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[Continued on next page]

(54) Title: USE OF A TYPE II ANTI-CD20 ANTIBODY WITH INCREASED ANTIBODY DEPENDENT CELLULAR CYTOTOXICITY (ADCC) IN COMBINATION WITH CYCLOPHOSPHAMIDE, VINCISTINE AND DOxorubicins FOR TREATING NON-HODGKIN’S LYMPHOMAS

Fig. 4

(57) Abstract: The present invention is directed to the use of an anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC) for the manufacture of a medicament for the treatment of cancer, especially of CD20 expressing cancers in combination with one or more chemotherapeutic agents selected from the group consisting of cyclophosphamide, vincristine and doxorubicin.
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USE OF A TYPE II ANTI-CD20 ANTIBODY WITH INCREASED ANTIBODY DEPENDENT CELLULAR CYTOTOXICITY (ADCC) IN COMBINATION WITH CYCLOPHOSPHAMIDE, VINCristINE AND DOxorubicine FOR TREATING NON-HODGKIN'S LYMPHOMAS

The present invention is directed to the use a type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC) for the manufacture of a medicament for the treatment of cancer, especially of CD20 expressing cancers in combination with a chemotherapeutic agent selected from the group consisting of cyclophosphamide, vincristine and doxorubicine.

**Background of the Invention**

The CD20 molecule (also called human B-lymphocyte-restricted differentiation antigen or Bp35) is a hydrophobic transmembrane protein with a molecular weight of approximately 35 kDa located on pre-B and mature B lymphocytes (Valentine, M.A., et al., J. Biol. Chem. 264(19) 1989 11282-1 1287; and Einfield, D.A., et al., EMBO J. 7(3) 1988 711-717). CD20 is found on the surface of greater than 90% of B cells from peripheral blood or lymphoid organs and is expressed during early pre-B cell development and remains until plasma cell differentiation. CD20 is present on both normal B cells as well as malignant B cells. In particular, CD20 is expressed on greater than 90% of B cell non-Hodgkin's lymphomas (NHL) (Anderson, K.C., et al., Blood 63(6) 1984 1424-1433)) but is not found on hematopoietic stem cells, pro-B cells, normal plasma cells, or other normal tissues (Tedder, T.F., et al., J. Immunol. 135(2) 1985 973-979).

The 85 amino acid carboxyl-terminal region of the CD20 protein is located within the cytoplasm. The length of this region contrasts with that of other B cell-specific surface structures such as IgM, IgD, and IgG heavy chains or histocompatibility antigens class II a or β chains, which have relatively short intracytoplasmic regions of 3, 3, 28, 15, and 16 amino acids, respectively (Komaromy, M., et al., NAR 11 (1983) 6775-6785). Of the last 61 carboxyl-terminal amino acids, 21 are acidic residues, whereas only 2 are basic, indicating that this region has a strong net negative charge. The GenBank Accession No. is NP-690605. It is thought that CD20 might be involved in regulating an early step(s) in the activation and differentiation process of B cells (Tedder, T.F., et al., Eur. J. Immunol. 16 (1986) 881-887) and could function as a calcium ion channel (Tedder, T.F., et al., J. Cell. Biochem. 14D (1990) 195).
There exist two different types of anti-CD20 antibodies differing significantly in their mode of CD20 binding and biological activities (Cragg, M.S., et al., Blood 103 (2004) 2738-2743; and Cragg, M.S., et al., Blood, 101 (2003) 1045-1052). Type I antibodies, as e.g. rituximab, are potent in complement mediated cytotoxicity, whereas type II antibodies, as e.g. Tositumomab (Bl), 11B8, AT80 or humanized B-LyI antibodies, effectively initiate target cell death via caspase-independent apoptosis with concomitant phosphatidylinerine exposure.

The sharing common features of type I and type II anti-CD20 antibodies are summarized in Table 1.

**Table 1:**

<table>
<thead>
<tr>
<th>Properties of type I and type II anti-CD20 antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>type I anti-CD20 antibodies</td>
</tr>
<tr>
<td>type I CD20 epitope</td>
</tr>
<tr>
<td>Localize CD20 to lipid rafts</td>
</tr>
<tr>
<td>Increased CDC (if IgG1 isotype)</td>
</tr>
<tr>
<td>ADCC activity (if IgG1 isotype)</td>
</tr>
<tr>
<td>Full binding capacity</td>
</tr>
<tr>
<td>Homotypic aggregation</td>
</tr>
<tr>
<td>Apoptosis induction upon cross-linking</td>
</tr>
</tbody>
</table>

**Summary of the Invention**

The invention comprises the use of a type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC) for the manufacture of a medicament for the treatment of CD20 expressing cancer in combination with one or more chemotherapeutic agents selected from the group consisting of cyclophosphamide, vincristine and doxorubicine.

The invention further comprises the use of a type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC) for the manufacture of
a medicament for the treatment of a patient suffering from a CD20 expressing cancer in combination with one or more chemotherapeutic agents selected from the group consisting of cyclophosphamide, vincristine and doxorubicine.

The invention further comprises a type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC) for the treatment of CD20 expressing cancer in combination with one or more chemotherapeutic agents selected from the group consisting of cyclophosphamide, vincristine and doxorubicine.

Preferably the treatment with the type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC) is in combination with cyclophosphamide and vincristine.

A pharmaceutical composition comprising both a type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC) and one or more chemotherapeutic agents selected from the group consisting of cyclophosphamide, vincristine and doxorubicine for use in CD20 expressing cancer, in particular in B-Cell Non-Hodgkin's lymphomas (NHL).

Preferably said type II anti-CD20 antibody is a glycoengineered, humanized B-Ly1 antibody.

**Detailed Description of the Invention**

The term "antibody" encompasses the various forms of antibodies including but not being limited to whole antibodies, human antibodies, humanized antibodies and genetically engineered antibodies like monoclonal antibodies, chimeric antibodies or recombinant antibodies as well as fragments of such antibodies as long as the characteristic properties according to the invention are retained. The terms "monoclonal antibody" or "monoclonal antibody composition" as used herein refer to a preparation of antibody molecules of a single amino acid composition. Accordingly, the term "human monoclonal antibody" refers to antibodies
displaying a single binding specificity which have variable and constant regions derived from human germline immunoglobulin sequences. In one embodiment, the human monoclonal antibodies are produced by a hybridoma which includes a B cell obtained from a transgenic non-human animal, e.g. a transgenic mouse, having a genome comprising a human heavy chain transgene and a light human chain transgene fused to an immortalized cell.

Preferably said type II anti-CD20 antibody is a monoclonal antibody.

The term "chimeric antibody" refers to a monoclonal antibody comprising a variable region, i.e., binding region, from one source or species and at least a portion of a constant region derived from a different source or species, usually prepared by recombinant DNA techniques. Chimeric antibodies comprising a murine variable region and a human constant region are especially preferred. Such murine/human chimeric antibodies are the product of expressed immunoglobulin genes comprising DNA segments encoding murine immunoglobulin variable regions and DNA segments encoding human immunoglobulin constant regions. Other forms of "chimeric antibodies" encompassed by the present invention are those in which the class or subclass has been modified or changed from that of the original antibody. Such "chimeric" antibodies are also referred to as "class-switched antibodies." Methods for producing chimeric antibodies involve conventional recombinant DNA and gene transfection techniques now well known in the art. See, e.g., Morrison, S.L., et al., Proc. Natl. Acad Sci. USA 81 (1984) 6851-6855; US 5,202,238 and US 5,204,244.

The term "humanized antibody" refers to antibodies in which the framework or "complementarity determining regions" (CDR) have been modified to comprise the CDR of an immunoglobulin of different specificity as compared to that of the parent immunoglobulin. In a preferred embodiment, a murine CDR is grafted into the framework region of a human antibody to prepare the "humanized antibody." See, e.g., Riechmann, L., et al., Nature 332 (1988) 323-327; and Neuberger, M.S., et al., Nature 314 (1985) 268-270. Particularly preferred CDRs correspond to those representing sequences recognizing the antigens noted above for chimeric and bifunctional antibodies.

The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. Human antibodies are well-known in the state of the

The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies isolated from a host cell such as a NSO or CHO cell or from an animal (e.g. a mouse) that is transgenic for human immunoglobulin genes or antibodies expressed using a recombinant expression vector transfected into a host cell. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences in a rearranged form. The recombinant human antibodies according to the invention have been subjected to in vivo somatic hypermutation. Thus, the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire in vivo.

As used herein, "specifically binding" or "binds specifically to" refers to an antibody specifically binding to the CD20 antigen. Preferably the binding affinity is of KD-value of $10^{-9}$ mol/1 or lower (e.g. $10^{-10}$ mol/1), preferably with a KD-value of $10^{-10}$ mol/1 or lower (e.g. $10^{-12}$ mol/1). The binding affinity is determined with a standard binding assay, such as Scatchard plot analysis on CD20 expressing cells.

The term "nucleic acid molecule", as used herein, is intended to include DNA molecules and RNA molecules. A nucleic acid molecule may be single-stranded or double-stranded, but preferably is double-stranded DNA.

The "constant domains" are not involved directly in binding the antibody to an antigen but are involved in the effector functions (ADCC, complement binding, and CDC).

The "variable region" (variable region of a light chain (VL), variable region of a heavy chain (VH)) as used herein denotes each of the pair of light and heavy chains which is involved directly in binding the antibody to the antigen. The domains of variable human light and heavy chains have the same general structure and each domain comprises four framework (FR) regions whose sequences are widely conserved, connected by three "hypervariable regions" (or complementarity
determining regions, CDRs). The framework regions adopt a b-sheet conformation and the CDRs may form loops connecting the b-sheet structure. The CDRs in each chain are held in their three-dimensional structure by the framework regions and form together with the CDRs from the other chain the antigen binding site. The antibody heavy and light chain CDR3 regions play a particularly important role in the binding specificity/affinity of the antibodies according to the invention and therefore provide a further object of the invention.

The terms "hypervariable region" or "antigen-binding portion of an antibody" when used herein refer to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region comprises amino acid residues from the "complementarity determining regions" or "CDRs". "Framework" or "FR" regions are those variable domain regions other than the hypervariable region residues as herein defined. Therefore, the light and heavy chains of an antibody comprise from N- to C-terminus the domains FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. Especially, CDR3 of the heavy chain is the region which contributes most to antigen binding. CDR and FR regions are determined according to the standard definition of Kabat, et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) and/or those residues from a "hypervariable loop".

The terms "CD20" and "CD20 antigen" are used interchangeably herein, and include any variants, isoforms and species homologs of human CD20 which are naturally expressed by cells or are expressed on cells transfected with the CD20 gene. Binding of an antibody of the invention to the CD20 antigen mediate the killing of cells expressing CD20 (e.g., a tumor cell) by inactivating CD20. The killing of the cells expressing CD20 may occur by one or more of the following mechanisms: Cell death/apoptosis induction, ADCC and CDC.

Synonyms of CD20, as recognized in the art, include B-lymphocyte antigen CD20, B-lymphocyte surface antigen Bl, Leu-16, Bp35, BM5, and LF5.

The term "anti-CD20 antibody" according to the invention is an antibody that binds specifically to CD20 antigen. Depending on binding properties and biological activities of anti-CD20 antibodies to the CD20 antigen, two types of anti-CD20 antibodies (type I and type II anti-CD20 antibodies) can be distinguished according to Cragg, M.S., et al., Blood 103 (2004) 2738-2743; and Cragg, M.S., et al., Blood 101 (2003) 1045-1052, see Table 2.
Table 2:
Properties of type I and type II anti-CD20 antibodies

<table>
<thead>
<tr>
<th>type I anti-CD20 antibodies</th>
<th>type II anti-CD20 antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>type I CD20 epitope</td>
<td>type II CD20 epitope</td>
</tr>
<tr>
<td>Localize CD20 to lipid rafts</td>
<td>Do not localize CD20 to lipid rafts</td>
</tr>
<tr>
<td>Increased CDC (if IgG1 isotype)</td>
<td>Decreased CDC (if IgG1 isotype)</td>
</tr>
<tr>
<td>ADCC activity (if IgG1 isotype)</td>
<td>ADCC activity (if IgG1 isotype)</td>
</tr>
<tr>
<td>Full binding capacity</td>
<td>Reduced binding capacity</td>
</tr>
<tr>
<td>Homotypic aggregation</td>
<td>Stronger homotypic aggregation</td>
</tr>
<tr>
<td>Apoptosis induction upon cross-linking</td>
<td>Strong cell death induction without cross-linking</td>
</tr>
</tbody>
</table>

One essential property of type I and type II anti-CD20 antibody is their mode of binding. Thus, type I and type II anti-CD20 antibody can be classified by the ratio of the binding capacities to CD20 on Raji cells (ATCC-No. CCL-86) of said anti-CD20 antibody compared to rituximab.

The type II anti-CD20 antibodies have a ratio of the binding capacities to CD20 on Raji cells (ATCC-No. CCL-86) of said anti-CD20 antibody compared to rituximab of 0.3 to 0.6, preferably of 0.35 to 0.55, more preferably 0.4 to 0.5. Examples of such type II anti-CD20 antibodies include e.g. tositumomab (Bl IgG2a), humanized B-LyI antibody IgGl (a chimeric humanized IgGl antibody as disclosed in WO 2005/044859), 11B8 IgGl (as disclosed in WO 2004/035607), and AT80 IgGl. Preferably said type II anti-CD20 antibody is a monoclonal antibody that binds to the same epitope as humanized B-LyI antibody (as disclosed in WO 2005/044859).

Type I anti-CD20 antibodies in contrast to the type II antibodies have a ratio of the binding capacities to CD20 on Raji cells (ATCC-No. CCL-86) of said anti-CD20 antibody compared to rituximab of 0.8 to 1.2, preferably of 0.9 to 1.1. Examples of such type I anti-CD20 antibodies include e.g. rituximab, 1F5 IgG2a (ECACC, hybridoma; Press, O.W., et al., Blood 69/2 (1987) 584-591), HI47 IgG3 (ECACC, hybridoma), 2C6 IgGl (as disclosed in WO 2005/103081), 2F2 IgGl (as disclosed
and WO 2004/035607 and WO 2005/103081) and 2H7 IgGl (as disclosed in WO 2004/056312).

The "ratio of the binding capacities to CD20 on Raji cells (ATCC-No. CCL-86) of an anti-CD20 antibodies compared to rituximab" is determined by direct immunofluorescence measurement (the mean fluorescence intensities (MFI) is measured) using said anti-CD20 antibody conjugated with Cy5 and rituximab conjugated with Cy5 in a FACSArray (Becton Dickinson) with Raji cells (ATCC-No. CCL-86), as described in Example No. 2, and calculated as follows:

\[
\text{Ratio of the binding capacities to CD20 on Raji cells (ATCC-No. CCL-86)} = \frac{\text{MFI(Cy5-anti-CD20 antibody)}}{\text{MFI(Cy5-rituximab)}} \times \frac{\text{Cy5-labeling ratio(Cy5-rituximab)}}{\text{Cy5-labeling ratio(Cy5-anti-CD20 antibody)}}
\]

MFI is the mean fluorescent intensity. The "Cy5-labeling ratio" as used herein means the number of Cy5-label molecules per molecule antibody.

Typically said type II anti-CD20 antibody has a ratio of the binding capacities to CD20 on Raji cells (ATCC-No. CCL-86) of said second anti-CD20 antibody compared to rituximab of 0.3 to 0.6, preferably 0.35 to 0.55, more preferably 0.4 to 0.5.

Said type II anti-CD20 antibody according to the invention, has increased antibody dependent cellular cytotoxicity (ADCC).

By "antibody having increased antibody dependent cellular cytotoxicity (ADCC)" or "antibody with increased antibody dependent cellular cytotoxicity (ADCC)" is meant an antibody, as that term is defined herein, having increased ADCC as determined by any suitable method known to those of ordinary skill in the art. One accepted in vitro ADCC assay is as follows:

1) the assay uses target cells that are known to express the target antigen recognized by the antigen-binding region of the antibody;

2) the assay uses human peripheral blood mononuclear cells (PBMCs), isolated from blood of a randomly chosen healthy donor, as effector cells;

3) the assay is carried out according to following protocol:
i) the PBMCs are isolated using standard density centrifugation procedures and are suspended at $5 \times 10^6$ cells/ml in RPMI cell culture medium;

ii) the target cells are grown by standard tissue culture methods, harvested from the exponential growth phase with a viability higher than 90%, washed in RPMI cell culture medium, labeled with 100 micro-Curies of $^{51}$Cr, washed twice with cell culture medium, and resuspended in cell culture medium at a density of $10^5$ cells/ml;

iii) 100 microliters of the final target cell suspension above are transferred to each well of a 96-well microtiter plate;

iv) the antibody is serially-diluted from 4000 ng/ml to 0.04 ng/ml in cell culture medium and 50 microliters of the resulting antibody solutions are added to the target cells in the 96-well microtiter plate, testing in triplicate various antibody concentrations covering the whole concentration range above;

v) for the maximum release (MR) controls, 3 additional wells in the plate containing the labeled target cells, receive 50 microliters of a 2% (VN) aqueous solution of non-ionic detergent (Nonidet, Sigma, St. Louis), instead of the antibody solution (point iv above);

vi) for the spontaneous release (SR) controls, 3 additional wells in the plate containing the labeled target cells, receive 50 microliters of RPMI cell culture medium instead of the antibody solution (point iv above);

vii) the 96-well microtiter plate is then centrifuged at 50 x g for 1 minute and incubated for 1 hour at 4°C;

viii) 50 microliters of the PBMC suspension (point i above) are added to each well to yield an effectoπtarget cell ratio of 25:1 and the plates are placed in an incubator under 5% CO2 atmosphere at 37°C for 4 hours;

ix) the cell-free supernatant from each well is harvested and the experimentally released radioactivity (ER) is quantified using a gamma counter;
x) the percentage of specific lysis is calculated for each antibody concentration according to the formula \((ER-MR)/(MR-SR) \times 100\), where ER is the average radioactivity quantified (see point ix above) for that antibody concentration, MR is the average radioactivity quantified (see point ix above) for the MR controls (see point V above), and SR is the average radioactivity quantified (see point ix above) for the SR controls (see point vi above);

4) "increased ADCC" is defined as either an increase in the maximum percentage of specific lysis observed within the antibody concentration range tested above, and/or a reduction in the concentration of antibody required to achieve one half of the maximum percentage of specific lysis observed within the antibody concentration range tested above. The increase in ADCC is relative to the ADCC, measured with the above assay, mediated by the same antibody, produced by the same type of host cells, using the same standard production, purification, formulation and storage methods, which are known to those skilled in the art, but that has not been produced by host cells engineered to overexpress GnTIII.

Said "increased ADCC" can be obtained by glycoengineering of said antibodies, that means enhance said natural, cell-mediated effector functions of monoclonal antibodies by engineering their oligosaccharide component as described in Umana, P., et al., Nature Biotechnol. 17 (1999) 176-180 and US 6,602,684.

The term "complement-dependent cytotoxicity (CDC)" refers to lysis of human tumor target cells by the antibody according to the invention in the presence of complement. CDC is measured preferably by the treatment of a preparation of CD20 expressing cells with an anti-CD20 antibody according to the invention in the presence of complement. CDC is found if the antibody induces at a concentration of 100 nM the lysis (cell death) of 20% or more of the tumor cells after 4 hours. The assay is performed preferably with \(^{51}\text{Cr}\) or Eu labeled tumor cells and measurement of released \(^{51}\text{Cr}\) or Eu. Controls include the incubation of the tumor target cells with complement but without the antibody.

Typically type II anti-CD20 antibodies of the IgGl isotype show characteristic CDC properties. Type II anti-CD20 antibodies have a decreased CDC (if IgGl isotype) compared to type I antibodies of the IgGl isotype. Preferably type II anti-CD20 antibodies are IgGl isotype antibodies.
The "rituximab" antibody (reference antibody; example of a type I anti-CD20 antibody) is a genetically engineered chimeric human gamma 1 murine constant domain containing monoclonal antibody directed against the human CD20 antigen. This chimeric antibody contains human gamma 1 constant domains and is identified by the name "C2B8" in US 5,736,137 (Anderson, K.C., et. al.) issued on April 17, 1998, assigned to IDEC Pharmaceuticals Corporation. Rituximab is approved for the treatment of patients with relapsed or refracting low-grade or follicular, CD20 positive, B cell non-Hodgkin's lymphoma. In vitro mechanism of action studies have shown that rituximab exhibits human complement—dependent cytotoxicity (CDC) (Reff, M.E., et. al., Blood 83(2) (1994) 435-445). Additionally, it exhibits significant activity in assays that measure antibody-dependent cellular cytotoxicity (ADCC).


Preferably the "humanized B-LyI antibody" has variable region of the heavy chain (VH) selected from group of SEQ ID No.3 to SEQ ID No.20 (B-HH2 to B-HH9 and B-HL8 to B-HL1 of WO 2005/044859 and WO 2007/031875). Especially preferred are Seq. ID No. 3, 4, 7, 9, 11, 13 and 15 (B-HH2, BHH-3, B-HH6, B-HH8, B-HL8, B-HL11 and B-HL13 of WO 2005/044859 and WO 2007/031875).

Preferably the "humanized B-LyI antibody" has variable region of the light chain (VL) of SEQ ID No. 20 (B-KV1 of WO 2005/044859 and WO 2007/031875).

Furthermore the humanized B-LyI antibody is preferably an IgGl antibody. Such humanized B-LyI antibodies according to the invention are glycoengineered (GE) in the Fc region according to the procedures described in WO 2005/044859, WO 2004/065540, WO 2007/031875, Umana, P., et al., Nature Biotechnol. 17 (1999) 176-180 and WO 99/154342. Such "glycoengineered, humanized B-LyI antibodies" have an altered pattern of glycosylation in the Fc region, preferably having a reduced level of fucose residues. Preferably at least 40% or more (in one embodiment between 40% and 60%, in another embodiment at least 50%, and in
still another embodiment at least 70% or more) of the oligosaccharides of the Fc region are non-fucosylated. Furthermore the oligosaccharides of the Fc region are preferably bisected.

The oligosaccharide component can significantly 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. Some generalizations between oligosaccharide structure and glycoprotein function can be made. 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, N., et al., Nature Biotechnol. 14 (1996) 975-81).

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 application. (Cumming, D.A., et al., Glycobiology 1 (1991) 115-30; Jenkins, N., et al., Nature Biotechnol. 14 (1996) 975-81). Bacteria very rarely glycosylate proteins, and like other types of common hosts, such as yeasts, filamentous fungi, insect and plant cells, yield glycosylation patterns associated with rapid clearance from the blood stream, undesirable immune interactions, and in some specific cases, reduced biological activity. 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. More recently, production from transgenic animals has also been tested. (Jenkins, N., et al., Nature Biotechnol. 14 (1996) 975-981).

All antibodies contain carbohydrate structures at conserved positions in the heavy chain constant regions, with each isotype possessing a distinct array of N-linked carbohydrate structures, which vary affect protein assembly, secretion or functional activity. (Wright, A., and Monison, S. L., Trends Biotech. 15 (1997) 26-32). The structure of the attached N-linked carbohydrate varies considerably,
depending on the degree of processing, and can include high-mannose, multiply-branched as well as biantennary complex oligosaccharides. (Wright, A., and Morrison, S. L., Trends Biotech. 15 (1997) 26-32). Typically, there is heterogeneous processing of the core oligosaccharide structures attached at a particular glycosylation site such that even monoclonal antibodies exist as multiple glycoforms. Likewise, it has been shown that major differences in antibody glycosylation occur between cell lines, and even minor differences are seen for a given cell line grown under different culture conditions. (Lifely, M. R., et al., Glycobiology 5(8) (1995) 813-22).

One way to obtain large increases in potency, while maintaining a simple production process and potentially avoiding significant, undesirable side effects, is to enhance the natural, cell-mediated effector functions of monoclonal antibodies by engineering their oligosaccharide component as described in Umana, P., et al., Nature Biotechnol. 17 (1999) 176-180 and US 6,602,684. IgGl type antibodies, the most commonly used antibodies in cancer immunotherapy, are glycoproteins that have a conserved N-linked glycosylation site at Asn297 in each CH2 domain. The two complex biantennary oligosaccharides attached to Asn297 are buried between the CH2 domains, forming extensive contacts with the polypeptide backbone, and their presence is essential for the antibody to mediate effector functions such as antibody dependent cellular cytotoxicity (ADCC) (Lifely, M. R., et al., Glycobiology 5 (1995) 813-822; Jefferis, R., et al., Immunol. Rev. 163 (1998) 59-76; Wright, A. and Morrison, S. L., Trends Biotechnol. 15 (1997) 26-32).

It was previously shown that overexpression in Chinese hamster ovary (CHO) cells of β(1,4)-N-acetylglucosaminyl transferase 111 ("GnTIII 7y), a glycosyltransferase catalyzing the formation of bisected oligosaccharides, significantly increases the in vitro ADCC activity of an antineuroblastoma chimeric monoclonal antibody (chCE7) produced by the engineered CHO cells. (See Umana, P., et al., Nature Biotechnol. 17 (1999) 176-180; and WO 99/154342, the entire contents of which are hereby incorporated by reference). The antibody chCE7 belongs to a large class of unconjugated monoclonal antibodies which have high tumor affinity and specificity, but have too little potency to be clinically useful when produced in standard industrial cell lines lacking the GnTIII enzyme (Umana, P., et al., Nature Biotechnol. 17 (1999) 176-180). That study was the first to show that large increases of ADCC activity could be obtained by engineering the antibody
producing cells to express GnTIII, which also led to an increase in the proportion of constant region (Fc)-associated, bisected oligosaccharides, including bisected, non-fucosylated oligosaccharides, above the levels found in naturally-occurring antibodies.

The term "expression of the CD20" antigen is intended to indicate an significant level of expression of the CD20 antigen in a cell, preferably on the cell surface of a T- or B- Cell, more preferably a B-cell, from a tumor or cancer, respectively, preferably a non-solid tumor. Patients having a "CD20 expressing cancer" can be determined by standard assays known in the art. E.g. CD20 antigen expression is measured using immunohistochemical (IHC) detection, FACS or via PCR-based detection of the corresponding mRNA.

The term "CD20 expressing cancer" as used herein refers to all cancers in which the cancer cells show an expression of the CD20 antigen. Such CD20 expressing cancer may be, for example, lymphomas, lymphocytic leukemias, lung cancer, non small cell lung (NSCL) cancer, bronchioloalviolar cell lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, gastric cancer, colon cancer, breast cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, prostate cancer, cancer of the bladder, cancer of the kidney or ureter, renal cell carcinoma, carcinoma of the renal pelvis, mesothelioma, hepatocellular cancer, biliary cancer, neoplasms of the central nervous system (CNS), spinal axis tumors, brain stem glioma, glioblastoma multiforme, astrocytomas, schwannomas, ependymonas, medulloblastomas, meningiomas, squamous cell carcinomas, pituitary adenoma, including refractory versions of any of the above cancers, or a combination of one or more of the above cancers.

Preferably CD20 expressing cancer as used herein refers to lymphomas (preferably B-Cell Non-Hodgkin's lymphomas (NHL)) and lymphocytic leukemias. Such lymphomas and lymphocytic leukemias include e.g. a) follicular lymphomas, b) Small Non-Cleaved Cell Lymphomas/ Burkitt's lymphoma (including endemic Burkitt's lymphoma, sporadic Burkitt's lymphoma and Non-Burkitt's lymphoma) c)
marginal zone lymphomas (including extranodal marginal zone B cell lymphoma (Mucosa-associated lymphatic tissue lymphomas, MALT), nodal marginal zone B cell lymphoma and splenic marginal zone lymphoma), d) Mantle cell lymphoma (MCL), e) Large Cell Lymphoma (including B-cell diffuse large cell lymphoma (DLCL), Diffuse Mixed Cell Lymphoma, Immunoblastic Lymphoma, Primary Mediastinal B-Cell Lymphoma, Angiocentric Lymphoma-Pulmonary B-Cell Lymphoma) f) hairy cell leukemia, g) lymphocytic lymphoma, Waldenstrom's macroglobulinemia, h) acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL)/ small lymphocytic lymphoma (SLL), B-cell prolymphocytic leukemia, i) plasma cell neoplasms, plasma cell myeloma, multiple myeloma, plasmacytoma j) Hodgkin's disease.

More preferably the CD20 expressing cancer is a B-Cell Non-Hodgkin's lymphomas (NHL). Especially the CD20 expressing cancer is a Mantle cell lymphoma (MCL), acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), B-cell diffuse large cell lymphoma (DLCL), Burkitt's lymphoma, hairy cell leukemia, follicular lymphoma, multiple myeloma, marginal zone lymphoma, post transplant lymphoproliferative disorder (PTLD), HIV associated lymphoma, Waldenstrom's macroglobulinemia, or primary CNS lymphoma.

The term "a method of treating" or its equivalent, when applied to, for example, cancer refers to a procedure or course of action that is designed to reduce or eliminate the number of cancer cells in a patient, or to alleviate the symptoms of a cancer. "A method of treating" cancer or another proliferative disorder does not necessarily mean that the cancer cells or other disorder will, in fact, be eliminated, that the number of cells or disorder will, in fact, be reduced, or that the symptoms of a cancer or other disorder will, in fact, be alleviated. Often, a method of treating cancer will be performed even with a low likelihood of success, but which, given the medical history and estimated survival expectancy of a patient, is nevertheless deemed to induce an overall beneficial course of action.

In one embodiment the treatment with the type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC) is in combination with cyclophosphamide and vincristine.

In another embodiment the treatment with the type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC) is in combination with doxorubicine.
In another embodiment the treatment with the type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC) is in combination with cyclophosphamide.

In another embodiment the treatment with the type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC) is in combination with cyclophosphamide, vincristine and doxorubicine.

The terms "co-administration", "co-administering " or " in combination" as used herein have the same meaning a refer to the administration of said type II anti-CD20 antibody and said chemotherapeutic agents as one single formulation or as two separate formulations. The co-administration can be simultaneous or sequential in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities. Said type II anti-CD20 antibody and said chemotherapeutic agents are co-administered either simultaneously or sequentially (e.g. via an intravenous (i.v.) through a continuous infusion (one for the antibody and eventually one for the chemotherapeutic agents; or the chemotherapeutic agents is administered orally). When both therapeutic agents are co-administered sequentially the dose is administered either on the same day in two separate administrations, or one of the agents is administered on day 1 and the second is co-administered on day 2 to day 7, preferably on day 2 to 4. Thus the term "sequentially" means within 7 days after the dose of the first antibody, preferably within 4 days after the dose of the first antibody; and the term "simultaneously" means at the same time. The terms "co-administration" with respect to the maintenance doses of the type II anti-CD20 antibody and the chemotherapeutic agents mean that the maintenance doses can be either co-administered simultaneously, if the treatment cycle is appropriate for both drugs, e.g. every week. Or the chemotherapeutic agents is e.g. administered e.g. every first to third day and type II anti-CD20 antibody is administered every week. Or the maintenance doses are co-administered sequentially, either within one or within several days.

It is self-evident that the antibodies are administered to the patient in a "therapeutically effective amount" (or simply "effective amount") which is the amount of the respective compound or combination that will elicit the biological or medical response of a tissue, system, animal or human that is being sought by the researcher, veterinarian, medical doctor or other clinician.
The amount of co-administration of said type II anti-CD20 antibody and said chemotherapeutic agents and the timing of co-administration will depend on the type (species, gender, age, weight, etc.) and condition of the patient being treated and the severity of the disease or condition being treated. Said type II anti-CD20 antibody and said chemotherapeutic agents are suitably co-administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 μg/kg to 50 mg/kg (e.g. 0.1-20 mg/kg) of said type II anti-CD20 antibody and 1 μg/kg to 50 mg/kg (e.g. 0.1-20 mg/kg) of said chemotherapeutic agents is an initial candidate dosage for co-administration of both drugs to the patient. If the administration is intravenous the initial infusion time for said type II anti-CD20 antibody or said chemotherapeutic agents may be longer than subsequent infusion times, for instance approximately 90 minutes for the initial infusion, and approximately 30 minutes for subsequent infusions (if the initial infusion is well tolerated).

The preferred dosage of said type II anti-CD20 antibody will be in the range from about 0.05mg/kg to about 30mg/kg. Thus, one or more doses of about 0.5mg/kg, 2.0mg/kg, 4.0mg/kg, 10mg/kg or 30mg/kg (or any combination thereof) may be co-administered to the patient. The preferred dosage of said chemotherapeutic agents will be in the range from 0.01 mg/kg to about 30 mg/kg, e.g. 0.1 mg/kg to 10.0mg/kg for bortezomib. Depending on the on the type (species, gender, age, weight, etc.) and condition of the patient and on the type of anti-CD20 antibody and chemotherapeutic agents, the dosage and the administration schedule of said anti-CD20 antibody can differ from the dosage of chemotherapeutic agents. E.g. the said anti-CD20 antibody may be administered e.g. every one to three weeks and said chemotherapeutic agents may be administered daily or every 2 to 10 days. An initial higher loading dose, followed by one or more lower doses may also be administered.

In a preferred embodiment, the medicament is useful for preventing or reducing metastasis or further dissemination in such a patient suffering from CD20 expressing cancer. The medicament is useful for increasing the duration of survival of such a patient, increasing the progression free survival of such a patient, increasing the duration of response, resulting in a statistically significant and clinically meaningful improvement of the treated patient as measured by the duration of survival, progression free survival, response rate or duration of
response. In a preferred embodiment, the medicament is useful for increasing the response rate in a group of patients.

May be used in the type II anti-CD20 antibody and chemotherapeutic agents combination treatment of CD20 expressing cancer. Such molecules are suitably present in combination in amounts that are effective for the purpose intended. Therefore in one embodiment, in the treatment with the type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC) in combination with one or more chemotherapeutic agents selected from the group consisting of cyclophosphamide, vincristine and doxorubicine, one additional corticosteroid, preferably prednisone, is administered.

In one embodiment the type II anti-CD20 antibody and chemotherapeutic agents combination treatment is used without such additional corticosteroids.

The use of the corticosteroid described above as in chemotherapeutic regimens is generally well characterized in the cancer therapy arts, and their use herein falls under the same considerations for monitoring tolerance and effectiveness and for controlling administration routes and dosages, with some adjustments. For example, the actual dosages of the chemotherapeutic agents and the corticosteroids may vary depending upon the patient's cultured cell response determined by using histoculture methods. Generally, the dosage will be reduced compared to the amount used in the absence of additional other agents.

Typical dosages of an effective the chemotherapeutic agents and/or the corticosteroids can be in the ranges recommended by the manufacturer, and where indicated by in vitro responses or responses in animal models, can be reduced by up to about one order of magnitude concentration or amount. Thus, the actual dosage will depend upon the judgment of the physician, the condition of the patient, and the effectiveness of the therapeutic method based on the in vitro responsiveness of the primary cultured malignant cells or histocultured tissue sample, or the responses observed in the appropriate animal models.

In the context of this invention, an effective amount of ionizing radiation may be carried out and/or a radiopharmaceutical may be used in addition to the type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC) and chemotherapeutic agent combination treatment of CD20 expressing cancer. The source of radiation can be either external or internal to the patient being
treated. When the source is external to the patient, the therapy is known as external beam radiation therapy (EBRT). When the source of radiation is internal to the patient, the treatment is called brachytherapy (BT). Radioactive atoms for use in the context of this invention can be selected from the group including, but not limited to, radium, cesium-137, iridium-192, americium-241, gold-198, cobalt-57, copper-67, technetium-99, iodine-123, iodine-131, and indium-111. It is also possible to label the antibody with such radioactive isotopes. Preferably the type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC) and chemotherapeutic agent combination treatment is used without such ionizing radiation.

Radiation therapy is a standard treatment for controlling unresectable or inoperable tumors and/or tumor metastases. Improved results have been seen when radiation therapy has been combined with chemotherapy. Radiation therapy is based on the principle that high-dose radiation delivered to a target area will result in the death of reproductive cells in both tumor and normal tissues. The radiation dosage regimen is generally defined in terms of radiation absorbed dose (Gy), time and fractionation, and must be carefully defined by the oncologist. The amount of radiation a patient receives will depend on various considerations, but the two most important are the location of the tumor in relation to other critical structures or organs of the body, and the extent to which the tumor has spread. A typical course of treatment for a patient undergoing radiation therapy will be a treatment schedule over a 1 to 6 week period, with a total dose of between 10 and 80 Gy administered to the patient in a single daily fraction of about 1.8 to 2.0 Gy, 5 days a week. In a preferred embodiment of this invention there is synergy when tumors in human patients are treated with the combination treatment of the invention and radiation. In other words, the inhibition of tumor growth by means of the agents comprising the combination of the invention is enhanced when combined with radiation, optionally with additional chemotherapeutic or anticancer agents. Parameters of adjuvant radiation therapies are, for example, contained in WO 99/60023.

The type II anti-CD20 antibodies are administered to a patient according to known methods, by intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, or intrathecal routes. Intravenous or subcutaneous administration of the antibodies is preferred.
The chemotherapeutic agents are administered to a patient according to known methods, e.g. by intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, or peroral routes. Intravenous, subcutaneous or oral administration of the chemotherapeutic agents is preferred.

The invention further comprises a kit comprising a type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC) and one or more chemotherapeutic agents selected from the group consisting of cyclophosphamide, vincristine and doxorubicine, for the combination treatment of a patient suffering from a CD20 expressing cancer. In a preferred embodiment, the kit containers may further include a pharmaceutically acceptable carrier. The kit may further include a sterile diluent, which is preferably stored in a separate additional container. The kit may further include a package insert comprising printed instructions directing the use of the combined treatment as a method for a CD20 expressing cancer disease, preferably a B-Cell Non-Hodgkin's lymphoma (NHL).

The term "package insert" refers to instructions customarily included in commercial packages of therapeutic products, which may include information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products.

In a preferred embodiment, the article of manufacture containers may further include a pharmaceutically acceptable carrier. The article of manufacture may further include a sterile diluent, which is preferably stored in a separate additional container.

As used herein, a "pharmaceutically acceptable carrier" is intended to include any and all material compatible with pharmaceutical administration including solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and other materials and compounds compatible with pharmaceutical administration. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the compositions.
Pharmaceutical Compositions:

Pharmaceutical compositions can be obtained by processing the type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC) and/or the chemotherapeutic agents selected from the group consisting of cyclophosphamide, vincristine and doxorubicine according to this invention with pharmaceutically acceptable, inorganic or organic carriers. Lactose, corn starch or derivatives thereof, talc, stearic acids or it's salts and the like can be used, for example, as such carriers for tablets, coated tablets, dragees and hard gelatine capsules. Suitable carriers for soft gelatine capsules are, for example, vegetable oils, waxes, fats, semi-solid and liquid polyols and the like. Depending on the nature of the active substance no carriers are, however, usually required in the case of soft gelatine capsules. Suitable carriers for the production of solutions and syrups are, for example, water, polyols, glycerol, vegetable oil and the like. Suitable carriers for suppositories are, for example, natural or hardened oils, waxes, fats, semi-liquid or liquid polyols and the like.

The pharmaceutical compositions can, moreover, contain preservatives, solubilizers, stabilizers, wetting agents, emulsifiers, sweeteners, colorants, flavorants, salts for varying the osmotic pressure, buffers, masking agents or antioxidants. They can also contain still other therapeutically valuable substances.

One embodiment of the invention is pharmaceutical composition comprising both said type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC) and one ore more chemotherapeutic agents selected from the group consisting of cyclophosphamide, vincristine and doxorubicine, in particular for use in CD20 expressing cancer.

Said pharmaceutical composition may further comprise one or more pharmaceutically acceptable carriers.

The present invention further provides a pharmaceutical composition, in particular for use in cancer, comprising (i) an effective first amount of a type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC), and (ii) an effective second amount of a one ore more chemotherapeutic agents selected from the group consisting of cyclophosphamide, vincristine and doxorubicine. Such composition optionally comprises pharmaceutically acceptable carriers and / or excipients.
Pharmaceutical compositions of the type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC) alone used in accordance with the present invention are prepared for storage by mixing an antibody having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

Pharmaceutical compositions of the chemotherapeutic agents selected from the group consisting of cyclophosphamide, vincristine and doxorubicine, depend on their pharmaceutical properties; e.g. for small chemical compounds such as e.g. bortezomib, one formulation could be e.g. the following:

a) Tablet Formulation (Wet Granulation):

<table>
<thead>
<tr>
<th>Item</th>
<th>Ingredients</th>
<th>mg/tablet</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Compound of formula (I)</td>
<td>5 25 100 500</td>
</tr>
<tr>
<td>2.</td>
<td>Lactose Anhydrous DTG</td>
<td>125 105 30 150</td>
</tr>
<tr>
<td>3.</td>
<td>Sta-Rx 1500</td>
<td>6 6 6 30</td>
</tr>
<tr>
<td>4.</td>
<td>Microcrystalline Cellulose</td>
<td>30 30 30 150</td>
</tr>
<tr>
<td>5.</td>
<td>Magnesium Stearate</td>
<td>1 1 1 1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>167 167 167 831</td>
</tr>
</tbody>
</table>
Manufacturing Procedure:
1. Mix items 1, 2, 3 and 4 and granulate with purified water.
2. Dry the granules at 50°C.
3. Pass the granules through suitable milling equipment.
4. Add item 5 and mix for three minutes; compress on a suitable press.

b) Capsule Formulation:

<table>
<thead>
<tr>
<th>Item</th>
<th>Ingredients</th>
<th>mg/capsule</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Compound of formula (I)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
</tr>
<tr>
<td>2.</td>
<td>Hydrous Lactose</td>
<td>159</td>
</tr>
<tr>
<td></td>
<td></td>
<td>123</td>
</tr>
<tr>
<td></td>
<td></td>
<td>148</td>
</tr>
<tr>
<td></td>
<td></td>
<td>---</td>
</tr>
<tr>
<td>3.</td>
<td>Corn Starch</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>70</td>
</tr>
<tr>
<td>4.</td>
<td>Talc</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>5.</td>
<td>Magnesium Stearate</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
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<td>5</td>
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<td></td>
<td></td>
<td>300</td>
</tr>
<tr>
<td></td>
<td></td>
<td>600</td>
</tr>
</tbody>
</table>

Manufacturing Procedure:
1. Mix items 1, 2 and 3 in a suitable mixer for 30 minutes.
2. Add items 4 and 5 and mix for 3 minutes.
3. Fill into a suitable capsule.

In one further embodiment of the invention the pharmaceutical compositions according to the invention are preferably two separate formulations for said type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC) and for the chemotherapeutic agents selected from the group consisting of cyclophosphamide, vincristine and doxorubicine. The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interracial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly- (methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano- particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped
articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (US 3,773,919), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D(-)-3-hydroxybutyric acid.

The formulations to be used for in vivo administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

The present invention further provides a method for the treatment of cancer, comprising administering to a patient in need of such treatment (i) an effective first amount of a type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC); and (ii) an effective second amount of one or more chemotherapeutic agents selected from the group consisting of cyclophosphamide, vincristine and doxorubicine.

As used herein, the term "patient" preferably refers to a human in need of treatment with type II anti-CD20 antibody (e.g. a patient suffering from CD20 expressing cancer) for any purpose, and more preferably a human in need of such a treatment to treat cancer, or a precancerous condition or lesion. However, the term "patient" can also refer to non-human animals, preferably mammals such as dogs, cats, horses, cows, pigs, sheep and non-human primates, among others.

The invention further comprises a type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC) for the treatment of CD20 expressing cancer in combination with one or more chemotherapeutic agents selected from the group consisting of cyclophosphamide, vincristine and doxorubicine.
The invention further comprises a type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC) for the treatment of a patient suffering from a CD20 expressing cancer in combination with one or more chemotherapeutic agents selected from the group consisting of cyclophosphamide, vincristine and doxorubicine.

The invention further comprises a type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC) and one or more chemotherapeutic agents selected from the group consisting of cyclophosphamide, vincristine and doxorubicine for use in the treatment of a patient suffering from a CD20 expressing cancer.

Preferably said type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC) is a glycoengineered, humanized B-LyI antibody.

Preferably the CD20 expressing cancer is a B-Cell Non-Hodgkin's lymphoma (NHL).

The following examples, sequence listing and figures are provided to aid the understanding of the present invention, the true scope of which is set forth in the appended claims. It is understood that modifications can be made in the procedures set forth without departing from the spirit of the invention.

**Description of the Sequence Listing:**

<table>
<thead>
<tr>
<th>SEQ ID NO:</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>amino acid sequence of variable region of the heavy chain (VH) of murine monoclonal anti-CD20 antibody B-LyI.</td>
</tr>
<tr>
<td>2</td>
<td>amino acid sequence of variable region of the light chain (VL) of murine monoclonal anti-CD20 antibody B-LyI.</td>
</tr>
<tr>
<td>3-19</td>
<td>amino acid sequences of variable region of the heavy chain (VH) of humanized B-LyI antibodies (B-HH2 to B-HH9, B-HL8, and B-HL10 to B-HL17)</td>
</tr>
<tr>
<td>20</td>
<td>amino acid sequences of variable region of the light chain (VL) of humanized B-LyI antibody B-KV1</td>
</tr>
</tbody>
</table>
Description of the Figures:

Figure 1
a) Synergistic antitumor activity of the combined treatment of a type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC) (B-HH6-B-KV1 GE) with cyclophosphamide and vincristine and
b) comparison with a combined treatment of a type I anti-CD20 antibody (rituximab) with cyclophosphamide and vincristine on WSU-DLCL2 human B-Cell Non-Hodgkin-Lymphoma (NHL).

Median values of tumor volume [mm$^3$] +/- IQR plotted on the y-axis; number of days after injection of tumor cells plotted on the x-axis. Legend: A)VeWele (circles), B) cyclophosphamide (25 mg/kg) and vincristine (0.25 mg/kg) once weekly (crosses), C) rituximab (30 mg/kg) once weekly (triangles), D) glycoengineered, humanized B-LyI (B-HH6-B-KV1 GE) (30 mg/kg) once weekly (squares), E) rituximab (30 mg/kg) with cyclophosphamide (25 mg/kg) and vincristine (0.25 mg/kg), once weekly (rhombuses) and F) glycoengineered, humanized B-LyI (B-HH6-B-KV1 GE) (30 mg/kg) with cyclophosphamide (25 mg/kg) and vincristine (0.25 mg/kg), once weekly (plus signs).

Figure 2
Mean Fluorescence Intensity (MFI, left y-axis) of type I anti-CD20 antibody (Cy5-rituximab = white bar) and type II anti-CD20 antibody (Cy5 - glycoengineered, humanized B-LyI B-HH6-B-KV1 GE = black bar) on Raji cells (ATCC-No. CCL-86); Ratio of the binding capacities to CD20 of type I anti-CD20 antibody (rituximab) and type II anti-CD20 antibody (B-HH6-B-KV1 GE) compared to rituximab (scaled on right y-axis).

Figure 3
a) Synergistic antitumor activity of the combined treatment of a type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC) (B-HH6-B-KV1 GE) with doxorubicine and
b) comparison with a combined treatment of a type I anti-CD20 antibody (rituximab) with doxorubicine on RL human follicular Non Hodgkin lymphoma (NHL).
Median values of tumor volume [mm$^3$] +/- IQR plotted on the y-axis; number of days after injection of tumor cells plotted on the x-axis. Legend: A) Vehicle (plus signs), B) doxorubicine (3 mg/kg) once weekly (crosses), C) rituximab (30 mg/kg) once weekly (triangles), D) glycoengineered, humanized B-IyI (B-HH6-B-KV1 GE) (30 mg/kg) once weekly (squares), E) rituximab (30 mg/kg) with doxorubicine (3 mg/kg), once weekly (rhombuses) and F) glycoengineered, humanized B-IyI (B-HH6-B-KV1 GE) (30 mg/kg) with doxorubicine (3 mg/kg), once weekly (circles).

**Figure 4**

a) Synergistic antitumor activity of the combined treatment of a type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC) (B-HH6-B-KV1 GE) with cyclophosphamide and

b) comparison with a combined treatment of a type I anti-CD20 antibody (rituximab) with cyclophosphamide on RL human follicular Non Hodgkin lymphoma (NHL).

Median values of tumor volume [mm$^3$] +/- IQR plotted on the y-axis; number of days after injection of tumor cells plotted on the x-axis. Legend: A) Vehicle (circles), B) cyclophosphoamide (50 mg/kg) once weekly (crosses), C) rituximab (30 mg/kg) once weekly (triangles), D) glycoengineered, humanized B-IyI (B-HH6-B-KV1 GE) (30 mg/kg) once weekly (squares), E) rituximab (30 mg/kg) with cyclophosphoamide (50 mg/kg), once weekly (rhombuses) and F) glycoengineered, humanized B-IyI (B-HH6-B-KV1 GE) (30 mg/kg) with cyclophosphoamide (50 mg/kg), once weekly (plus signs).
Experimental Procedures

Example 1

Antitumor activity of combined treatment of a type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC) (B-HH6-B-KV1 GE) with cyclophosphamide and vincristine.

Test agents:
Type II anti-CD20 antibody B-HH6-B-KV1 GE (= humanized B-LyI, glycoengineered B-HH6-B-KV1, see WO 2005/044859 and WO 2007/031875) was provided as stock solution (c=9.4 mg/ml) from GlycArt, Schlieren, Switzerland. Antibody buffer included histidine, trehalose and polysorbate 20. Antibody solution was diluted appropriately in PBS from stock for prior injections.

Rituximab was provided by Hoffmann La Roche, Basel.

Cyclophosphamide and vincristine were purchased as clinical formulation from Baxter Oncology GmbH, Halle, Germany or medac, Gesellschaft fur klinische Spezialpraparate mbH, Hamburg, Germany, respectively. Dilution was adjusted from reconstituted stock solution.

Cell lines and culture conditions:
WSU-DLCL2 human Non-Hodgkin-Lymphoma (NHL) cells were kindly provided from Hoffmann-La Roche, Inc., Nutley, NJ, USA. The tumor cell line was routinely cultured in RPMI medium (PAA, Laboratories, Austria) supplemented with 10% fetal bovine serum (PAA Laboratories, Austria) and 2 mM L-glutamine, at 37°C in a water-saturated atmosphere at 5% CO₂. Passage 4 was used for transplantation. Cells were co-injected with Matrigel.

Animals:
Female SCID beige mice; age 7-8 weeks at arrival (purchased from Charles River, Sulzfeld, Germany) were maintained under specific-pathogen-free condition with daily cycles of 12 h light /12 h darkness according to committed guidelines (GV-Solas; Felasa; TierschG). Experimental study protocol was reviewed and approved by local government. After arrival animals were maintained in the quarantine part of the animal facility for one week to get accustomed to new environment and for observation. Continuous health monitoring was carried out on regular basis. Diet
food (Provimi Kliba 3337) and water (acidified pH 2.5-3) were provided ad libitum.

Monitoring:
Animals were controlled daily for clinical symptoms and detection of adverse effects. For monitoring throughout the experiment body weight of animals was documented two times weekly and tumor volume was measured by caliper after staging.

Treatment of animals:
Animal treatment started at the day of randomisation 9 days after cell transplantation. Glycoengineered, humanized type II anti-CD20 antibody B-HH6-B-KV1 GE or Rituximab were administered as single agents i.v. q7d on study day 9, 15, 23, 30, 37, 44, 51 and 58 at the indicated dosage of 30 mg/kg. The corresponding vehicle was administered on the same days. Cyclophosphamide and vincristine were given i.v. once weekly on day 9, 15, 23, 30, 37, 44, 51 and 58 at 25 mg/kg or 0.25 mg/kg, respectively. In the combination therapy groups, both antibodies were administered 24 hours after the chemotherapeutic agents on day 10, 16, 24, 31, 38, 45, 52 and 59.

Tumor growth inhibition study in vivo:
On day 35 after cell transplantation, there was a significant tumor growth inhibition of 73%, 85%, 66%, 94% or 90% in the animals given rituximab, anti-CD20 antibody B-HH6-B-KV1 GE, chemotherapy, combination of chemotherapy and anti-CD20 antibody or combination of chemotherapy and rituximab, respectively, compared to the control group. At the end of the experiment, a significantly better tumor growth inhibition was observed in the chemotherapy/anti-CD20 antibody B-HH6-B-KV1 GE combination group as compared to the chemotherapy/rituximab combination group.

The effect of the different treatments until the end of the study on day 64 after cell transplantation was demonstrated by the Tumor Growth Delay value (T-C, where T is the median time in days required for the treatment group tumors to reach a predetermined size of 1500 mm$^3$, and C is the median time in days for the control group tumors to reach the same size). Results are shown in following table:
Table 3:
Tumor growth delay of the treatment groups compared to control group in days

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<td>Rituximab (30 mg/kg)</td>
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<td>3</td>
<td>anti-CD20 antibody B-HH6-B-KV1 GE (30 mg/kg)</td>
<td>19</td>
</tr>
<tr>
<td>4</td>
<td>Cyclophosphamide (25 mg/kg) Vincristine (0.25 mg/kg)</td>
<td>14</td>
</tr>
<tr>
<td>5</td>
<td>Cyclophosphamide (25 mg/kg) Vincristine (0.25 mg/kg) anti-CD20 antibody B-HH6-B-KV1 GE (30 mg/kg)</td>
<td>38</td>
</tr>
<tr>
<td>6</td>
<td>Cyclophosphamide (25 mg/kg) Vincristine (0.25 mg/kg) Rituximab (30 mg/kg)</td>
<td>28</td>
</tr>
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</table>

Example 2
Determination of the ratio of the binding capacities to CD20 on Raji cells (ATCC-No. CCL-86) of type II anti-CD20 antibody compared to rituximab

Raji cells (ATCC-No. CCL-86) were maintained in culture in RPMI-1640 medium (PanBiotech GmbH, Cat.-No. PO4-18500) containing 10% FCS (Gibco, Cat-No. 10500-064). The type II anti-CD20 antibody B-HH6-B-KV1 GE (glycoengineered, humanized B-LyI antibody) and rituximab were labeled using Cy5 Mono NHS ester (Amersham GE Healthcare, Catalogue No. PA15101) according to the manufacturer's instructions. Cy5-conjugated rituximab had a labeling ratio of 2.0 molecules Cy5 per antibody. Cy5-conjugated B-HH6-B-KV1 GE had a labeling ratio of 2.2 molecules Cy5 per antibody. In order to determine and compare the binding capacities and mode of both antibodies, binding curves (by titration of Cy5-conjugated Rituximab and Cy5-conjugated B-HH6-B-KV1 GE) were generated by direct immunofluorescence using the Burkitt's lymphoma cell line Raji (ATCC-No. CCL-86). Mean fluorescence intensities (MFI) for were analyzed as EC50 (50% of maximal intensity) for Cy5-conjugated Rituximab and Cy5-conjugated B-HH6-B-KV1 GE, respectively. 5*105 cells per sample were
stained for 30 min at 4°C. Afterwards, cells were washed in culture medium. Propidium iodide (PI) staining was used to exclude dead cells. Measurements were performed using the FACSArray (Becton Dickinson), Propidium iodide (PI) was measured at Far Red A and Cy5 at Red-A. Figure 2 shows Mean Fluorescence Intensity (MFI) for binding at EC50 (50% of maximal intensity) of Cy5-labeled B-HH6-B-KV1 GE (black bar) and Cy5-labeled rituximab (white bar).

Then the ratio of the binding capacities to CD20 on Raji cells (ATCC-No. CCL-86) is calculated according to the following formula:

\[
\text{Ratio of the binding capacities to CD20 on Raji cells (ATCC-No. CCL-86)} = \frac{\text{MFI}(\text{Cy5-anti-CD20 antibody})}{\text{MFI}(\text{Cy5-rituximab})} \times \frac{\text{Cy5 labeling ratio (Cy5-rituximab)}}{\text{Cy5 labeling ratio (Cy5-anti-CD20 antibody)}}
\]

\[= \frac{\text{MFI}(\text{B-HH6-B-KV1 })}{\text{MFI}(\text{Cy5-rituximab})} \times \frac{\text{Cy5 labeling ratio (Cy5-rituximab)}}{\text{Cy5 labeling ratio (B-HH6-B-KV1 )}}
\]

\[= \frac{207}{433} \times \frac{2.2}{2.0} = 0.44
\]

Thus B-HH6-B-KV1 GE as a typical type II anti-CD20 antibody shows reduces binding capacity compared to rituximab.

**Example 3**

Antitumor activity of combined treatment of a type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC) (B-HH6-B-KV1 GE) with doxorubicin.

**Test agents:**

Type II anti-CD20 antibody B-HH6-B-KV1 GE (= humanized B-Ly1, glycoengineered B-HH6-B-KV1, see WO 2005/044859 and WO 2007/031875) was provided as stock solution (c=9.4 mg/ml) from GlycArt, Schlieren, Switzerland. Antibody buffer included histidine, trehalose and polysorbate 20. Antibody solution was diluted appropriately in PBS from stock for prior injections.

Rituximab was provided by Hoffmann La Roche, Basel.
Doxorubicine was purchased as clinical formulation from Hexal, Holzkirchen, Germany. Dilution is adjusted from reconstituted stock solution.

**Cell lines and culture conditions:**

RL human follicular Non Hodgkin lymphoma cells were kindly provided from Dr. Charles Dumontet, INSERM 590, Lyon, France. The tumor cell line was routinely cultured in RPMI medium (PAA, Laboratories, Austria) supplemented with 10% fetal bovine serum (PAA Laboratories, Austria) and 2 mM L-glutamine, at 37°C in a water-saturated atmosphere at 5% CO2. Passage 2 was used for transplantation.

**Animals:**

Female SCID beige mice; age 7-8 weeks at arrival (purchased from Charles River, Sulzfeld, Germany) were maintained under specific-pathogen-free condition with daily cycles of 12 h light /12 h darkness according to committed guidelines (GV-Solas; Felasa; TierschG). Experimental study protocol was reviewed and approved by local government. After arrival animals were maintained in the quarantine part of the animal facility for one week to get accustomed to new environment and for observation. Continuous health monitoring was carried out on regular basis. Diet food (Provimi Kliba 3337) and water (acidified pH 2.5-3) were provided ad libitum.

**Monitoring:**

Animals were controlled daily for clinical symptoms and detection of adverse effects. For monitoring throughout the experiment body weight of animals was documented two times weekly and tumor volume was measured by caliper after staging.

**Treatment of animals:**

Animal treatment started at the day of randomisation 14 days after cell transplantation. Glycoengineered, humanized type II anti-CD20 antibody B-HH6-B-KVI GE or Rituximab were administered as single agents i.v. q7d on study day 14, 21, 28, 36 and 42 at the indicated dosage of 30 mg/kg or 60 mg/kg. The corresponding vehicle was administered on the same days as well as Doxorubicin which was given i.v. once weekly at 3 mg/kg. In the combination therapy groups, doxorubicin was administered i.v. once weekly on day 15, 22, 29, 37 and 43 at 3 mg/kg and Rituximab was administered i.v. once weekly on the same days at 30 mg/kg in the combination therapy group.
Example 4

Antitumor activity of combined treatment of a type II anti-CD20 antibody (B-HH6-B-KV1 GE) and Cyclophosphamide in the RL cell line.

Test agents:

Type II anti-CD20 antibody B-HH6-B-KV1 GE (= humanized B-LyI, glycoengineered B-HH6-B-KV1, see WO 2005/044859 and WO 2007/031875) was provided as stock solution (c=9.4 mg/ml) from GlycArt, Schlieren, Switzerland. Antibody buffer included histidine, trehalose and polysorbate 20. Antibody solution was diluted appropriately in PBS from stock for prior injections.

Type I anti-CD20 antibody Rituximab was provided as stock solution (c=10 mg/ml) from Hoffmann La Roche, Basel, Switzerland. Buffer contains polysorbat 80, Sodiumchloride and Sodiumcitrat.

Cyclophosphamide was purchased as clinical formulation from Baxter Oncology GmbH, Halle, Germany or medac, Gesellschaft fur klinische Spezialpraparate mbH, Hamburg, Germany, respectively. Dilution was adjusted from reconstituted stock solution.

Cell lines and culture conditions:
The RL human follicular Non Hodgkin lymphoma cell line is routinely cultured in RPMI 1640 medium supplemented with 10% fetal calf serum and antibiotics. RL cells grow in suspension and form clusters. Exponential growing cells were injected subcutaneously in SCID mice.

Animals:
Animals used were 6-week old females, SCID mice, provided by Charles River (L’Arbresle, France) with IPSOS status. Animals were housed at least one week before injection of RL cells. Cages contained 5 animals.

Monitoring:
Animals were controlled daily for clinical symptoms and detection of adverse effects. For monitoring throughout the experiment body weight of animals was documented two times weekly and tumor volume was measured by caliper after staging. Study exclusion criteria for animals were described and approved by the local Experimental Animal Committee.
Treatment of animals:

Treatment started 31 days after cell transplantation at randomisation. Humanized type II anti-CD20 antibody B-HH6-B-KV1 GE, vehicle or rituximab were given i.v. once weekly to animals at a dosage of 30 mg/kg, (day 31, 38, 45 and 52). Cyclophosphamide was injected on the same days at a dose of 50 mg/kg. The antibody dilutions were prepared freshly from stock before use.

Tumor growth inhibition study in vivo:

On day 66 after cell transplantation, there was a significant tumor growth inhibition of 54%, 85% or 91% in the animals given the combinations of Rituximab and Cyclophosphamide, anti-CD20 antibody B-HH6-B-KV1 GE and Rituximab or anti-CD20 antibody B-HH6-B-KV1 GE and cyclophosphamide. Thus, the combination treatment of anti-CD20 antibody B-HH6-B-KV1 GE and cyclophosphamide yielded the best antitumor activity compared to the treatment with cyclophosphamide alone.
Patent Claims

1. Use of a type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC) for the manufacture of a medicament for the treatment of CD20 expressing cancer in combination with one or more chemotherapeutic agents selected from the group consisting of cyclophosphamide, vincristine and doxorubicine.

2. Use of a type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC) for the manufacture of a medicament for the treatment of a patient suffering from a CD20 expressing cancer in combination with one or more chemotherapeutic agents selected from the group consisting of cyclophosphamide, vincristine and doxorubicine.

3. Use according to any one of claims 1 or 2, characterized in that the treatment with the type II anti-CD20 antibody is in combination with cyclophosphamide and vincristine.

4. Use according to any one of claims 1 or 2, characterized in that the treatment with the type II anti-CD20 antibody is in combination with doxorubicine.

5. Use according to any one of claims 1 or 2, characterized in that the treatment with the type II anti-CD20 antibody is in combination with cyclophosphamide.

6. Use according to any one of claims 1 or 2, characterized in that the treatment with the type II anti-CD20 antibody is in combination with cyclophosphamide, vincristine and doxorubicine.

7. Use according to any one of claims 1 to 5, characterized in that said type II anti-CD20 antibody is a glycoengineered, humanized B-LyI antibody.

8. Use according to any one of claims 1 to 6, characterized in that the CD20 expressing cancer is a B-Cell Non-Hodgkin’s lymphoma (NHL).

9. Use according to any one of claims 1 to 7, characterized in that one additional corticosteroid, preferably prednisone, is administered.
10. A pharmaceutical composition comprising both a type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC) and one or more chemotherapeutic agents selected from the group consisting of cyclophosphamide, vincristine and doxorubicine for use in CD20 expressing cancer, in particular in B-Cell Non-Hodgkin's lymphomas (NHL).

11. A type II anti-CD20 antibody with increased antibody dependent cellular cytotoxicity (ADCC) for the treatment of a patient suffering from a CD20 expressing cancer in combination with one or more chemotherapeutic agents selected from the group consisting of cyclophosphamide, vincristine and doxorubicine.

12. A type II anti-CD20 antibody according to claim 11, characterized in that said type II anti-CD20 antibody is a glycoengineered, humanized B-LyI antibody.

13. A type II anti-CD20 antibody according to claim 12, characterized in that the CD20 expressing cancer is a B-Cell Non-Hodgkin's lymphoma (NHL).
Fig. 2

![Bar graph showing mean fluorescence intensity (MFI) and ratio of binding capacities for Cy5-Rituximab type I and Cy5-humanized B-Ly1 type II on Raji cells.](image)
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

INV. A61K39/395 C07K16/28 A61P35/00

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

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

C07K A61K

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, FSTA, WPI Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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Further documents are listed in the continuation of Box C.  

* A document defining the general state of the art which is not considered to be of particular relevance
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Date of the actual completion of the international search: 23 July 2009

Date of mailing of the international search report: 31/07/2009

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: Habedanck, Robert
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<td>UMANA P ET AL: &quot;Novel 3(rd) generation humanized type IICD20 antibody with glycoengineered fc and modified elbow hinge for enhanced ADCC and superior apoptosis induction&quot; BLOOD, W.B.SAUNDERS COMPANY, ORLANDO, FL, vol. 108, no. 11, PART 1, 9 December 2006 (2006-12-09), page 72A, XP008087672 ISSN: 0006-4971</td>
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