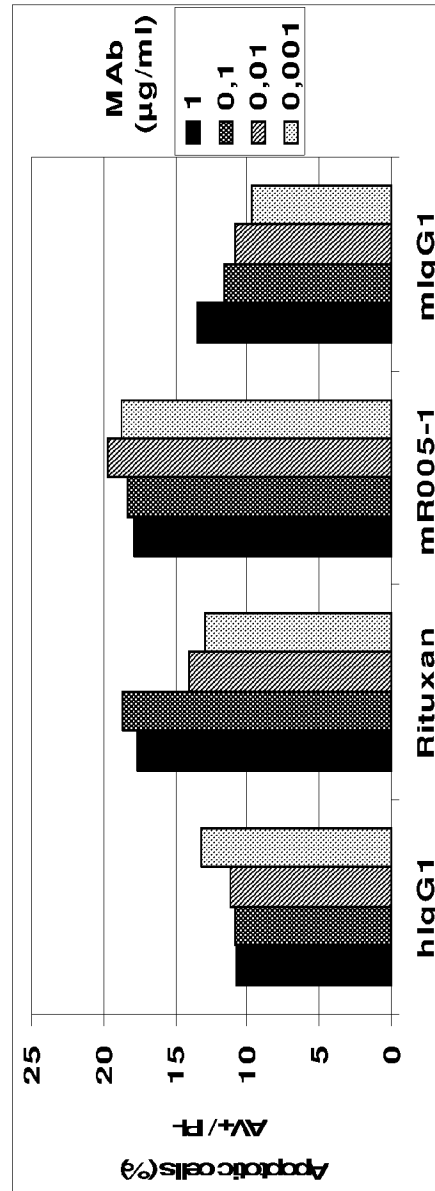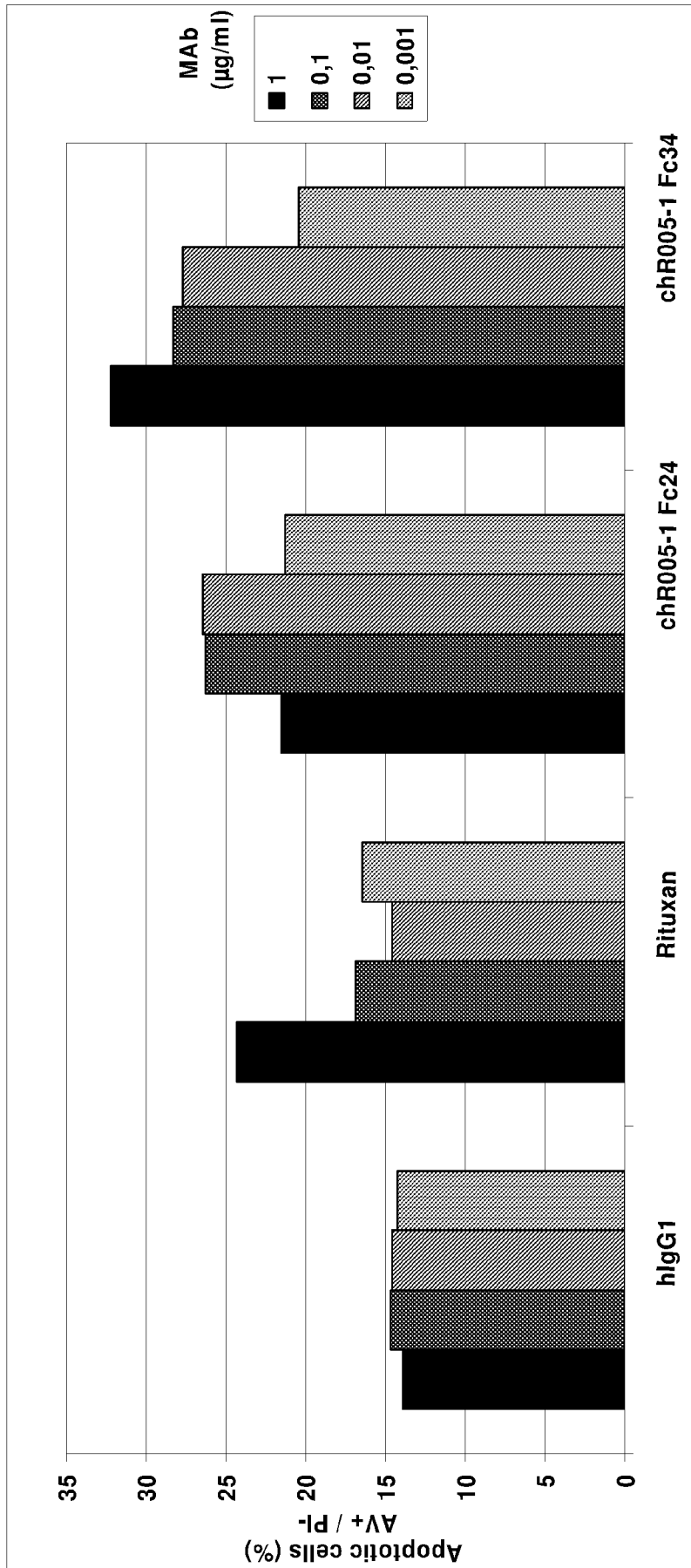
Figure 24

Figure 25
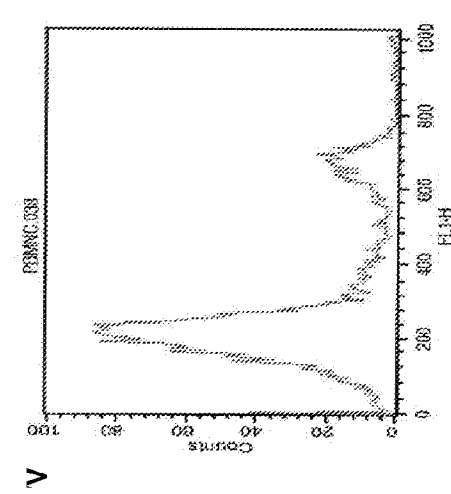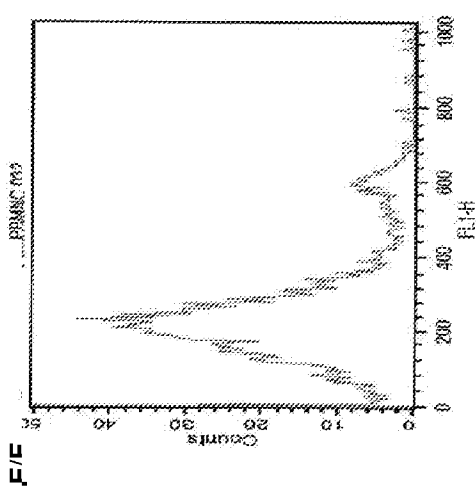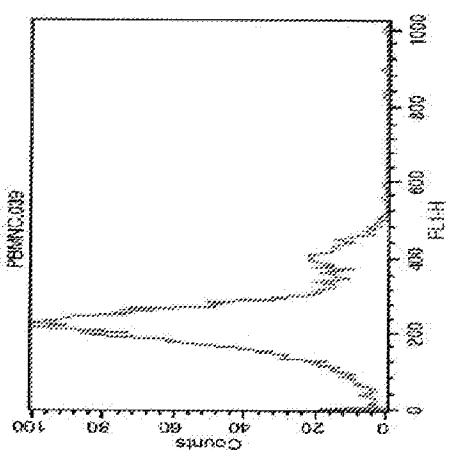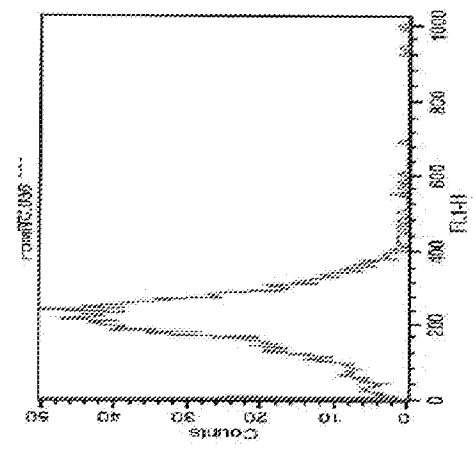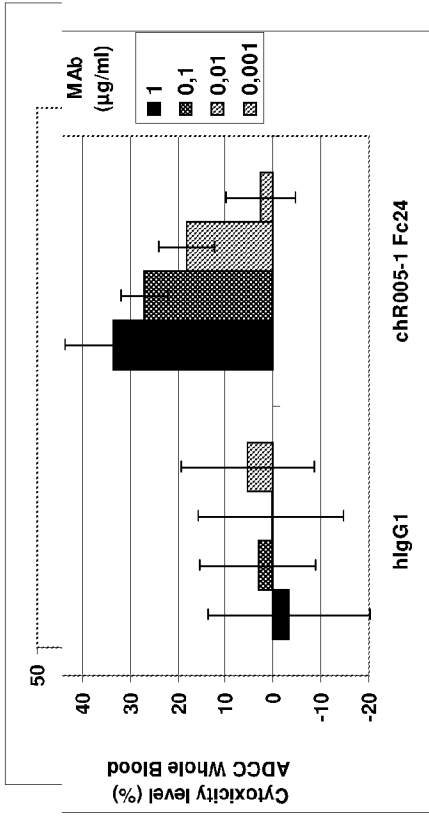
Figure 26
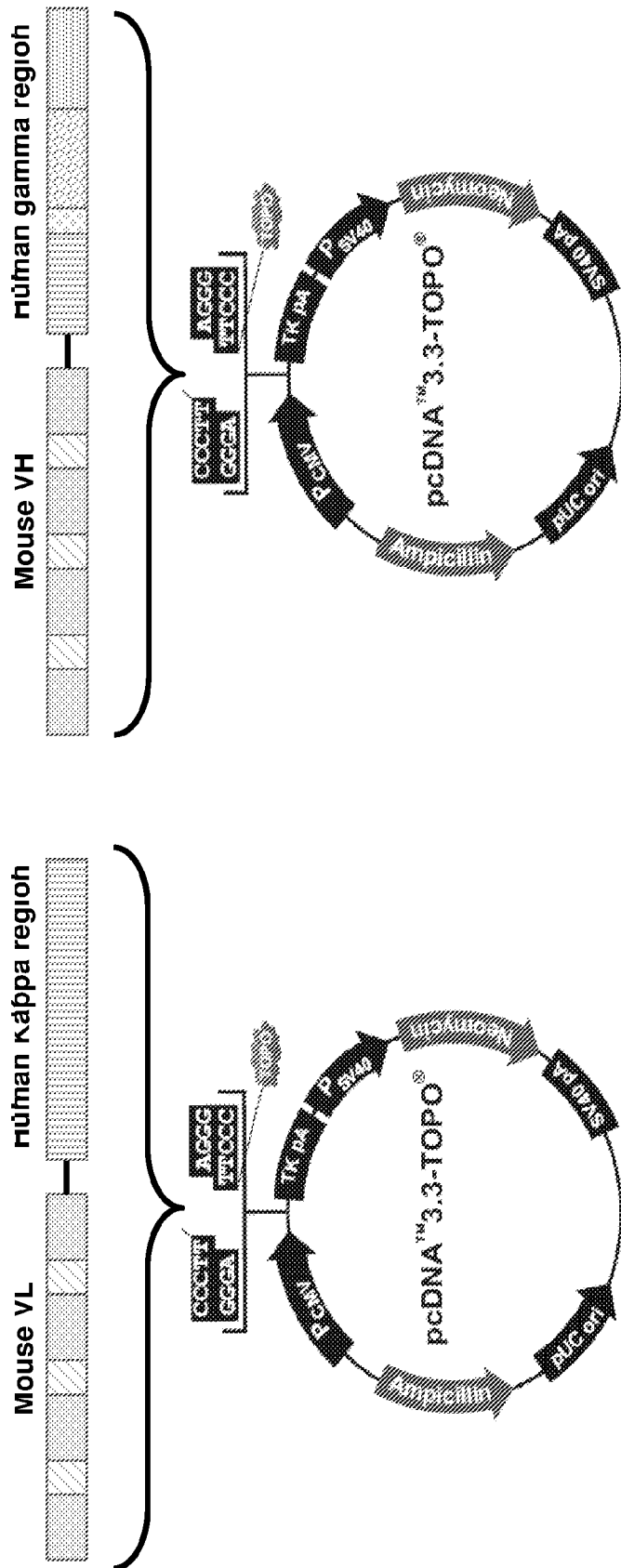
Figure 27
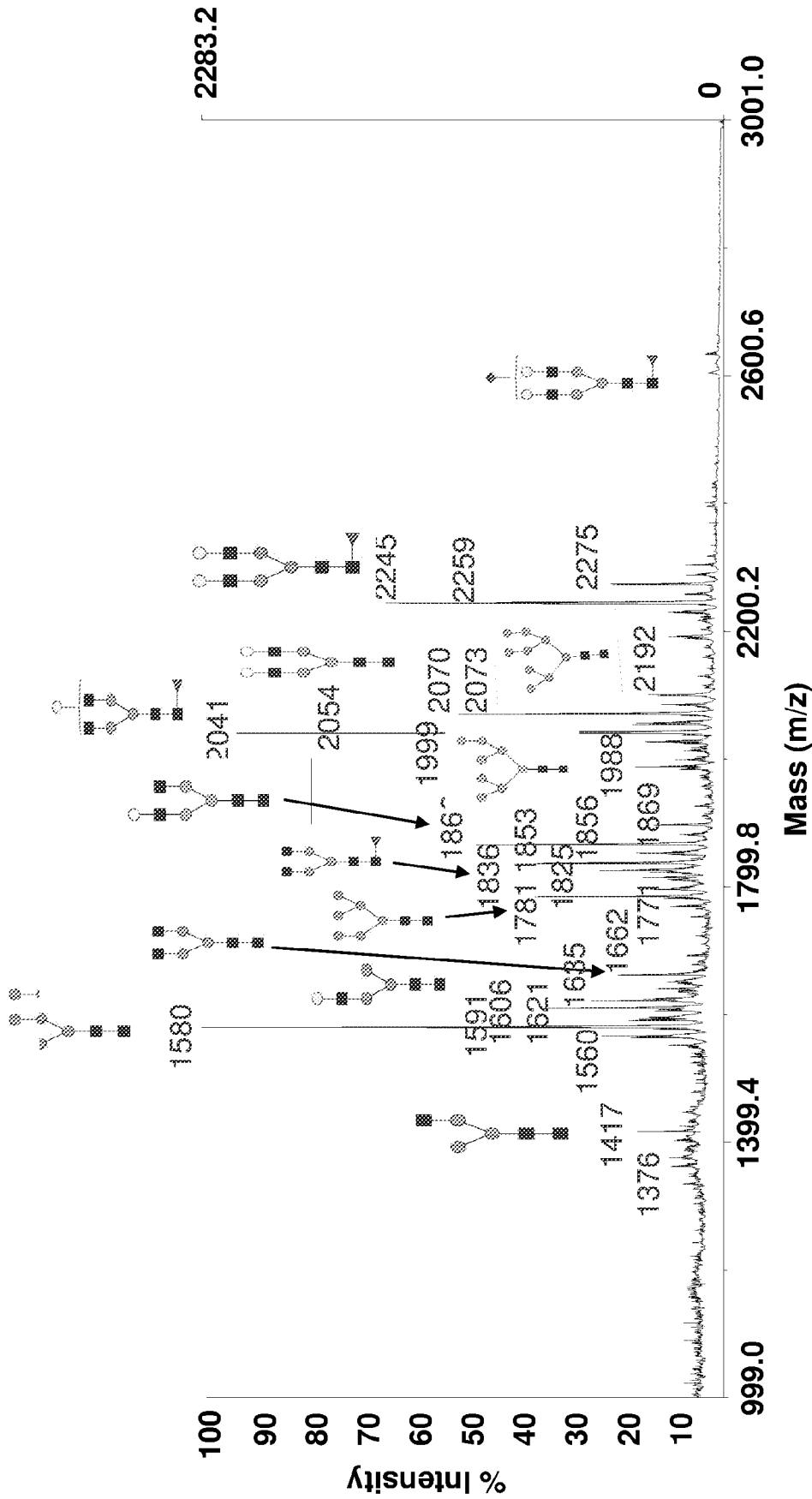
Figure 28
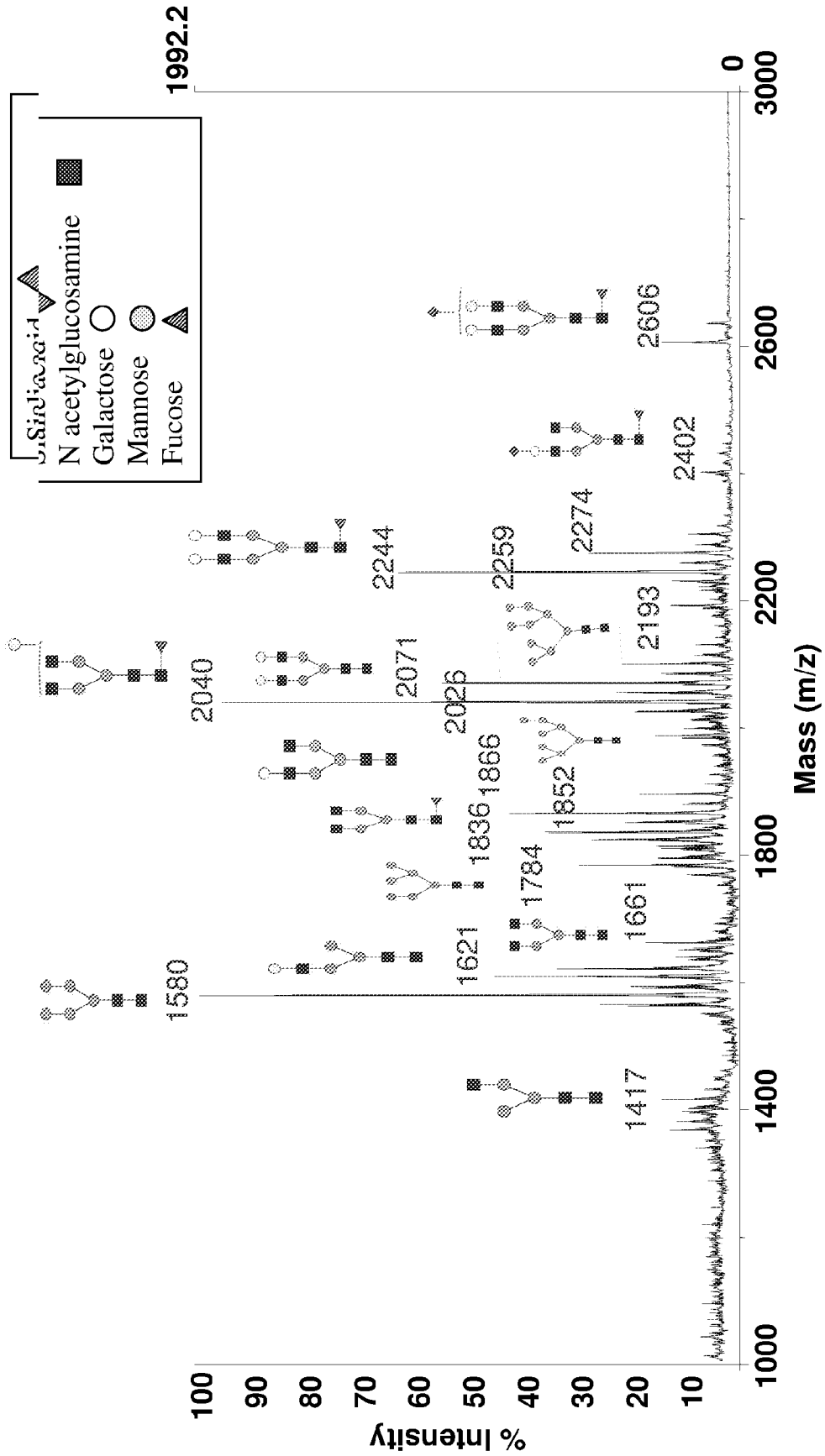
Figure 29

Figure 30

| Masse théorique [M+Na]$^+$ permeth	cnru05-1-1-c0 %RELATIF	cnru05-1-1-c24 %RELATIF	cnru05-1-1-c34 %RELATIF	Interprétation
1375,69	0,0	0,6	1,4	(Man)$_4$(GlcNac)$_2$
1416,71	1,3	2,5	2,5	(GlcNAc)$_1$ + (Man)$_3$(GlcNAc)$_2$
1579,78	0,0	19,2	17,3	(Man)$_5$(GlcNAc)$_2$
1590,84	1,2	3,2	2,9	(GlcNAc)$_1$ + (Man)$_3$(GlcNAc)$_2$ **(Fuc)$_1$**
1620,81	2,8	3,7	4,9	(GlcNAc)$_1$ + (Man)$_4$(GlcNAc)$_2$
1661,84	2,4	2,7	3,3	(GlcNAc)$_2$ + (Man)$_3$(GlcNAc)$_2$
1783,88	0,0	5,7	6,0	(Man)$_6$(GlcNAc)$_2$
1794,90	0,0	1,2	2,6	(Gal)$_1$(GlcNAc)$_1$ + (Man)$_3$(GlcNAc)$_2$ **(Fuc)$_1$**
1824,92	0,0	3,0	2,9	(Gal)$_1$ (GlcNAc)$_1$ + (Man)$_4$(GlcNAc)$_2$
1835,93	28,0	7,2	5,2	(GlcNAc)$_2$ **(Fuc)$_1$** + (Man)$_3$(GlcNAc)$_2$
1865,94	6,5	8,0	7,4	(GlcNAc)$_2$ (Fuc)$_1$ + (Man)$_3$(GlcNAc)$_2$ hypermeth ou (Gal)$_1$ (GlcNAc)$_2$ + (Man)$_3$(GlcNAc)$_2$
1987,98	0,0	1,9	2,3	(Man)$_7$(GlcNAc)$_2$
2040,03	38,3	16,8	16,1	(Gal)$_1$(GlcNAc)$_2$ **(Fuc)$_1$** + (Man)$_3$(GlcNAc)$_2$
2070,04	6,2	8,6	8,8	(Gal)$_1$(GlcNAc)$_2$ (Fuc)$_1$ + (Man)$_3$(GlcNAc)$_2$ hypermeth ou (Gal)$_2$ (GlcNAc)$_2$ + (Man)$_3$(GlcNAc)$_2$
2192,09	0,0	2,2	1,5	(Man)$_8$(GlcNAc)$_2$
2244,12	12,4	11,2	11,6	(Gal)$_2$ (GlcNAc)$_2$ **(Fuc)$_1$** + (Man)$_3$(GlcNAc)$_2$
2396,19	0,0	0,9	0,8	(Man)$_9$(GlcNAc)$_2$
2401,20	0,0	0,6	1,0	(Gal)$_1$ (GlcNAc)$_2$ **(Fuc)$_1$** (NeuAc)$_1$+ (Man)$_3$(GlcNAc)$_2$
2605,30	0,8	1,0	1,4	(Gal)$_2$ (GlcNAc)$_2$ **(Fuc)$_1$** (NeuAc)$_1$+ (Man)$_3$(GlcNAc)$_2$
2966,47	0,0	0,0	0,0	(Gal)$_2$ (GlcNAc)$_2$ **(Fuc)$_1$** (NeuAc)$_2$+ (Man)$_3$(GlcNAc)$_2$

Figure 31
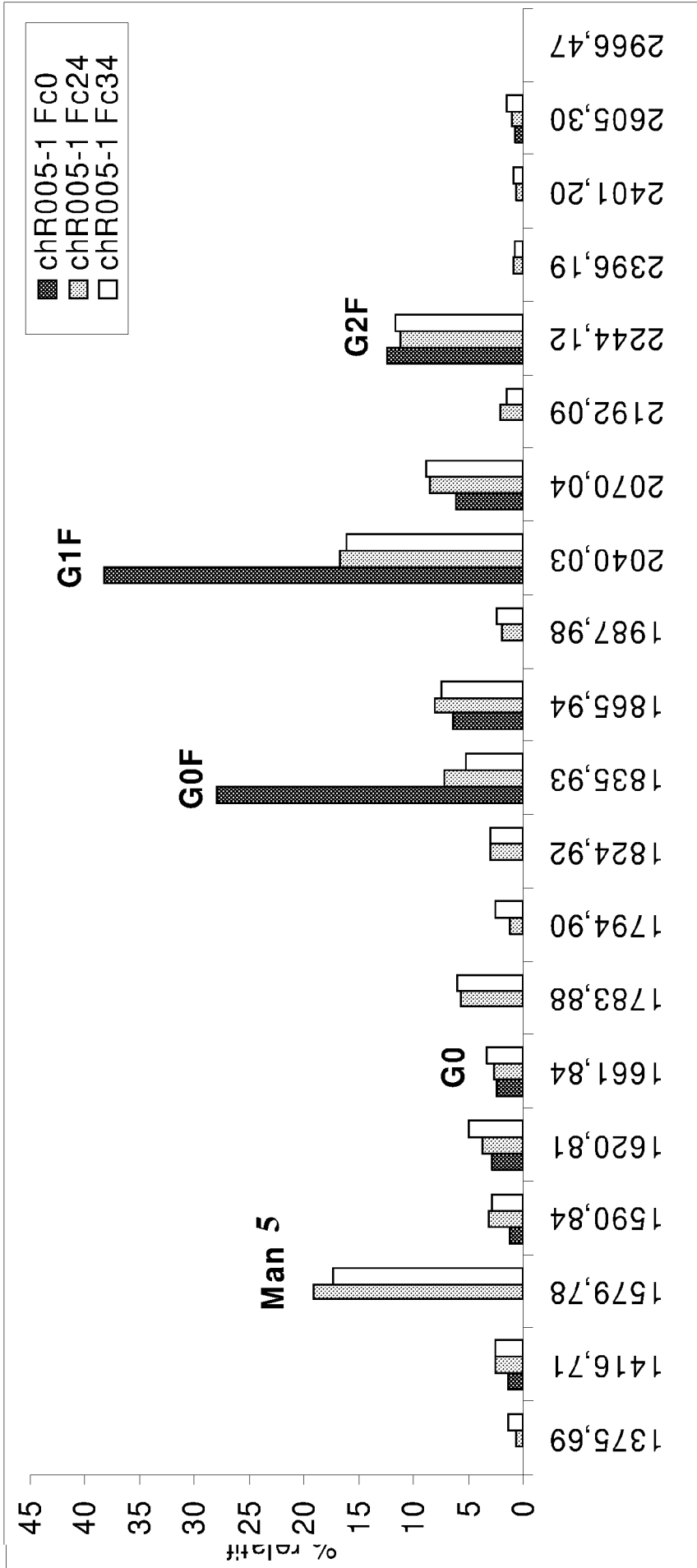
Figure 32

Theoric Mass [M+Na]+ permeth	chR005-1 Fc0 %relatif	chR005-1 Fc6 %relatif	chR005-1 Fc9 %relatif	chR005-1 Fc7 %relatif	chR005-1 Fc18 %relatif	chR005-1 Fc19 %relatif	chR005-1 Fc23 %relatif	chR005-1 Fc28 %relatif	chR005-1 Fc29 %relatif	chR005-1 Fc30 %relatif	chR005-1 Fc20 %relatif	chR005-1 Fc24 %relatif	chR005-1 Fc34 %relatif
1375,69	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,6	0,3	0,7
1416,71	2,7	2,7	3,4	1,9	2,1	4,3	4,2	1,4	4,8	4,1	4,0	5,5	5,8
1579,78	0,0	0,0	0,0	1,4	4,5	23,4	39,6	0,0	24,6	44,5	38,7	35,6	32,9
1590,84	0,8	0,0	0,0	1,2	3,0	6,6	5,8	0,7	7,1	6,4	5,4	5,8	5,7
1620,81	0,7	0,0	0,7	2,1	3,2	3,5	2,8	0,0	2,9	2,7	3,0	3,2	3,5
1661,84	1,4	0,0	1,2	2,3	0,0	2,0	2,7	0,0	2,3	2,1	2,3	3,8	3,4
1783,88	0,0	0,0	0,0	0,0	0,0	3,0	7,5	0,0	3,4	7,3	8,0	6,7	8,3
1794,90	0,0	0,0	0,0	1,4	0,0	1,6	1,4	0,0	2,1	0,8	1,5	1,5	1,5
1824,92	0,0	0,0	0,0	1,1	2,0	1,8	2,5	0,0	2,0	1,9	2,6	2,2	2,1
1835,93	68,2	75,4	72,0	26,2	26,8	19,3	15,3	76,6	20,9	13,9	13,6	14,5	12,6
1865,94	0,0	0,0	0,0	11,3	0,0	2,6	0,0	0,0	2,6	1,9	1,9	2,4	2,4
1987,98	0,0	0,0	0,0	0,0	0,0	1,0	4,1	0,0	1,0	3,0	4,4	3,5	4,7
2040,03	25,0	21,2	21,9	31,2	30,5	21,7	8,6	20,5	17,6	8,6	8,1	8,9	8,7
2070,04	0,0	0,0	0,0	12,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
2192,08	0,0	0,0	0,0	0,0	0,0	0,5	2,1	0,0	0,6	1,5	2,3	2,2	3,5
2244,12	1,2	0,7	0,7	6,9	18,6	6,1	2,0	0,8	5,2	1,3	1,7	1,9	1,7
2396,19	0,0	0,0	0,0	0,0	0,0	0,0	0,4	0,0	0,0	0,0	0,4	0,3	1,3
2401,20	0,0	0,0	0,0	0	3,4	1,8	0,4	0,0	1,8	0,0	0,5	0,8	0,3
2605,30	0,0	0,0	0,0	0,9	5,3	0,7	0,4	0,0	0,7	0,0	0,3	0,2	0,2
2966,47	0,0	0,0	0,0	0,0	0,7	0,0	0,2	0,0	0,0	0,0	0,0	0,0	0,0

Figure 33

(A)

(B)

Figure 34

(A)

(B)
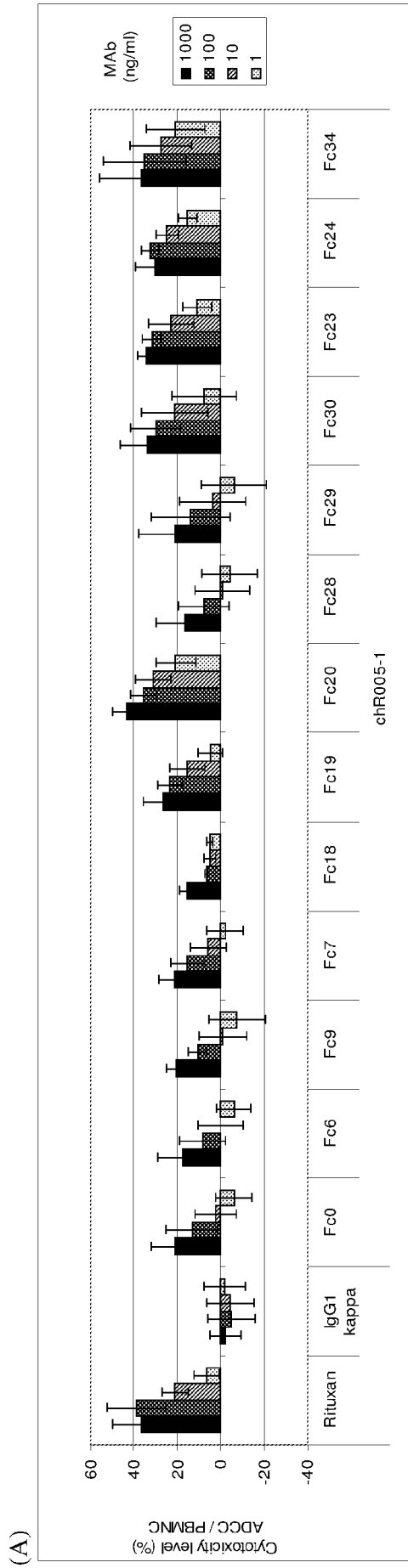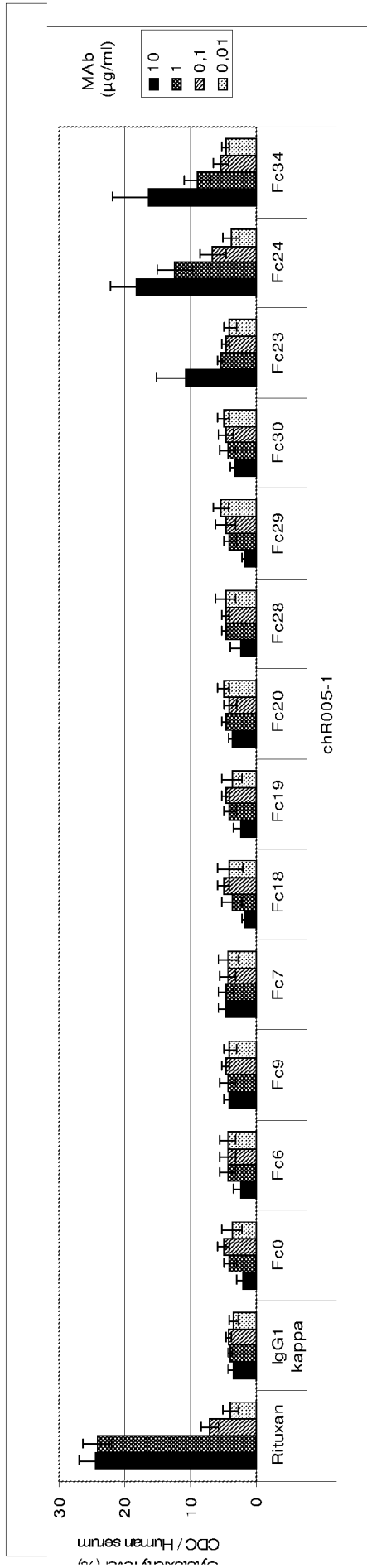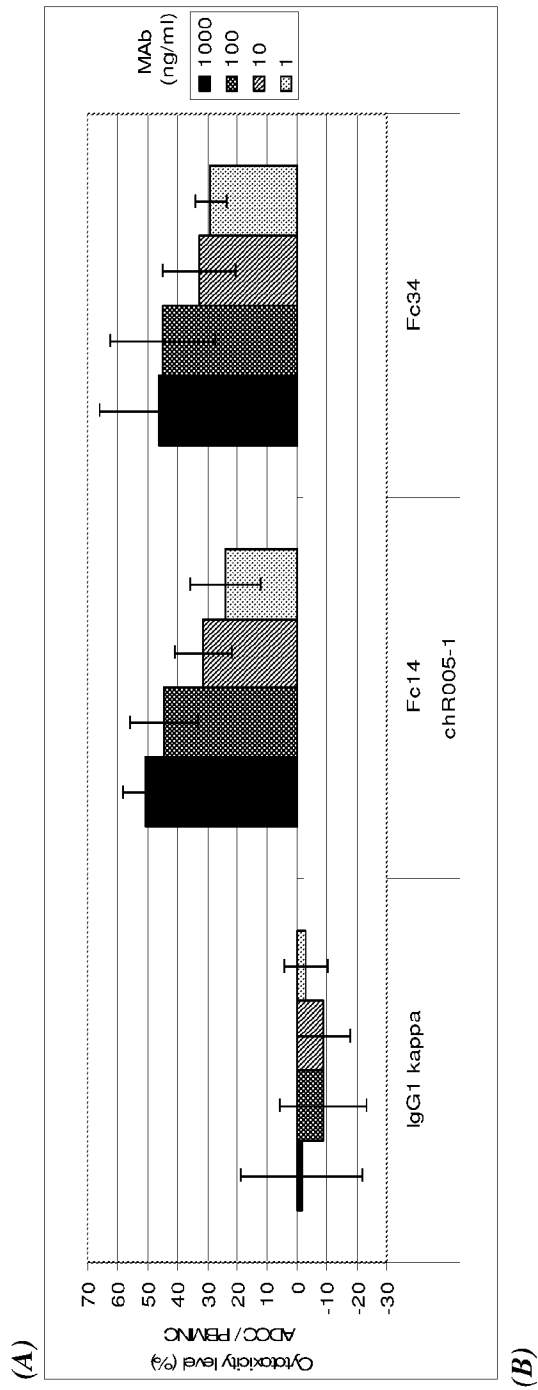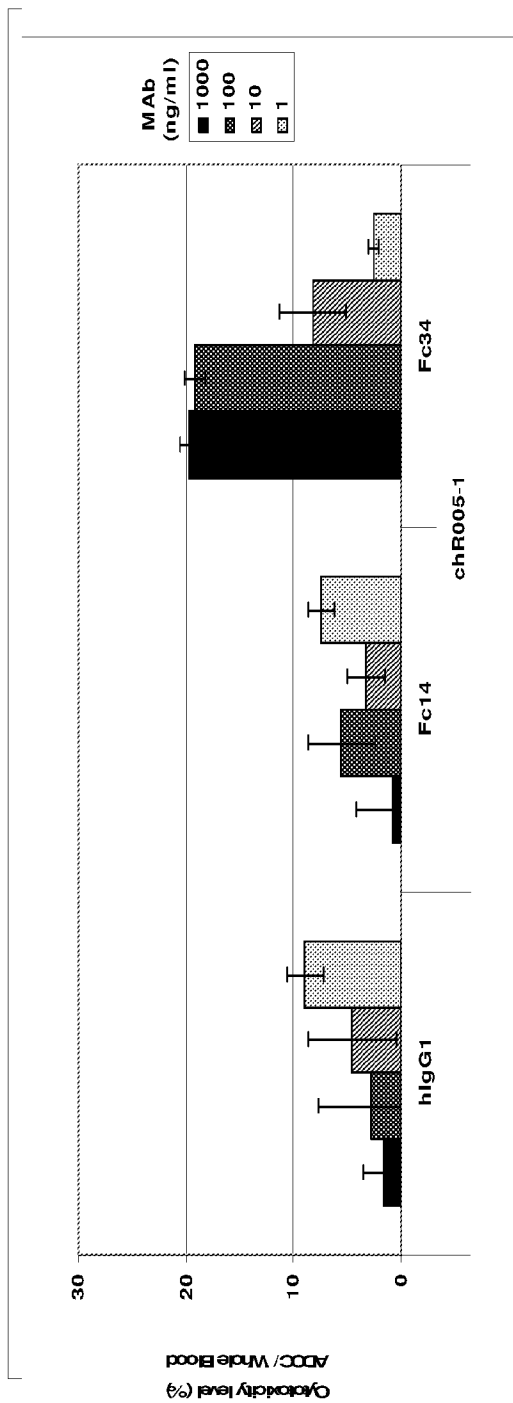
Figure 35

Figure 36

(A)

B)
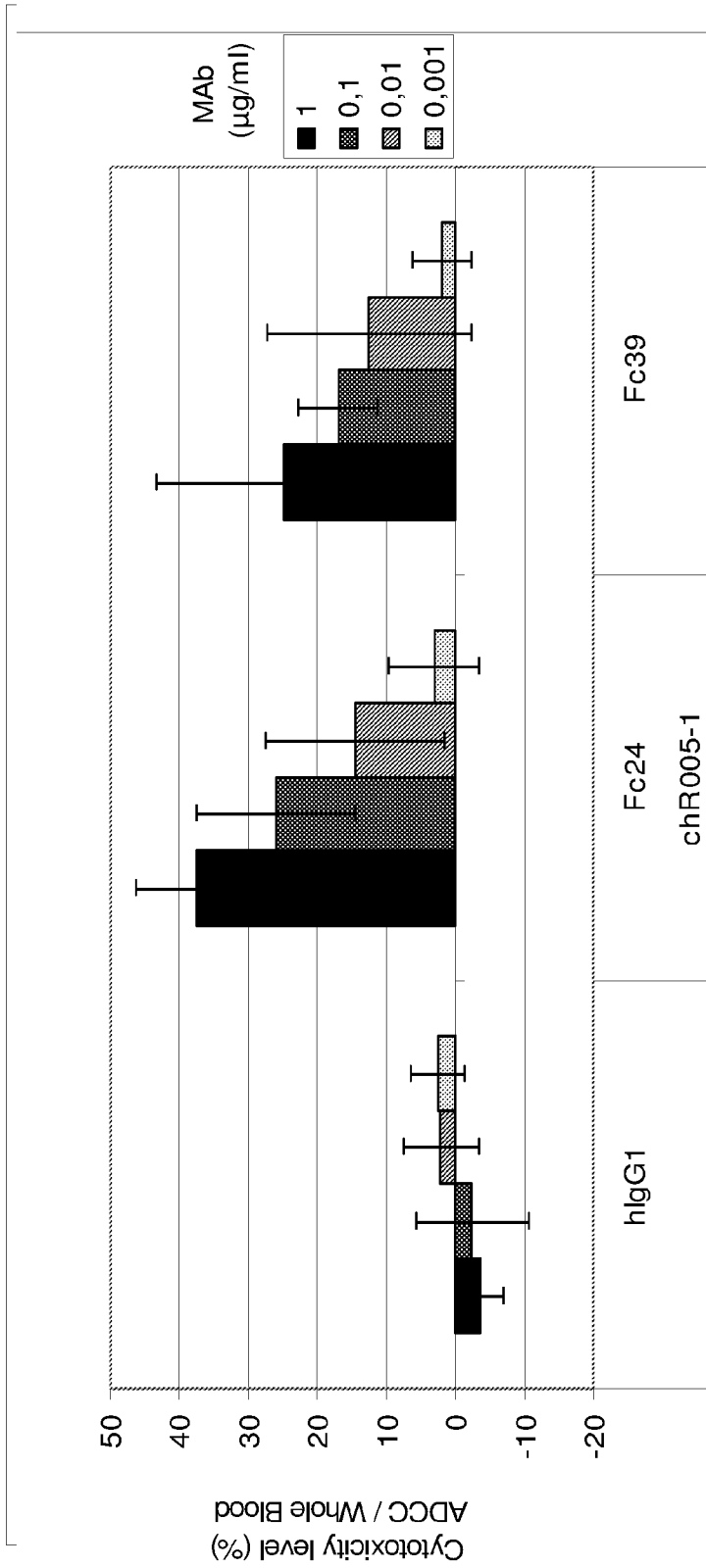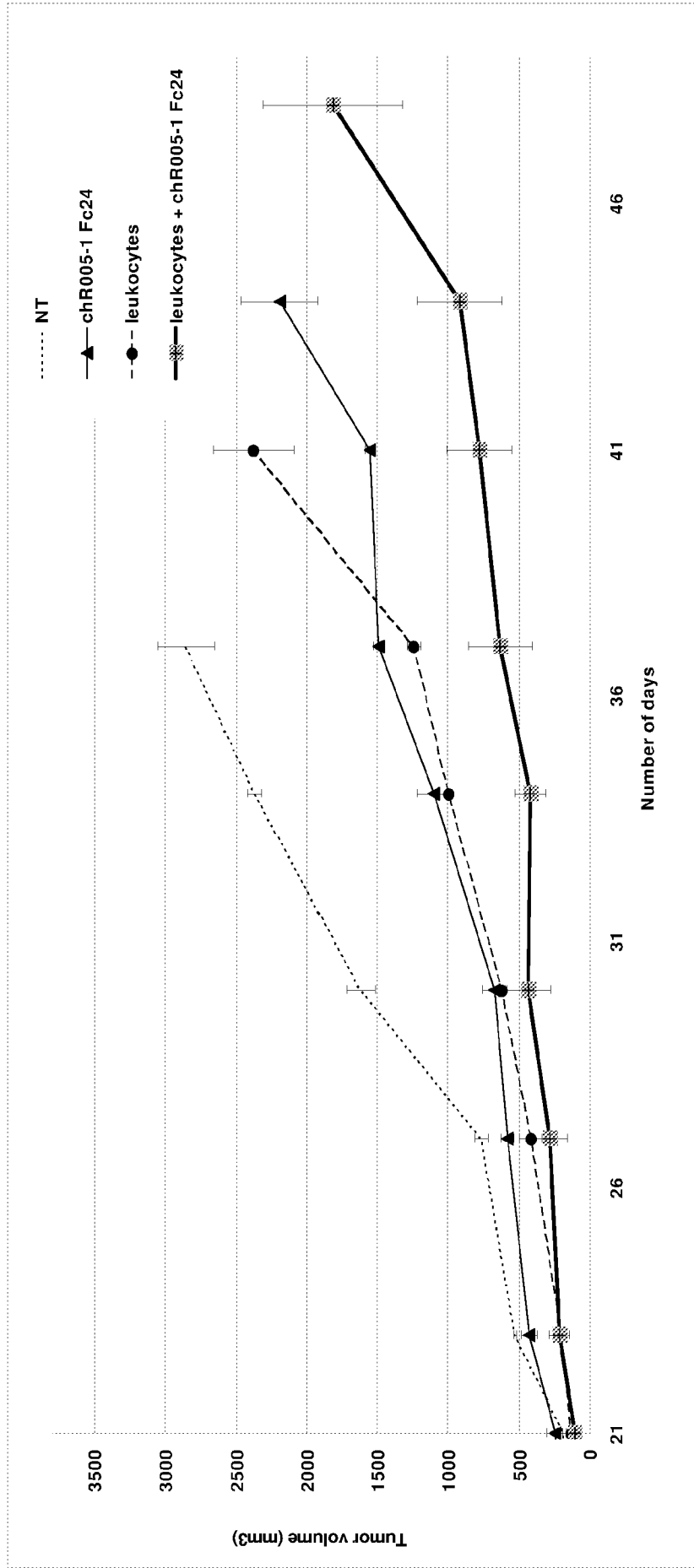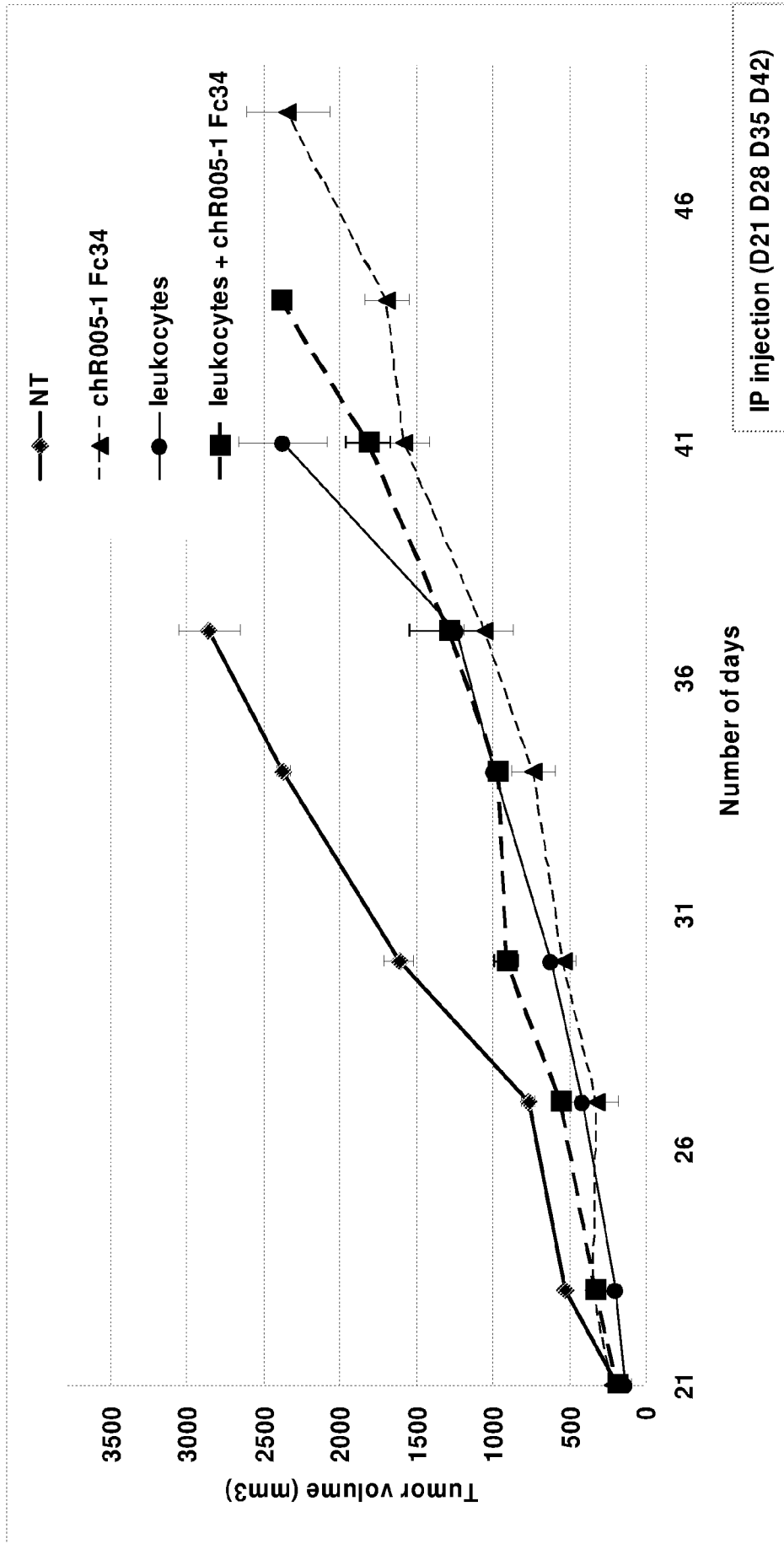

Figure 37
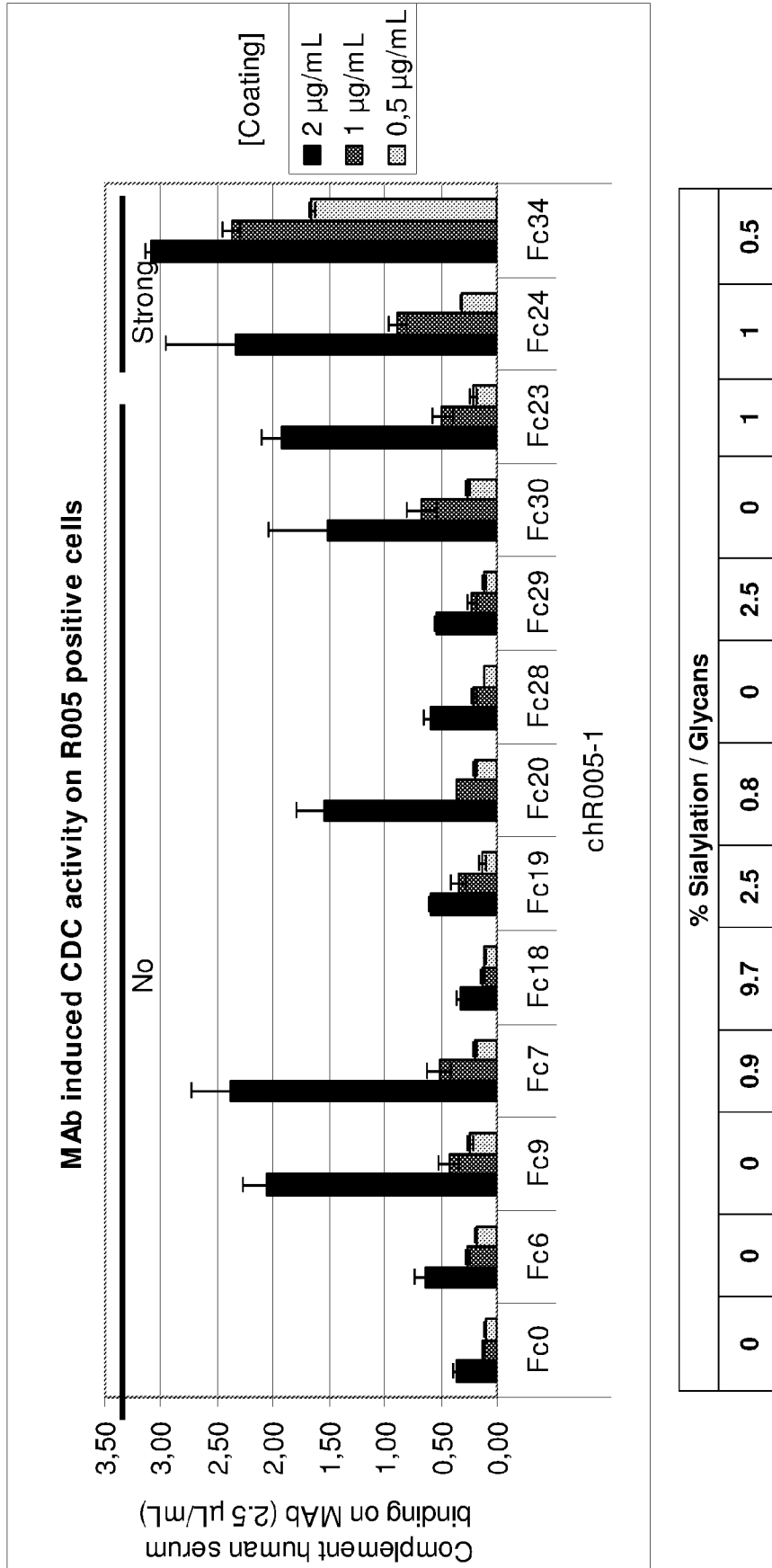
Figure 38
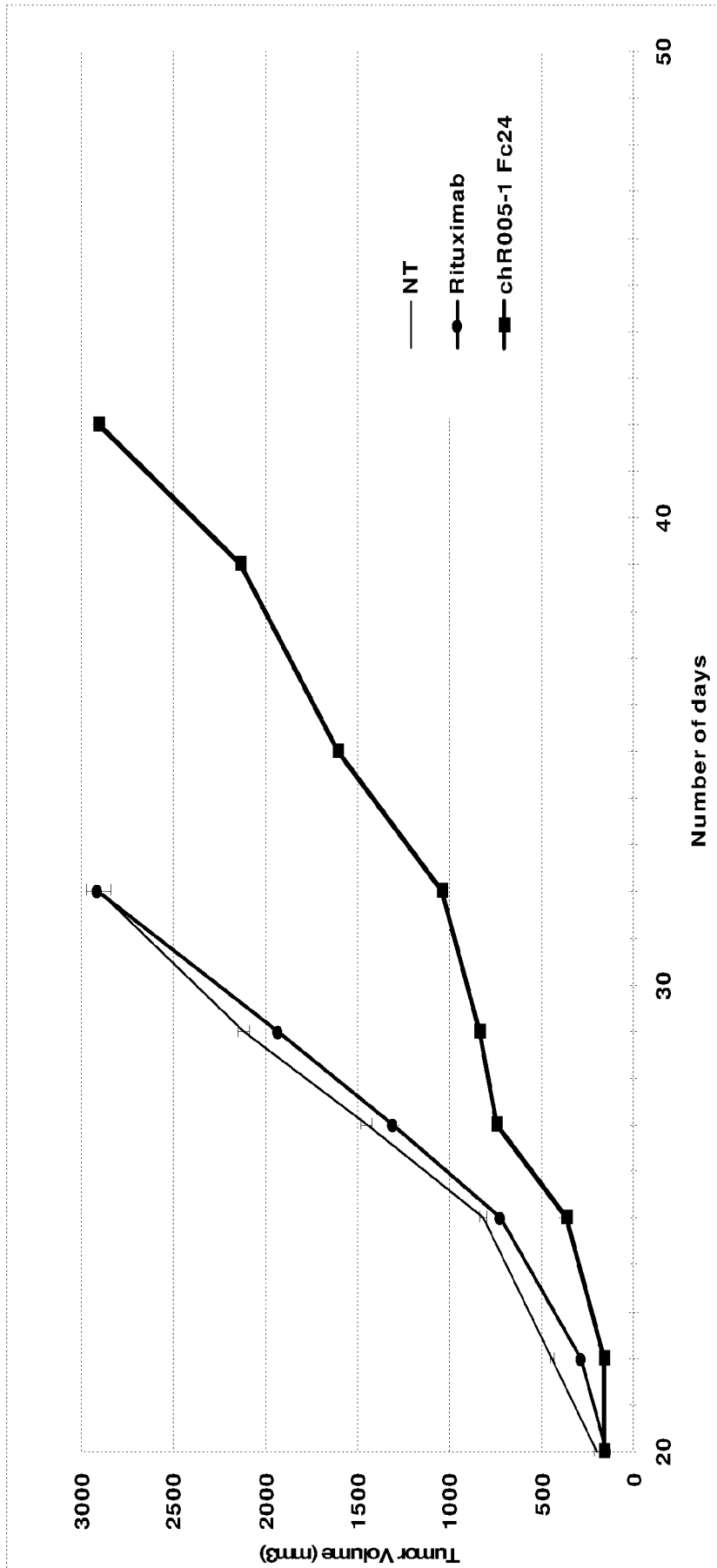
Figure 39
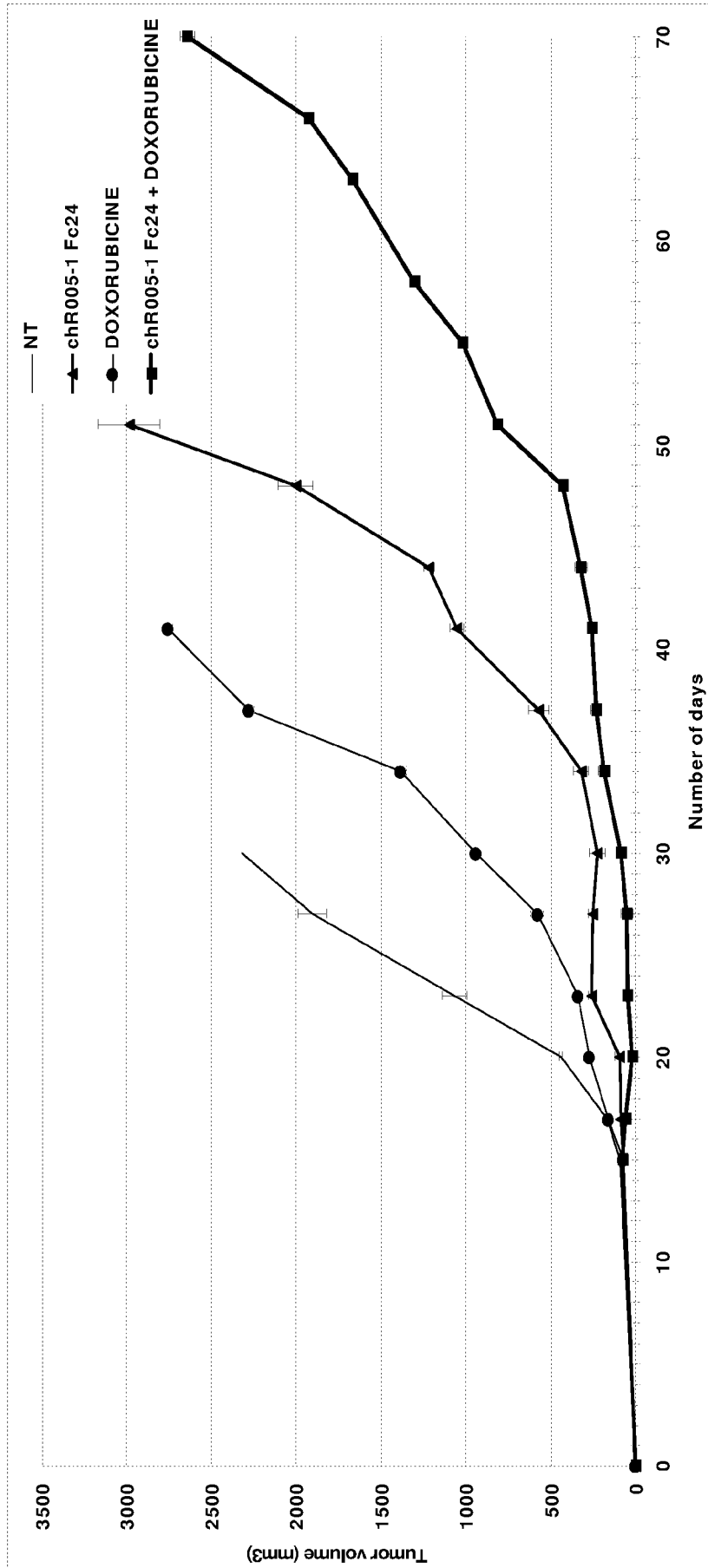
Figure 40

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K47/48   C07K16/28
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HORTON HOLLY M ET AL: "Potent in vitro and in vivo activity of an Fc-engineered anti-CD19 monoclonal antibody against lymphoma and leukemia", CANCER RESEARCH, vol. 68, no. 19, October 2008 (2008-10), pages 8049-8057, XP009145044, ISSN: 0008-5472 the whole document ----- -/--	1-21

[X] Further documents are listed in the continuation of Box C.   [X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 October 2011	13/10/2011

| Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016 | Authorized officer Chapman, Rob |

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Form PCT/ISA/210 (second sheet) (April 2005)

page 1 of 4

C(Continuation).	DOCUMENTS CONSIDERED TO BE RELEVANT	
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	AWAN FARRUKH T ET AL: "CD19 targeting of chronic lymphocytic leukemia with a novel Fc-domain-engineered monoclonal antibody", BLOOD, AMERICAN SOCIETY OF HEMATOLOGY, US, vol. 115, no. 6, 1 February 2010 (2010-02-01), pages 1204-1213, XP009143421, ISSN: 0006-4971 the whole document	1-21
Y	PINA M CARDARELLI ET AL: "A nonfucosylated human antibody to CD19 with potent B-cell depletive activity for therapy of B-cell malignancies", CANCER IMMUNOLOGY, IMMUNOTHERAPY, SPRINGER, BERLIN, DE, vol. 59, no. 2, 6 August 2009 (2009-08-06), pages 257-265, XP019757711, ISSN: 1432-0851 the whole document	1-21
Y	STAVENHAGEN JEFFREY B ET AL: "Fc optimization of therapeutic antibodies enhances their ability to kill tumor cells in vitro and controls tumor expansion in vivo via low-affinity activating fc gamma receptors", CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER REREARCH, US, vol. 67, no. 18, 1 September 2007 (2007-09-01), pages 8882-8890, XP002489883, ISSN: 0008-5472, DOI: DOI:10.1158/0008-5472.CAN-07-0696 the whole document	1-21
Y	US 7 183 387 B1 (PRESTA LEONARD [US]) 27 February 2007 (2007-02-27) the whole document	1-21
Y	WO 2008/031056 A2 (MEDIMMUNE INC [US]; DAMSCHRODER MELISSA [US]; KIENER PETER [US]; WU HE) 13 March 2008 (2008-03-13) the whole document	1-21
Y	WO 2008/022152 A2 (XENCOR INC [US]; BERNETT MATTHEW J [US]; CHU SEUNG YUP [US]; DESJARLAI) 21 February 2008 (2008-02-21) the whole document	1-21
Y	US 2009/098124 A1 (STAVENHAGEN JEFFREY B [US]) 16 April 2009 (2009-04-16) the whole document	1-21

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Form PCT/ISA/210 (continuation of second sheet) (April 2005)

C(Continuation).	DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	STROHL ET AL: "Optimization of Fc-mediated effector functions of monoclonal antibodies", CURRENT OPINION IN BIOTECHNOLOGY, LONDON, GB, vol. 20, no. 6, 1 December 2009 (2009-12-01), pages 685-691, XP026778879, ISSN: 0958-1669, DOI: DOI:10.1016/J.COPBIO.2009.10.011 [retrieved on 2009-11-04] the whole document -----	1-21
A	MASUDA ET AL: "Enhanced binding affinity for FcgammaRIIIa of fucose-negative antibody is sufficient to induce maximal antibody-dependent cellular cytotoxicity", MOLECULAR IMMUNOLOGY, PERGAMON, GB, vol. 44, no. 12, 17 April 2007 (2007-04-17), pages 3122-3131, XP022031617, ISSN: 0161-5890, DOI: DOI:10.1016/J.MOLIMM.2007.02.005 the whole document -----	1-21
A	MOORE GREGORY L ET AL: "Engineered Fc variant antibodies with enhanced ability to recruit complement and mediate effector functions", MABS, vol. 2, no. 2, March 2010 (2010-03), pages 181-189, XP009143498, ISSN: 1942-0862 the whole document -----	1-21
A	BEERS STEPHEN A ET AL: "Antigenic modulation limits the efficacy of anti-CD20 antibodies: implications for antibody selection", BLOOD, AMERICAN SOCIETY OF HEMATOLOGY, US, vol. 115, no. 25, 1 June 2010 (2010-06-01) , pages 5191-5201, XP009145013, ISSN: 0006-4971 the whole document -----	1-21

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JASSAL R ET AL: "Sialylation of human IgG-Fc carbohydrate by transfected rat alpha2,6-sialyltransferase", BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, ACADEMIC PRESS INC. ORLANDO, FL, US, vol. 286, no. 2, 17 August 2001 (2001-08-17), pages 243-249, XP002574380, ISSN: 0006-291X, DOI: DOI:10.1006/BBRC.2001.5382 the whole document -----	1-21
A	LUND J ET AL: "Multiple interactions of IgG with its core oligosaccharide can modulate recognition by complement and human Fc gamma receptor I and influence the synthesis of its oligosaccharide chains", JOURNAL OF IMMUNOLOGY, AMERICAN ASSOCIATION OF IMMUNOLOGISTS, US, vol. 157, no. 11, 1 December 1996 (1996-12-01), pages 4963-4969, XP002484003, ISSN: 0022-1767 the whole document -----	1-21

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Box No. I   Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1.   With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a.   (means)

   ☐   on paper

   ☒   in electronic form

   b.   (time)

   ☒   in the international application as filed

   ☐   together with the international application in electronic form

   ☐   subsequently to this Authority for the purpose of search

2.   ☐   In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3.   Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
US 7183387	B1	27-02-2007	US	2007009523 A1	11-01-2007
			US	2010166749 A1	01-07-2010
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			KR	20090059149 A	10-06-2009
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			EP	1999470 A2	10-12-2008
			JP	2009529331 A	20-08-2009
			SG	170110 A1	29-04-2011
			WO	2007106707 A2	20-09-2007