(12)

Patent Application

(54) **Title:**
HUMANISED ANTI-CD52 ANTIBODIES

(57) **Abstract:**
The present invention relates to novel humanised antibodies against human CD52 and their use in methods of treating or preventing human diseases.

(51) **International Patent Classification:**
C07K 16/28 (2006.01)

(21) **International Application Number:**
PCT/EP2012/060345

(22) **International Filing Date:**
1 June 2012 (01.06.2012)

(25) **Filing Language:** English

(26) **Publication Language:** English

(30) **Priority Data:**
1109238.4 1 June 2011 (01.06.2011) GB
61/492,005 1 June 2011 (01.06.2011) US

(71) **Applicant** *(for all designated States except US)*: **ANTI-TOPE LTD** [GB/GB]; Babraham Institute, Babraham, Cambridgeshire CB2 4AT (GB).

(72) **Inventors; and**
(75) **Inventors/Applicants** *(for US only)*: **JONES, Timothy David** [GB/GB]; 27 Brick Row, Babraham, Cambridge, Cambridgeshire CB22 3AJ (GB). **HOLGATE, Robert George Edward** [GB/GB]; 96 Princess Mews, Royston, Hertfordshire SG8 9BL (GB). **CARR, Francis Joseph**

[GB/GB]; Birchlea, the holdings, Balmedie, Aberdeenshire AB23 8WU (GB).

(74) **Agents: CHAPMAN, Paul William** et al.; Kilburn & Strode LLP, 20 Red Lion Street, London, Greater London WC1R 4PJ (GB).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,

[Continued on next page]

(54) **Title:** HUMANISED ANTI-CD52 ANTIBODIES

Figure 1a: Human CD52 expression vector

(57) **Abstract**: The present invention relates to novel humanised antibodies against human CD52 and their use in methods of treating or preventing human diseases.

WO 2012/164063 A1

MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

HUMANISED ANTI-CD52 ANTIBODIES

The present invention relates to novel humanised antibodies against human CD52 and their use in methods of treating or preventing human diseases.
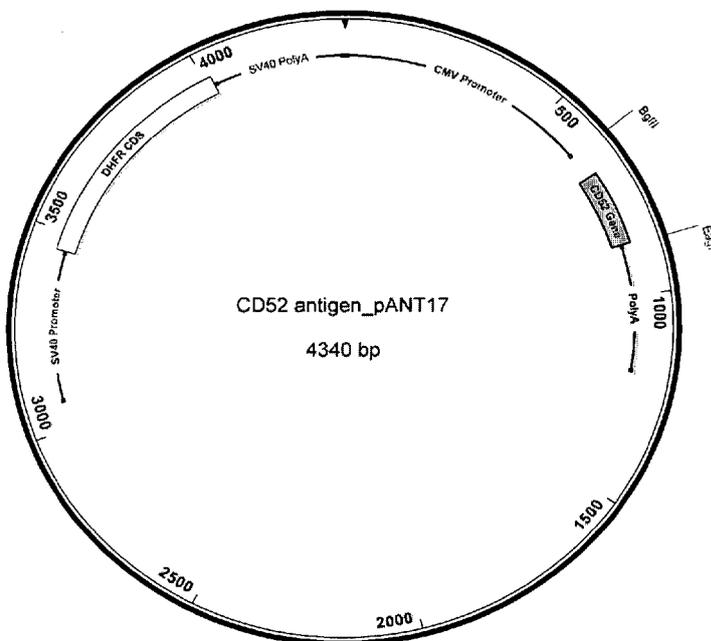
BACKGROUND OF THE INVENTION

CD52 is a glycosylated, glycosylphosphatidylinositol (GPI)-anchored cell surface protein found in abundance on a variety of normal and malignant lymphoid cells especially B and T cells (Gilleece et al, Blood 82 807-812 (1993); Hale et al, J Biol Regul Homeost Agents, 15 p386- 391 (2001); Rodig et al, Clin Cancer Res 12, p7174-7179 (2006)). CD52 is expressed at lower levels on myeloid cells such as monocytes, macrophages and dendritic cells (DC) with little expression found on mature natural killer (NK) cells, neutrophils, and hematological stem cells. CD52 is also produced by epithelial cells in the epididymis and duct deferens, and is acquired by sperm during passage through the genital tract (Hale et al, *ibid*,; Domagala et al, Med Sci Monit 7 p325- 331 (2001)). The exact biological function of CD52 remains unclear but some evidence suggests that it may be involved in T cell migration and co-stimulation (Masuyama et al, J Exp Med 189 979-989 (1999); Watanabe et al, Clin Immunol 120 247-259 (2006)).

Campath-1H (alemtuzumab, Campath®, MabCampath®) is a humanised anti-human CD52 monoclonal antibody that exhibits potent *in vitro* antibody-dependent cell mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). CD52 is present on at least 95% of all human peripheral blood lymphocytes and monocytes/macrophages (Hale G. et al., The CAMPATH-1 antigen (CD52). Tissue Antigens 1990,35:118-127). Campath-1H recognizes an epitope which consists of the carboxy terminal four amino acids of the mature CD52 protein and a portion of the negatively charged GPI anchor. Due to its significant cytotoxic effects, Campath-1H is capable of depleting CD52 positive cells *in vivo* and it is approved for front line and third line treatment of chronic lymphocytic leukemia (CLL). Campath-1H has been evaluated for its utility in the treatment of several autoimmune diseases, including rheumatoid arthritis, vasculitis, myositis, Wegener's disease and diabetes. The most advanced studies of Campath-1H are in treating relapsing remitting multiple sclerosis (MS). These studies showed a significant improvement in time to relapse compared to human interferon beta-1a (Rebif® (i e , interferon beta- Ia)).

A major limitation of Campath-1H is immunogenicity whereby antibodies are induced in up to
70% of patients (Therapeutic Monoclonal Antibodies: From Bench to Clinic, ed. Zhiqiang An
(2009) ISBN: 978-0-470-11791-0). In order to improve the clinical utility of anti-CD52
antibodies, there is a major need for improved anti-CD52 antibodies which are not associated
with significant immunogenicity in patients.

SUMMARY OF THE INVENTION

The invention relates to humanized immunoglobulins that have binding specificity for human
CD52 (huCD52). The invention also provides humanised antibodies that bind to human CD52
with an equilibrium dissociation constant (Kd) of at least 10^{-8}M. The invention also provides
humanised antibodies that specifically bind to human CD52 having an antibody heavy chain of
either IgG1, IgG2, IgG3 or IgG4, or using a mutated IgG constant region especially a constant
region which enhances ADCC (antibody-dependant cellular cytotoxicity) or CDC (complement-
dependant cytotoxicity). The invention also provides humanised antibodies wherein the antibody
light chain is a kappa light chain. The humanised antibody can be encoded by human IgG heavy
chain and human kappa light chain nucleic acids that encode protein sequences in their variable
regions as set forth in SEQ ID NO:20 through SEQ ID NO:28.

The present invention also provides humanised antibodies that specifically bind to human CD52
whereby the antibody variable regions have been selected or modified to exclude one or more
human CD4+ T cell epitopes. The present invention also provides humanised antibodies that
specifically bind to human CD52 whereby the antibody variable regions have been formed
primarily by fusing segments of sequences entirely derived from existing human antibody
variable region sequences.

The present invention also provides humanised anti-CD52 antibodies of the invention
comprising heavy chain CDR1, CDR2, and CDR3 amino acid sequences, "RYGMS" (SEQ ID
NO.5), "MMKTKGGRTYYPDSVKG" (SEQ ID NO.6) and "DGYY" (SEQ ID NO. 7),
respectively, and light chain CDR1, CDR2, and CDR3 amino acid sequences,
"KSSQSLLHSDGKTYLN" (SEQ ID NO.8), "LVSKLDS" (SEQ ID NO.9), and

"WQGTHLWT" (SEQ ID NO. 10), respectively. The present invention also provides

65   humanised anti-CD52 antibodies of the invention comprising heavy chain variable region amino acid sequences corresponding to SEQ ID NOS:20 to 24 for the heavy chain and SEQ ID NOS:25 to 28 for the light chain. In a preferred embodiment of the invention, a humanised anti-CD52 antibody of the present invention comprising heavy chain variable region amino acid sequences corresponding to SEQ ID NO:22 for the heavy chain and SEQ ID NO: 28 is provided.

70

Humanised antibodies of the present invention can be composed of any of the above CDR sequences SEQ ID NO.5 to SEQ ID NO.10 and minor variants of these CDR sequences where alterations of one or more amino acids does not significantly reduce binding to human CD52.

Humanised antibodies can be created by joining together the CDR sequences with sequences

75   from human variable region frameworks where such framework sequences are derived from single or multiple other human antibody variable region framework sequences. Commonly such human variable region framework sequences will include one or more mutations which contribute to optimal or improved binding of the humanised antibodies to CD52. In a preferred embodiment of the present invention, such human variable region framework sequences in the

80   humanised antibodies are derived entirely from sequences in other human antibody variable regions as described in of EP1844074 . These sequences comprise joined segments of sequences from other human antibody variable regions, together with human constant regions. In particular, such humanised antibodies also contain CDR sequences derived entirely from CDR sequences in other human antibody variable regions including joined segments of CDR

85   sequences from other human CDRs together with human constant regions, thus creating humanised antibodies in which the variable region sequences are derived entirely from sequences in other human antibody variable regions together with human constant regions, this creating a "fully human" antibody.

90   The invention also provides humanised antibodies that specifically bind to human CD52, wherein said humanised antibody is produced by a prokaryotic or eukaryotic cell, especially from a mammalian cell line, especially CHO or NS0 cells. The invention also provides a humanised antibody that specifically binds to human CD52 that is a Fab fragment or a single chain Fv (scFv). The invention also provides multispecific proteins including at least one

95   humanised antibody from the sequences SEQ ID NOS:20 to 24 for the heavy chain and SEQ ID
      NOS:25 to 28 for the light chain whereby the multispecific protein specifically binds to human
      CD52 and, additionally, binds or interacts with one or more other molecules.   Different
      antibodies or proteins may be included in each multispecific antibody can be linked to each other
      either covalently or non-covalently.

100

      The invention provides a pharmaceutical composition comprising a humanised antibody (either
      as a proteinaceous antibody or a gene encoding the antibody) that specifically binds to human
      CD52 and a pharmaceutically acceptable carrier.   The pharmaceutical composition can further
      comprise one or more chemotherapeutic agents either linked or unlinked to the humanised

105   antibody.

      The invention provides a method for treatment of CLL and other leukemias; several autoimmune
      diseases including multiple sclerosis, rheumatoid arthritis, vasculitis, myositis, Wegener's disease
      and diabetes; and organ transplant rejection and graft-vs-host disease, in each case comprising

110   administering to the patient an effective dosage of a humanised antibody (either as a
      proteinaceous antibody or a gene encoding the antibody) that specifically binds to human CD52,
      wherein the antibody causes the destruction or apoptosis of CD52+ target cells such as B and T
      cells.   In addition, the invention also provides a method for diagnosis of the above mentioned
      diseases, for example by administration of humanised antibody attached to a detectable label and

115   determination of binding of the humanised antibody *in* vivo to provide a basis for detection of
      CD52+ cells, for example in localised tumour masses or in inflammatory lesions.   Alternatively
      the humanised antibodies of the present invention may be used for *in vitro* tests for CD52+ cells
      as a means for detection of disease and also for *in vitro* tests for antibodies which may bind to
      the humanised antibodies used therapeutically.   Accordingly, such humanised antibodies of the

120   invention can be used as diagnostic or therapeutic agents *in vivo* and *in vitro*.

      The humanised antibodies of the invention can encompass various antibody isotypes, or mixtures
      thereof, such as IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgAsec, IgD, IgE or mutated forms
      of these IgGs such as mutations which enhance binding to Fc receptors (for example, Horton et

125   al., Blood 116 (2010) p3004-3012) or to complement (for example, Natsume et al., Cancer Res

68 (2008) p3863-3872). Typically humanised antibodies include IgG1 heavy chain constant regions and κ light chain constant regions. The humanised antibodies can be full-length (e.g. IgG1/κ antibody) or can include only an antigen-binding portion (e.g., a Fab, F(ab')2, Fv or a scFv fragment).

130

Some humanised anti-CD52 antibodies of the present invention can be characterised by one or more of the following properties: a) specificity for human CD52 (specifically binding to human CD52); b) a binding affinity to human CD52 with an equilibrium dissociation constant (Kd) of at least 10^{-8}M.

135

In another aspect, the invention provides nucleic acid molecules encoding the humanised antibodies, or antigen-binding portions, of the invention. Accordingly, recombinant expression vectors that include the antibody-encoding nucleic acids of the invention, and host cells transfected with such vectors, are also encompassed by the invention, as are methods of making

140   the antibodies of the invention by culturing these host cells.

Anti-human CD52 humanised monoclonal antibodies of the invention, or antigen binding portions thereof (e.g., Fab), can be derivatised or linked to another functional molecule, e.g., another peptide or protein (e.g., a Fab' fragment). For example, an antibody or antigen-binding

145   portion of the humanised antibodies of the invention can be functionally linked (e.g., by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities. For example, the humanised anti-CD52 antibody, or antigen binding fragment thereof, can be conjugated to a therapeutic moiety, e.g., a cytotoxic drug, an enzymatically active toxin, or a fragment thereof, a radioisotope, a therapeutic nucleic acid, or a

150   small molecule anti-cancer drug. The antibodies of the invention can also be conjugated to cytotoxic pharmaceuticals, e.g., radiolabeled with a cytotoxic agents such as, e.g. 131I, or can be coupled to a ribosome inactivating protein, e.g. pseudomonas exotoxin (PE38 fragment, plant or bacterial toxins such as ricin, the α-chain of ricin, saporin, pokeweed antiviral protein, diphtheria toxin, or *Pseudomonas* exotoxin A (Kreitman and Pastan (1998) Adv. Drug Delivery Rev.

155   31:53.).

In another aspect, the present invention provides compositions, e.g., pharmaceutical and diagnostic compositions, comprising a pharmaceutically acceptable carrier and at least one humanised monoclonal antibody of the invention, or an antigen-binding portion thereof, which

160   specifically binds to human CD52. Some compositions may also comprise a combination of the humanised antibodies or antigen-binding portions of the invention. Such compositions may also comprise combinations with one or more other biologically active molecules as separate molecules, for example, a combination of at least one humanised monoclonal antibody of the invention and another biologically active molecule, or may combine combinations with one or

165   more other biologically active molecules in the same molecule, for example as a bispecific or multispecific molecule either as a combination of two or more humanised antibodies of the invention or as a combination with one or more other biologically active molecules.

For *in vivo* methods, the humanised antibodies, or antigen-binding portions thereof (or a

170   bispecific or multispecific molecule of the invention) can be administered to a human subject suffering from a disease related to CD52+ cells, or to a disease that can be ameliorated or prevented by treatment with the humanised antibodies of the invention.

Humanised monoclonal antibody compositions of the invention also can be administered in

175   combination with other known therapies, e.g., an anti-cancer therapy, a therapy for an autoimmune disease such as rheumatoid arthritis, or a therapy for multiple sclerosis. Accordingly, the invention provides a method for treating cancer or inflammatory diseases in a subject comprising administering a therapeutically effective amount of a pharmaceutical composition of a humanised antibody together with a pharmaceutical carrier to the subject.

180
In yet another aspect, the present invention provides a method using antibodies of the invention for detecting in vitro or in vivo the presence of human CD52 antigen in a sample, e.g., for diagnosing a human CD52-related disease. In some methods, this is achieved by contacting a sample to be tested, along with a control sample, with a humanised monoclonal antibody of the

185   invention, or an antigen-binding portion thereof (or a bispecific or multispecific molecule), under conditions that allow for formation of a complex between the antibody and human CD52. Complex formation is then detected (e.g., using an ELISA) in the test samples, and any

statistically significant increase in the formation of complexes between the test and control samples is indicative the presence of human CD52 antigen in the test sample.

190   It will be understood by those skilled in the art that the humanised antibodies of the present invention will have additional uses or compositions beyond those described herein, in all cases where the humanised antibody binds to human CD52 antigen whereby such uses and compositions shall be considered to be within the scope of the invention. It will be understood

195   by those skilled in the art that the variable region sequences of the humanised antibodies of the present invention (SEQ ID NO:20 through SEQ ID NO:28) or CDRs of the humanised antibodies of the present invention (SEQ ID NO:5 through SEQ ID NO:10) may be subject to variations which do not significantly change the properties of the humanised antibodies of the present invention whereby such variants shall be considered to be within the scope of the

200   invention. In addition, such variations either within the variable region or CDR sequences of the humanised antibodies should be considered to be within the scope of the present invention where such variations have significant homology to the humanised sequences of the present invention. For example, a variant nucleic acid may be determined to be within the scope of the invention where this includes sequences containing or substantially identical to SEQ ID NO:11 through

205   SEQ ID NO:19 as determined by its ability to hybridise under stringent conditions to a nucleic acid of the present invention. In one embodiment, a nucleic acid sequence can be determined to be within the scope of the invention (e.g., is substantially identical to SEQ ID NO:11 through SEQ ID NO:19) by its ability to hybridise under stringent conditions to a nucleic acid within the scope of the invention (such as SEQ ID NO:11 through SEQ ID NO:19). The term "hybridise"

210   refers to the binding, duplexing, or hybridising of a molecule to a particular nucleotide sequence under stringent hybridisation conditions when that sequence is present in a complex mixture (e.g. total cellular or library DNA or RNA), wherein the particular nucleotide sequence is detected at least at about 10 times background. Stringent hybridisation conditions will be selected, for example, to be 5-10°C lower than the thermal melting point (Tm) for the specific sequence at a

215   defined ionic strength pH. It will also be understand that humanised antibodies of the present invention may be modified in the heavy chain constant regions in order to enhance ADCC and CDC. For enhancement of ADCC, fucose-depleted forms of the humanized antibodies may be produced by expression of the antibodies in certain mammalian cells including a variant CHO

line, Lec13 (Shields et al., J Biol Chem 277 (2002) p26733–26740), a rat hybridoma cell line,
YB2/0 (Shinkawa et al., J Biol Chem 278 (2003) p3466–3473), and a FUT8 (a-1,6-
fucosyltransferase) knockout CHO cell line (Yamane-Ohnuki et al., Biotechnol Bioeng 87
(2004) p614–622). Alternatively mutations in the heavy chain constant regions may be used to
enhance ADCC such as described by Shields et al., J Biol Chem 276 (2001) p6591-6604 and
Lazar et al,. Proc Natl Acad Sci U S A 2006; 103 (2006) p4005–4010. Alternatively mutations
in the heavy chain constant regions may be used to enhance CDC, for example using antibodies
of mixed human IgG1 / IgG3 isotype (Natsume et al., ibid).

It will be understood by those skilled in the art, from precedent elsewhere especially from
clinical studies with Campath-1H (Zhiqiang An, *ibid*), that antibodies which bind to human
CD52 antigen are fundamentally immunogenic in patients, probably due to the inherent
cytotoxicity of anti-CD52 antibodies which acts as co-stimulatory signal to CD4+ T cell epitopes
from the antibodies, thus resulting in CD4+ T helper cell responses and immunogenicity. It will
therefore be understood by those skilled in the art that the antibodies of the present invention are
surprisingly devoid of such CD4+ T helper cell responses as determined by *in vitro* studies with
human blood (cf Example 9) and that such anti-CD52 antibodies with low CD4+ T cell
responses (<=4% T cell responses in human T cell assays) are novel.

BRIEF DESCRIPTION OF THE DRAWINGS

Within the figure legends, the nomenclature 2E8 or ANT01 is used interchangeably for mouse,
chimeric or humanised antibodies derived from the 2E8 mouse monoclonal antibody.

Figure 1 shows the plasmid vectors used for expression of chimeric and humanised antibodies in
mammalian cells comprising pANT17 for heavy chains and pANT13 for light chains.

Figure 2 shows a flow cytometry analysis of binding of the 2E8 mouse monoclonal antibody to
NS0 cells transfected with human CD52 compared to binding to NS0 CD52-. Staining was with
anti-mouse IgG-PE conjugated antibody with signal derived from PE on the Y axis.

250

Figure 3 shows a flow cytometry analysis of binding of dilutions of chimeric 2E8 to Hut78 cells compared to Campath-1H. Staining was with anti-human IgG-PE conjugated antibody.

Figure 4 shows a competition flow cytometry analysis using Campath-1H-PE in competition

255   with chimeric 2E8 and Campath-1H for binding to Hut78 cells.

Figure 5 shows the mean cytotoxicity from 5 human PBMC samples used as effector cells in an ADCC assay for chimeric 2E8 and Campath-1H with REH target cells.

260   Figure 6 – as Figure 5 except for 2 individual PBMC with dilutions of chimeric 2E8 and Campath-1H with high expressing REH target cells.

Figure 7 – as Figure 6 except using human complement for CDC assays with dilutions of chimeric 2E8 and Campath-1H and high expressing REH target cells.

265

Figure 8 shows a competition CD52 peptide ELISA for binding of humanised 2E8 variants in competition with biotinylated chimeric 2E8.

Figure 9 shows a flow cytometry analysis for binding of dilutions of humanised variants and

270   Campath-1H to REH cells.

Figure 10 shows the mean cytotoxicity from 4 human PBMC samples used as effector cells in an ADCC assay for humanised 2E8 variants and Campath-1H with REH target cells.

275   Figure 11 shows the CDC for humanised 2E8 variants and Campath-1H with high CD52 expressing REH target cells and human complement.

Figure 12 shows a competition flow cytometry analysis using Campath-1H-PE in competition with chimeric 2E8, Campath-1H and selected variants for binding to REH cells.

280

Figure 13 shows the direct cytotoxic effects of anti-human CD52 antibodies on REH cells as measured by apoptosis and necrosis.

285   Figure 14 shows the Kaplan–Meier plot for SCID mice transplanted with Raji human Burkitt lymphoma cells following treatment with Campath-1H and the lead humanised 2E8 variant VH3/VK4 (V region SEQ ID NOS. 22 and 28).

EXAMPLES

290   Commercially available reagents referred to in the Examples were used according to manufacturer's instructions unless otherwise indicated. The source of cells identified in the Examples and throughout the specification by ECACC accession numbers is the European Collection of Cell Cultures (ECACC), Salisbury, England.   Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by

295   one of ordinary skill in the art to which this invention belongs.   Exemplary methods and materials are described below although methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention.   The materials, methods, and examples are illustrative only and not intended to be limiting in scope.

300   **EXAMPLE 1: GENERATION OF MOUSE MONOCLONAL ANTIBODIES**

CD52 peptide (GQNDTSQTSSPSC) was custom synthesised and conjugated to either KLH or BSA via a maleimidocaproyl-N-Hydroxysuccinimide linker (Mimotopes, Wirral, Cheshire UK) leaving the peptide N-terminus free. Raji and HuT78 cells were obtained from ECACC. CD52-expressing NS0 cell lines were generated as follows: DNA encoding human CD52 (NCBI

305   Reference Sequence: NM_001803.2) (full length sequence including the N-terminal signal peptide, the C-terminal displaced GPI-anchor signal peptide and the mature GPI-anchored surface peptide) was PCR amplified and subcloned into pANT antibody expression vectors (Figure 1a) via BglII and EagI sites. Transcription of the CD52 gene was under the control of the CMV I/E promoter (US5168062 and US5385839, University of Iowa).   The pANT

310   expression plasmid contained a mutant *dhfr* minigene (Simonsen & Levinson 1983, PNAS **80**:2495-2499) under the control of a SV40 promoter and polyA sequence for selection in

eukaryotic cells as well as a β-lactamase (Ap^R) gene for prokaryotic selection and a pMB1 origin of replication for propagation in prokaryotic cells. The expression plasmid was propagated in *E. coli* XL1-blue (Stratagene Cat. No. 200130). Stable CD52 expressing cell lines were obtained by transfecting NS0 cells by electroporation and placing cells under selection with 200nM methotrexate. Cells were grown and expanded then tested by flow cytometry for CD52 expression. High CD52 expressing cell lines were frozen down and used for immunising mice as described below.

Female Balb/c mice were primary immunised by intraperoneal (i.p.) injection either of 50ug of CD52 peptide-KLH conjugate in Complete Freund's Adjuvant (CFA), or primary immunised with 1 x 10^6 RAJI cells expressing CD52 in phosphate-buffered saline (PBS). After four weeks, all mice were boosted by i.p. injection of 10^6 HUT-78 cells in PBS with an additional booster injection two weeks later. Four weeks later, all mice received a third boost with 3 x 10^6 NS0 cells expressing CD52 in PBS i.p. Two subsequent boosts of 10^7 NS0 cells expressing CD52 in PBS were injected i.p at two weekly intervals and some mice were given a further boost of 5ug of CD52 peptide-KLH.

Three days prior to myeloma fusion, the two mice showing the highest antibody titre were given an i.p. boost of 10^7 NS0 cells expressing CD52 in PBS. On the day of fusion, both mice were sacrificed, spleens were removed, and the cells from each entire spleen were pooled, washed in serum-free culture medium and split into two equal samples. Half of the spleen cells were fused to F0 myeloma cells and half were fused to P3X63Ag8U.1 myeloma cells by PEG-mediated fusion. Plates 1-4 contained the F0 fused cells and plates 5-8 contained the P3X63Ag8U.1 cells. The complete fusion medium consisted of DMEM, 2% L-glutamine, 1% penicillin-streptomycin, 10% fetal bovine serum, 5% BriClone hybridoma cloning medium (National Institute for Cellular Biotechnology, Dublin, Ireland) and Hypoxanthine-Aminopterin-Thymidine (HAT). The resulting fusions were seeded into 96-well plates at 200ul per well. The remaining non-plated fused cells were stabilized in culture for up to three days, then frozen and stored in liquid nitrogen. The plated fusion cells were cultured at 37°C in 5% CO$_2$ for two weeks, transferred to 96-well plates, and tested for the presence of secreted anti-CD52 antibodies using the CD52 peptide-KLH ELISA as described below. Cells from 24 immunopositive wells were expanded in

culture and tested for CD52-specific antibody by CD52 peptide ELISA, NS0-CD52 cell-based ELISA and by flow cytometry.

345

For the CD52 peptide ELISA, ELISA plates (VWR, Lutterworth, UK) were coated overnight at 4°C with 100ul/well of either CD52 peptide-KLH, CD52 peptide-BSA, KLH only or BSA only at 0.5µg/ml in PBS. Plates were washed and blocked with 150ul/well PBS containing 2% BSA. Cell culture supernatants or purified antibodies were diluted in PBS/2% BSA and 100ul added to

350   each plate followed by incubation for 1 hour at room temperature. Plates were washed three times with PBS-Tween (0.05%) and incubated for 1 hour with 100ul/well goat anti-mouse Ig (Fab-specific) conjugated to Horseradish Peroxidase (Sigma-Aldrich). Plates were washed three times with PBS-Tween following which SigmaFast OPD substrate (Sigma-Aldrich) was added and incubated at room temperature in the dark to allow colour to develop. The reaction was

355   stopped by adding 50 µl of 3M HCl. Plates were read at 490nm using a Dynex plate reader (Dynex, Worthing, UK). CD52 peptide-specific hybridomas were those that bound to CD52 peptide-KLH and CD52 peptide-BSA but not to either KLH only or BSA only

For the NS0-CD52 cell-based ELISA, $3x10^5$cells/well (NS0 wild-type or NS0 cells expressing

360   CD52) were plated out in a V-bottom 96 well plate. The plate was centrifuged, supernatants were removed and the plate blotted on absorbent paper. Hybridoma samples were diluted 1 in 2 in FACS buffer (D-PBS containing 1%BSA and 0.05% sodium azide) and 100µl transferred to each of two plates containing either NS0 (Plate 1) or NS0-CD52 (Plate 2) cells. After incubation at room temperature for 1hr, the plates were washed twice by centrifuging the plates and

365   resuspending the cells in 200µl FACS buffer between centrifuging. After centrifugation, cells were resuspended in 100µl FACS Buffer containing anti-mouse IgG (Fab specific) (Sigma) diluted 1:500. After incubation for 1hr at room temperature, plates were washed twice by centrifuging and resuspending the cells in PBS. After centrifugation, cells were resuspended in 50µl PBS and transferred to an ELISA plate. 100µl TMB substrate (Invitrogen) was added and

370   incubated at room temperature in the dark to allow the colour to develop. The reaction was stopped by adding 50 µl of 3M HCl. Plates were read at 450nm using Dynex plate reader. CD52-specific clones were those that bound to NS0-CD52 cells specifically when compared with NS0 wild-type cells.

375   For flow cytometry, 3×10^5 cells NS0-CD52 or wild-type NS0 were stained using a 1 in 2 dilution

of anti-CD52 hybridomas antibodies together with a 1 in 100 dilution of anti-mouse IgG-PE

conjugated antibody (Sigma). Mouse IgG (Sigma) was also included as a separate control for the

different murine isotypes present within the hybridomas. Cells were stained for 1 hour at 4°C.

An anti-mouse IgG-PE conjugated antibody only control was also included. Cells were washed

380   twice with FACS buffer and finally resuspended in FACS buffer and flow cytometry performed

using a Beckton Dickinson FACSCalibur (Becton Dickinson, Oxford, UK). Instrument settings

were determined by analysis of relevant isotype control antibodies.

From results of CD52 peptide ELISA, NS0-CD52 cell-based ELISA and flow cytometry,

385   huCD52 specific hybridomas were cloned, expanded in culture, frozen as parental stocks and

stored in liquid nitrogen. Each of the selected hybridomas was diluted in cloning medium and

plated into 96-well plates at a cell density of one cell per three wells. Cloning medium consisted

of DMEM, 2% L-glutamine, 1% penicillin-streptomycin, 10% fetal bovine serum, 5% BriClone

hybridoma cloning medium and hypoxanthine-thymidine (HT). Cultures were maintained at

390   37°C in 5% CO_2 for 2 weeks with the cloned cells receiving fresh medium after one week in

culture. Two weeks after cloning, supernatants from all seeded wells were transferred to new

96-well plates and tested for the presence of anti-CD52 antibodies using the CD52 peptide

ELISA and flow cytometry as described previously. Positive wells were expanded in culture and

retested. Positive cells were further expanded and tested for antibody isotype. Anti-CD52

395   positive subclones were frozen, stored in liquid nitrogen and used for monoclonal antibody

production for further studies.

Monoclonal antibodies were isotyped using the Rapid ELISA Mouse Antibody Isotyping

Kit (Perbio, Cramlington, UK). Antibodies were purified on a 1ml Protein A-sepharose column

400   (GE Healthcare, Little Chalfont, UK). Prior to purification, both the tubing and the Protein A

column were depyrogenated using 0.4M NaOH. The column was re-equilibrated with 20 column

volumes of PBS pH 7.4. Hybridoma cell culture supernatants were harvested, adjusted to 1x

PBS pH 7.4 using 10x PBS and filter sterilised. Filtered supernatant was pumped through on the

Protein A-sepharose column at 0.5 ml/min. The column was washed with 1x PBS pH 7.4 and

405   IgG was eluted using sterile 0.1M Sodium Citrate pH3, with 0.9 ml fractions collected and neutralised with 0.1ml of sterile 1M Tris-HCl pH 9. Under sterile conditions, the product was buffer exchanged into PBS pH 7.4 to remove any elution buffer and concentrate the sample. After concentration, antibodies were quantified by OD280nm using an extinction coefficient, Ec (0.1%) of 1.4. Purified antibodies were analysed by SDS-PAGE using a Novex NuPAGE

410   electrophoresis system with 4-12% NuPage gel (Invitrogen, Paisley, UK) and MES running buffer. 1 μg of antibody was prepared with 4xNuPAGE sample buffer plus beta-mercaptoethanol and heated. The gel was stained with InstantBlue staining solution (Expedeon, Cambridge, UK) and molecular size were estimated by comparing stained bands to PageRuler™ Plus Prestained Protein Ladder (Fermentas, York, UK). Two bands were identified for each antibody with no

415   detectable contamination present. Purified antibodies were testing using the CD52 peptide flow cytometry as described above. From flow cytometry analysis (Figure 2), the lead monoclonal antibody designated 2E8 was shown to bind selectively to NS0-CD52 cells.

EXAMPLE 2 – VARIABLE REGION GENE SEQUENCING

420   Total RNA was extracted from 2E8 hybridoma cells using the RNAqueous-4PCR Kit (Ambion, Warrington, UK) and used to synthesis cDNA. Murine immunoglobulin heavy and kappa light chain variable (V) region fragments were amplified by PCR using degenerate mouse leader sequence primers (Sigma) and unique constant domain primers (Sigma) as shown in Table 1. The resulting PCR fragments were subcloned into the pGEM-T Easy I vector system (Promega,

425   Southampton, UK) and inserts were sequenced using the vector-specific primer, M13Forward (Sigma) All DNA sequencing was performed by Geneservice Ltd, Cambridge, UK). The resultant V region nucleotide sequences are shown as SEQ ID No.1 and SEQ ID No.2 and corresponding amino acid sequences as SEQ ID No.3 and SEQ ID No.4 for heavy and light chain V regions respectively.

430

Sequence	Name-Pool
ATGRASTTSKGGYTMARCTKGRTTT	MuIgV$_H$5'-A
ATGRAATGSASCTGGGTYWTYCTCTT	MuIgV$_H$5'-B
ATGGACTCCAGGCTCAATTTAGTTTTCCT	MuIgV$_H$5'-C
ATGGCTGTCYTRGBGCTGYTCYTCTG	MuIgV$_H$5'-C

ATGGVTTGGSTGTGGAMCTTGCYATTCCT	MuIgV$_H$5'-C
ATGAAATGCAGCTGGRTYATSTTCTT	MuIgV$_H$5'-D
ATGGRCAGRCTTACWTYYTCATTCCT	MuIgV$_H$5'-D
ATGATGGTGTTAAGTCTTCTGTACCT	MuIgV$_H$5'-D
ATGGGATGGAGCTRTATCATSYTCTT	MuIgV$_H$5'-E
ATGAAGWTGTGGBTRAACTGGRT	MuIgV$_H$5'-E
ATGGRATGGASCKKIRTCTTTMTCT	MuIgV$_H$5'-E
ATGAACTTYGGGYTSAGMTTGRTTT	MuIgV$_H$5'-F
ATGTACTTGGGACTGAGCTGTGTAT	MuIgV$_H$5'-F
ATGAGAGTGCTGATTCTTTTGTG	MuIgV$_H$5'-F
ATGGATTTTGGGCTGATTTTTTTTATTG	MuIgV$_H$5'-F
CCAGGGRCCARKGGATARACIGRTGG	MuIgGV$_H$3'-2
ATGRAGWCACAKWCYCAGGTCTTT	MuIgkV$_L$5'-A
ATGGAGACAGACACACTCCTGCTAT	MuIgkV$_L$5'-B
ATGGAGWCAGACACACTSCTGYTATGGGT	MuIgkV$_L$5'-C
ATGAGGRCCCCTGCTCAGWTTYTTGGIWTCTT	MuIgkV$_L$5'-D
ATGGGCWTCAAGATGRAGTCACAKWYYCWGG	MuIgkV$_L$5'-D
ATGAGTGTGCYCACTCAGGTCCTGGSGTT	MuIgkV$_L$5'-E
ATGTGGGGAYCGKTTTYAMMCTTTTCAATTG	MuIgkV$_L$5'-E
ATGGAAGCCCCAGCTCAGCTTCTCTTCC	MuIgkV$_L$5'-E
ATGAGIMMKTCIMTTCAITTCYTGGG	MuIgkV$_L$5'-F
ATGAKGTHCYCIGCTCAGYTYCTIRG	MuIgkV$_L$5'-F
ATGGTRTCCWCASCTCAGTTCCTTG	MuIgkV$_L$5'-F
ATGTATATATGTTTGTTGTCTATTTCT	MuIgkV$_L$5'-F
ATGAAGTTGCCTGTTAGGCTGTTGGTGCT	MuIgkV$_L$5'-G
ATGGATTTWCARGTGCAGATTWTCAGCTT	MuIgkV$_L$5'-G
ATGGTYCTYATVTCCTTGCTGTTCTGG	MuIgkV$_L$5'-G
ATGGTYCTYATVTTRCTGCTGCTATGG	MuIgkV$_L$5'-G
ACTGGATGGTGGGAAGATGGA	MuIgkV$_L$3'-1

Table 1

435

Sequences of the 2E8 hypervariable regions (CDRs) were as follows;

SEQ ID NO. 5 CDRH1   RYGMS

440   SEQ ID NO. 6 CDRH2   MMKTKGGRTYYPDSVKG

SEQ ID NO. 7 CDRH3 DGYY

SEQ ID NO. 8 CDRL1 KSSQSLLHSDGKTYLN

SEQ ID NO. 9 CDRL2 LVSKLDS

SEQ ID NO. 10 CDRL3 WQGTHLWT

445

EXAMPLE 3 – GENERATION OF CHIMERIC ANTIBODY

The heavy and light chain V region sequences of the 2E8 monoclonal antibody were PCR amplified and subcloned into pANT antibody expression vectors (Figure 1b) with heavy and light chain V regions cloned into pANT17 and pANT13 respectively. Heavy chain V region
450   genes were cloned into pANT17 via MluI and HindIII sites in frame with the human γ1 heavy chain gene (G1m3 (G1m(f)) allotype) and light chain V region genes were cloned into pANT13 via BssHII and BamHI sites in frame with the human kappa light chain constant region gene (Km3 allotype). Transcription of both heavy and light chain genes was under the control of the CMV I/E promoter (US5168062 and US5385839, University of Iowa) and the pANT17 plasmid
455   contained a mutant *dhfr* minigene (Simonsen & Levinson 1983, PNAS **80**:2495-2499) under the control of a SV40 promoter and polyA sequence for selection in eukaryotic cells. Both pANT17 and pANT13 contained a β-lactamase (Ap^R) gene for prokaryotic selection and a pMB1 origin of replication for propagation in prokaryotic cells. All plasmids were propagated in *E. coli* XL1-blue (Stratagene Cat. No. 200130). Primers used to amplify the variable region genes for cloning
460   into the pANT expression vectors are shown in Table 2.

Sequence	Name
ctgttgctacgcgtgtccactccGAGGTGCACCTGATGGAG	2E8 VH 5′
ctgccccagaaagcttaccTGAGGAGACTGTGAGAGTG	2E8 VH 3′
ggctcccaggcgcgcgatgtGATGTTTTGATGACCCAGAC	2E8 VK 5′
gaattgcgggatccaactgaggaagcaaagtttaaattctactcacgTTTGATTTCCAGTTTGGTGCC	2E8 VK 3′

Table 2

465

The heavy and light chain expression constructs were then co-transfected either transiently into HEK293 cells by calcium phosphate-based transfection or stably transfected into NS0 cells by

electroporation. Secreted antibody was purified from the cell culture supernatants by Protein A chromatography. As shown in Figure 3 using flow cytometry analysis as detailed in Example 1,

470   dilutions of the 2E8 antibody exhibited an improved binding profile to CD52+ HuT78 cells compared to Campath-1H. As shown in Figure 4, the 2E8 antibody exhibited an improved competitive binding profile by flow cytometry analysis when competed with Campath-1H for binding to CD52+ HuT78 cells.

475   **EXAMPLE 4 – ANTIBODY-DEPENDENT CELL-MEDIATED CYTOTOXICITY (ADCC)**

ADCC assays were performed with the 2E8 monoclonal antibody as follows. Target cells (either REH or Raji cells) were harvested and preloaded with 25μM (final) Calcein-AM (Sigma). After incubation with Calcein for 1hr at 37°C, cells were washed in media to remove unincorporated

480   Calcein. $1x10^4$ target cells were added to a clear V-bottomed plate containing 2E8 or control antibodies at 50μg/ml final as in Figure 5 or as depicted in Figure 6, and incubated for 1hr to pre-opsonise the target cells. PBMCs (effector cells) were isolated from healthy community donor buffy coats (from blood drawn within 24 hours) obtained from the UK National Blood Transfusion Service (Addenbrooke's Hospital, Cambridge, UK) and according to approval

485   granted by Addenbrooke's Hospital Local Research Ethics Committee. PBMCs were isolated from buffy coats by Lymphoprep (Axis-Shield, Dundee, UK) density centrifugation. $5x10^5$ effector cells were added to each well of the plate containing target cells and antibody in a final volume of 250μl (50:1 ratio of effector to target cells). Samples were incubated for 4hr at 37°C / 5% CO_2. After 4 hours, Triton X-100 was added to the control wells containing cells (effector

490   and/or target cells) to establish the maximum lysis control. Following centrifugation, 150μl media was transferred from each well to a 96 well clear-bottom black-walled plate and the plate fluorescence was measured at 520nm. Results were expressed as:

$$\% \text{ Cytotoxicity} = \frac{(\text{test sample signal minus background Calcein-AM release})}{(\text{maximum target cell lysis signal minus background Calcein-AM release})} \times 100$$

495

Figure 5 shows the mean ADCC for PBMC from 5 human donors for chimeric 2E8 ('ANT01') and Campath-1H for target CD52+ REH cells showing a significantly enhanced ADCC for

500   chimeric 2E8. Subsequently a high CD52 expressing variant REH cell line was isolated by
        FACS which exhibited approximately 2x the binding of Campath-1H compared to the parent
        REH cells. Figure 6 shows a dilution series of chimeric 2E8 and Campath-1H for ADCC on
        high CD52+ REH cells using PBMC from two individual donors. This also shows the
        significantly enhanced ADCC profile for chimeric 2E8 compared to Campath-1H.

505

        ## EXAMPLE 5 – COMPLEMENT-DEPENDENT CYTOTOXICITY (CDC)

        CDC assays were performed on the 2E8 monoclonal antibody as follows. Target cells (either
        REH or Raji cells) were harvested and 5×10^4 cells/well were added to a black-walled, clear flat-
        bottomed 96 well plate. 2E8 or control antibodies for final concentrations as shown in Figure 7

510   were added together with either active or heat inactivated (@60°C for 30min) human serum
        (Pathway Diagnostics Ltd, Dorking, UK) per well (25% final serum concentration). Samples
        were incubated for 3hr at 37°C / 5% CO_2. After 3 hours, Triton X-100 was added to the control
        cell containing wells to establish the maximum lysis control. Prestoblue (10x) cell viability
        reagent (Invitrogen) was diluted with assay growth media and added to each well to obtain a

515   final 1 in 10 dilution of PrestoBlue. After incubation for 1hr at 37°C / 5% CO_2, the plate
        fluorescence was measured at 590nm. Results were expressed as:

        % Cell Viability = (Sample minus background release) x 100
                         ───

520
                          (max readout (No lysis) minus background readout)

        Figure 7 shows a significantly enhanced CDC profile for chimeric 2E8 compared to Campath-
        1H.

525

        ## EXAMPLE 6 – GENERATION OF HUMANISED ANTIBODIES

        Humanised antibodies were generated using methods described in EP1844074 (Antitope Ltd).
        Structural models of the mouse 2E8 V regions were produced using Swiss PDB and analysed in
        order to identify important amino acids that were likely to be important for the CD52 binding

530   properties of the antibody ('constraining residues'). A database of human V region sequences
        was used to identify segments of human V region sequences containing each of the constraining

residues to be used in design of the humanised antibodies. Typically two or more alternative V region sequence segments were used to provide each constraining residue resulting in a large range of possible sequences of humanised anti-CD52 V region sequences for 2E8. These

535   sequences were then analysed for the prediction of non-germline MHC class II peptide binding by *in silico* analysis as described in Fothergill et al. (WO9859244, assignee Eclagen Ltd) and also for known CD4+ T-cell epitopes using databases including "The Immune Epitope Database and Analysis Resource", http://www.immuneepitope.org/. V region sequences with predicted non-germline MHC class II binding peptides, or with significant hits against T cell epitope

540   databases were discarded. This resulted in a reduced set of V region sequences. Selected combinations of V region sequence segments were then combined to produce humanised heavy and light chain variable region amino acid sequences. Five heavy chains and four light chain sequences (designated VH1 to VH5, and Vκ1 to Vκ4 respectively) were selected for 2E8 (SEQ ID No.s 20 to 24 and 25 to 28 respectively).

545

DNA encoding humanised variant V regions were synthesised and subcloned into the expression vectors pANT17 and pANT13 (Figure 1) as described in Example 3. All combinations of humanised VH and Vκ chains (i.e. a total of 20 pairings for 2E8) were transiently transfected into HEK293 and also transfected into NS0 cells, and antibody was purified by protein A

550   chromatography from the culture supernatants as described in Example 3.

EXAMPLE 7 – ANALYSIS OF HUMANISED ANTIBODIES

The binding of HEK-derived and NS0-derived 2E8 humanised variants to CD52 peptide was assessed in a competition ELISA against the parent chimeric antibody. The parental 2E8

555   chimeric antibody was biotinylated using Biotin Tag™ Micro Biotinylation kit (Sigma–Aldrich). 96 well MaxiSorp plates (Nunc) were coated with 0.025μg/ml CD52 peptide-KLH in Dulbecco's PBS (PAA Laboratories, Yeovil, UK) (100μl final volume) at 4°C overnight. Plates were blocked with Dulbecco's PBS-2% BSA for 1 hour at room temperature. Plates were washed 3 times with wash buffer (0.05% Tween20 in Dulbecco's-PBS). Test humanised antibodies at

560   various concentrations were premixed with biotinylated parent chimeric antibody (0.035μg/ml final concentration) and then added to the CD52 peptide-KLH plate (100μl final volume). All samples were tested in duplicate. Plates were incubated for 1h at room temperature and washed

3 times with wash buffer. 100µl of a 1 in 1000 dilution of Streptavidin HRP (Sigma-Aldrich) was added and incubated for 1 hour at room temperature. Plates were washed 3 times with wash
565   buffer and 100µl of TMB substrate (Invitrogen) was added and incubated at room temperature in the dark to allow the colour to develop. The reaction was stopped by adding 50 µl of 3M HCl. Plates were read at 450nm using Dynex plate reader.

As shown in Figure 8, all lead humanised 2E8 variants displayed competitive binding profiles
570   similar to the parent chimeric antibody. Humanised variants were subsequently tested for binding by flow cytometry as detailed in Example 1, for ADCC as in Example 4, and for CDC as in Example 5. As shown in Figure 9, the humanised variants exhibited an improved binding profile by flow cytometry to Campath-1H for binding to REH cells. As shown in Figures 10 and 11, the humanised variants also exhibited improved ADCC and CDC profile using REH target cells for
575   ADCC at a target:effector cell ratio of 50:1 or the high CD52+ REH cell line for CDC (as in Example 4) compared to Campath-1H.

EXAMPLE 8 – GENERATION OF scFv's and Fab's

Humanised 2E8 variants from Example 6 were converted into scFv's and cloned into M13 phage
580   display vectors as described in Benhar I. and Reiter Y., Current Protocols in Immunology, Unit 10.19B, Wiley Online Library, May 2002 (http://www.currentprotocols.com/protocol/im1019b) using the pCANTAB5E vector RPAS Expression Module (Amersham Pharmacia Biotech, Little Chalfont, UK). Humanised VH and VK genes were amplified using primers which provided terminal SfiI and NotI restriction sites, an internal Gly4Ser linker and a C terminal his6 tag. The
585   scFv constructs were inserted into the pCANTAB5E vector as SfiI-NotI fragments and transformed into E.coli HB2151 resulting in scFv exported to the periplasm and partially to the growth medium. scFv's were purified from growth medium by nickel-chelate affinity chromatography using HIS-Select HF Cartridges (Sigma-Aldrich). Purified 2E8 scFv's were tested in a competition assay as detailed in Example 7 for binding to CD52 peptide and all
590   humanised scFvs exhibited competitive binding to CD52 peptide. Humanised 2E8 variants from Example 6 were also converted into Fab's using the method used for scFv's except that amplified humanised VH and VK genes were further amplified with CH1 and Cκ constant region genes to form VH-CH1 and VK-Cκ fragments which were further amplified with primers to join

these fragments with a 22 amino acid pelB leader sequence (Lei S.P. et al., J Bacteriol. 169

595 (1987) p4379–4383) between the upstream VH-CH1 and downstream VK-Cκ gene fragments
resulting in a dicistronic Fab gene. Fab's from humanised 2E8 variants were generated and
purified as above for scFv's and tested in CD52 peptide competition assay as detailed in
Example 7. All humanised Fab's exhibited competitive binding to CD52 peptide.

600 **EXAMPLE 9 – ANALYSIS OF CD4+ T CELL RESPONSES**

PBMCs were isolated from healthy community donor buffy coats (from blood drawn within 24
hours) obtained from the UK National Blood Transfusion Service (Addenbrooke's Hospital,
Cambridge, UK) and according to approval granted by Addenbrooke's Hospital Local Research
Ethics Committee. PBMCs were isolated from buffy coats by Lymphoprep (Axis-shield,

605 Dundee, UK) density centrifugation and CD8$^+$ T cells were depleted using CD8$^+$ RosetteSep™
(StemCell Technologies Inc, London, UK). Donors were characterized by identifying HLA-DR
haplotypes using an HLA SSP-PCR based tissue-typing kit (Biotest, Solihull, UK). T cell
responses to control antigens including the recall antigen tetanus toxin were also determined
(KLH Pierce, Cramlingtom, UK and peptides derived from Influenza A and Epstein Barr

610 viruses). PBMC were then frozen and stored in liquid nitrogen until required.

To prepare monocyte derived dendritic cells (DC), 50 different donor PBMCs were selected to
provide a distribution with frequencies of HLA-DR and HLA-DQ allotypes similar to the
frequencies in the overall world population. PBMCs were revived in AIM-V® culture medium

615 and CD14$^+$ cells isolated using Miltenyi CD14 Microbeads and LS columns (Miltenyi Biotech,
Oxford, UK). Monocytes were resuspended in AIM-V® supplemented with 1000U/ml IL-4 and
1000U/ml GM-CSF ("DC culture media") to 4-6x10^6 PBMC/ml and then distributed in 24 well
plates (2ml final culture volume). Cells were fed on day 2 by half volume DC culture media
change. By day 3, monocytes had differentiated to semi-mature DC which were pre-incubated

620 with either 40ug/ml of Campath-1H, chimeric 2E8 antibody, humanised 2E8 antibodies,
100μg/ml KLH or media only. Semi-mature DC were incubated with antigen for 24 hours after
which excess test antibody was removed by washing the cells twice and resuspending in DC
culture media supplemented with 50ng/ml TNF-α (Peprotech, London, UK). DCs were fed on
day 7 by a half volume DC culture media (supplemented with 50ng/ml TNFα) change before

625   harvesting mature DC on day 8. The harvested mature DC were counted and viability assessed
       using trypan blue dye exclusion. The DC were then γ-irradiated (4000 rads) and resuspended at
       $2x10^5$ cells per ml in AIM-V media before use in the ELISpot and proliferation assays.
       Additionally, on day 8, fresh CD4+ T cells were also prepared. To purify CD4+ T cells, PBMCs
       were revived in AIM-V® culture medium and $CD4^+$ cells isolated using Miltenyi CD4
630   Microbeads and LS columns (Miltenyi Biotech, Oxford, UK) and resuspended in AIM-V®
       media at $2x10^6$ cells/ml.

       On day 8, T cell proliferation assays were established whereby $1x10^5$ autologous $CD4^+$ T cells
       were added to $1x10^4$ humanised 2E8 or chimeric 2E8 antibody loaded DC (ratio of 10:1) in 96
635   well U-bottomed plates, with AIM-V® media added to a final volume 200ul/well). On day 14,
       assay plates were pulsed with 1uCi [3H] (Perkin Elmer, Beaconsfield, UK) per well in 25ul
       AIMV for 6 hours before harvesting onto filter mats (Perkin Elmer) using a TomTec Mach III
       (Hamden CT, USA) cell harvester. Counts per minute (cpm) for each well were determined by
       Meltilex™ (Perkin Elmer) scintillation counting on a 1450 Microbeta Wallac Trilux Liquid
640   Scintillation Counter (Perkin Elmer) in paralux, low background counting. Counts per minute for
       each antibody sample were normalised to the media only control.

       For ELISpot assays, ELISpot plates (Millipore, Watford, UK) were coated with 100µl/well IL-2
       capture antibody (R&D Systems, Abingdon, UK) in PBS. Plates were then washed twice in
645   PBS, incubated overnight in block buffer (1% BSA (Sigma) in PBS) and washed in AIM V®
       medium. On day 8, $1x10^5$ autologous $CD4^+$ T cells were added to $1x10^4$ antigen loaded DC
       (ratio of 10:1) in 96 well ELISpot plates. All preparations were tested in sextuplet cultures. For
       each donor PBMC, a negative control (AIM V® medium alone), no cells control and a PHA
       (10ug/ml) positive control were also included.
650
       After a further 7 day incubation period, ELISpot plates were developed by three sequential
       washes in dH_2O and PBS prior to the addition of 100µl filtered biotinylated detection antibody
       (R&D Systems, Abingdon, UK) in PBS/1% BSA. Following incubation at 37°C for 1.5 hour,
       plates were further washed three times in PBS and 100µl filtered streptavidin-AP (R&D
655   Systems) in PBS/1% BSA was added for 1 hour (incubation at room temperature).

Streptavidin-AP was discarded and plates were washed four times in PBS. BCIP/NBT (R&D Systems) was added to each well and incubated for 30 minutes at room temperature. Spot development was stopped by washing the wells and the backs of the wells three times with dH$_2$O. Dried plates were scanned on an Immunoscan™ Analyser and spots per well (spw) were

660   determined using Immunoscan™ Version 4 software.

For both proliferation and IL-2 ELISpot assays, results were expressed as a Stimulation Index (SI) defined as the ratio of cpm (proliferation assay) or spots (ELISpot assay) for the test antibody against a medium-only control using a threshold of SI equal to or greater than 2

665   (SI≥2.0) for positive T cell responses. The data showed that both Campath-1H and chimeric 2E8 antibody induced T cell responses in 10 or more of the 50 donor PBMCs tested (>=20%) whilst none of the humanised 2E8 antibodies induced T cell responses in more than 2 of 50 donors (<=4%) demonstrating the effectiveness of the humanisation process in removing T cell responses from the V regions.

670

EXAMPLE 10 – DIRECT CYTOXICITY ASSAY

Direct cytotoxic effects of anti-human CD52 antibodies were assessed using Annexin V / Propidium Iodide co-staining as markers of apoptosis and necrosis respectively. 1×10^5 REH cells were plated in the presence of 100 μg/ml anti-human CD52 test antibodies or an isotype matched

675   control antibody +/- 100μg/ml F(ab')^2 crosslinking antibody (Jackson ImmunoResearch, Cat no. 109-006-008) (600ul final vol). Cells were incubated for 72hrs before washing in PBS/2%BSA followed by co-staining with Annexin V/Propidium Iodide according to manufacturers recommended protocol (Invitrogen, Cat no. V13245). Scatterplots were generated using FACS analysis and divided into three regions for quantitation of live cells (unstained), apoptotic cells

680   (FL1, Annexin V positive) and necrotic cells (FL1, Annexin V positive & FL3, Propidium Iodide positive). As shown in Figure 13, the humanised 2E8 antibodies exhibited increased apoptosis and necrosis compared to Campath-1H of REH target cells, with % necrotic cells of >40% from the humanised antibodies compared to 19.9% for Campath-1H.

685

EXAMPLE 11 - TUMOUR ANIMAL MODEL

A tumour animal model was used for *in vivo* analysis of anti-human CD52 antibodies in inhibiting tumour growth. In the model, Raji human Burkitt lymphoma cells were transplanted

690 into SCID mice and the animals treated with anti-human CD52 antibodies. 7 week old female Fox Chase SCID Mice (Charles River, Morrisville, North Carolina, USA) were injected with 1 x 10^6 Raji cells (American Type Culture Collection, 0.2 mL cell suspension) via a bolus tail-vein (i.v.) injection. Anti-human CD52 test antibodies or an isotype matched control antibody were each administered intraperitoneally (i.p.) once daily on alternate days for seven doses, starting

695 three days after tumour cell injection. The dosing volume of 10 mL/kg (0.20 mL/20 g mouse) was scaled to the body weight of each animal, as determined twice weekly. The results shown in Figure 14 demonstrated an improved survival rate at both 1 and 10mg/kg doses by the lead VH3/VK4 anti-CD52 antibody (V region SEQ IDs: 22 and 28) compared to Campath 1H.

700

Sequences

>SEQ ID No. 1

2E8 Mouse VH DNA

705 GAGGTGCACCTGATGGAGTCTGGGGGAGGCTTAGTGCAGCCTGGAGGGTCCCTGAGACTCTCCTGTGC
AGCCTCTGGATTCACTTTCAGTAGGTATGGCATGTCTTGGGTTCGCCAGACTCCAGACAAGAGGCTGG
AGTTGGTCGCAATGATGAAAACTAAAGGTGGTAGGACCTATTATCCAGACAGTGTGAAGGGCCGATTC
ACCATTTCCAGAGACAATGCCAAGAACTCCCTGTACCTGCAAATGAGCAGTCTGAAGTCTGAGGACAC
AGCCATCTATTTCTGTGCAAGTGATGGTTACTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCA

710

>SEQ ID No. 2

2E8 Mouse VK DNA

GATGTTTTGATGACCCAGACTCCACTCACTTTGTCGGTAACCATTGGACAACCAGCCTCCATCTCTTGC

AAGTCAAGTCAGAGCCTCTTACATAGTGATGGAAAGACATATTTGAATTGGTTGTTACAGAGGCCAGG

715 CCAGTCTCCAAAGCGCCTAATCTATCTGGTGTCTAAACTGGACTCTGGAGTCCCTGACAGGTTCACTGG
CAGTGGATCAGGGACAGATTTCACACTGAAAATCAGCAGAGTGGAGGCTGAGGATTTGGGAATTTATT
ATTGCTGGCAAGGTACACATTTGTGGACGTTCGGTGGAGGCACCAAACTGGAAATCAAA

>SEQ ID No. 3

720 2E8 Mouse VH amino acid
EVHLMESGGGLVQPGGSLRLSCAASGFTFSRYGMSWVRQTPDKRLELVAMMKTKGGRTYYPDSVKGRFT
ISRDNAKNSLYLQMSSLKSEDTAIYFCASDGYYWGQGTTLTVSS

>SEQ ID No. 4

725 2E8 Mouse VK amino acid
DVLMTQTPLTLSVTIGQPASISCKSSQSLLHSDGKTYLNWLLQRPGQSPKRLIYLVSKLDSGVPDRFTGSGS
GTDFTLKISRVEAEDLGIYYCWQGTHLWTFGGGTKLEIK

>SEQ ID No. 5

730 2E8 VH CDR1 amino acid
RYGMS

>SEQ ID No. 6

2E8 VH CDR2 amino acid
MMKTKGGRTYYPDSVKG

735

>SEQ ID No. 7

2E8 VH CDR3 amino acid
DGYY

740 >SEQ ID No. 8

2E8 VK CDR1 amino acid
KSSQSLLHSDGKTYLN

>SEQ ID No. 9

745 2E8 VK CDR2 amino acid
LVSKLDS

>SEQ ID No. 10

2E8 VK CDR3 amino acid

750   WQGTHLWT

           >SEQ ID No. 11
           2E8 Humanised VH Variant 1 DNA
           GAGGTGCACCTGGTGGAATCCGGCGGAGGACTGGTGCAGCCTGGCGGCTCCCTGAGACTGTCTTGCGCCGCCTC
755   CGGCTTCACCTTCTCCAGATACGGCATGTCCTGGGTCCGACAGGCCCCTGGCAAGGGCCTGGAACTGGTGGCCA
           TGATGAAGACCAAGGGCGGCAGAACCTACTACCCCGACTCCGTGAAGGGCCGGTTCACCATCTCCCGGGACAAC
           GCCAAGAACTCCCTGTACCTGCAGATGTCCTCCCTGAAGGCCGAGGACACCGCCATCTACTTTTGCGCCTCCGA
           CGGCTACTACTGGGGCCAGGGCACCACCGTGACCGTGTCATCA

760   >SEQ ID No. 12
           2E8 Humanised VH Variant 2 DNA
           GAGGTGCACCTGGTGGAATCCGGCGGAGGACTGGTGCAGCCTGGCGGCTCCCTGAGACTGTCTTGCGCCGCCTC
           CGGCTTCACCTTCTCCAGATACGGCATGTCCTGGGTCCGACAGGCCCCTGGCAAGGGCCTGGAACTGGTGGCCA
           TGATGAAGACCAAGGGCGGCAGAACCTACTACCCCGACTCCGTGAAGGGCCGGTTCACCATCTCCCGGGACAAC
765   GCCAAGAACTCCCTGTACCTGCAGATGAACTCCCTGCGGGCCGAGGACACCGCCATCTACTTTTGCGCCTCCGA
           CGGCTACTACTGGGGCCAGGGCACCACCGTGACCGTGTCATCA

           >SEQ ID No. 13
           2E8 Humanised VH Variant 3 DNA
770   GAGGTGCACCTGGTGGAATCCGGCGGAGGACTGGTGCAGCCTGGCGGCTCCCTGAGACTGTCTTGCGCCGCCTC
           CGGCTTCACCTTCTCCAGATACGGCATGTCCTGGGTCCGACAGGCCCCTGGCAAGGGCCTGGAACTGGTGGCCA
           TGATGAAGACCAAGGGCGGCAGAACCTACTACCCCGACTCCGTGAAGGGCCGGTTCACCATCTCCCGGGACAAC
           GCCAAGAACTCCCTGTACCTGCAGATGAACTCCCTGCGGGCCGAGGACACCGCCATCTACTACTGCGCCTCCGA
           CGGCTACTACTGGGGCCAGGGCACCACCGTGACCGTGTCATCA
775
           >SEQ ID No. 14
           2E8 Humanised VH Variant 4 DNA
           GAGGTGCACCTGGTGGAATCCGGCGGAGGACTGGTGCAGCCTGGCGGCTCCCTGAGACTGTCTTGCGCCGCCTC
           CGGCTTCACCTTCTCCAGATACGGCATGTCCTGGGTCCGACAGGCCCCTGGCAAGGGCCTGGAACTGGTGGCCA
780   TGATGAAGACCAAGGGCGGCAGAACCTACTACCCCGACTCCGTGAAGGGCCGGTTCACCATCTCCCGGGACAAC
           GCCAAGAACTCCCTGTACCTGCAGATGAACTCCCTGCGGGCCGAGGACACCGCCGTGTACTACTGCGCCTCCGA
           CGGCTACTACTGGGGCCAGGGCACCACCGTGACCGTGTCATCA

           >SEQ ID No. 15
785   2E8 Humanised VH Variant 5 DNA
           GAGGTGCACCTGGTGGAATCCGGCGGAGGACTGGTGCAGCCTGGCGGCTCCCTGAGACTGTCTTGCGCCGCCTC
           CGGCTTCACCTTCTCCAGATACGGCATGTCCTGGGTCCGACAGGCCCCTGGCAAGGGACTGGAATGGGTGGCCA
           TGATGAAGACCAAGGGCGGCAGAACCTACTACCCCGACTCCGTGAAGGGCCGGTTCACCATCTCCCGGGACAAC
           GCCAAGAACTCCCTGTACCTGCAGATGAACTCCCTGCGGGCCGAGGACACCGCCATCTACTACTGCGCCTCCGA
790   CGGCTACTACTGGGGCCAGGGCACCACCGTGACCGTGTCATCA

           >SEQ ID No. 16
           2E8 Humanised VK Variant 1 DNA
           GACGTGCTGATGACCCAGACCCCCCTGACCCTGTCCGTGACCCTGGGCCAGCCTGCCTCCATCTCCTGCAAGTC
795   CTCCCAGTCCCTGCTGCACTCCGACGGCAAGACCTACCTGAACTGGCTGCAGCAGCGGCCTGGCCAGTCCCCCA
           AGCGGCTGATCTACCTGGTGTCCAAGCTGGACTCCGGCGTGCCCGACAGATTCACCGGCTCTGGCTCCGGCACC
           GACTTCACCCTGAAGATCTCCCGGGTGGAAGCCGAGGACGTGGGCATCTACTACTGCTGGCAGGGCACCCATCT
           GTGGACCTTCGGCGGAGGCACAAAGGTGGAAATCAAA

800   >SEQ ID No. 17

2E8 Humanised VK Variant 2 DNA
GACGTGCTGATGACCCAGACCCCCCTGACCCTGTCCGTGACCCTGGGCCAGCCTGCCTCCATCTCCTGCAAGTC
CTCCCAGTCCCTGCTGCACTCCGACGGCAAGACCTACCTGAACTGGCTGCAGCAGCGGCCTGGCCAGTCTCCTC
GGCGGCTGATCTACCTGGTGTCCAAGCTGGACTCCGGCGTGCCCGACAGATTCACCGGCTCTGGCTCCGGCACC
805   GACTTCACCCTGAAGATCTCCCGGGTGGAAGCCGAGGACGTGGGCATCTACTACTGCTGGCAGGGCACCCATCT
GTGGACCTTCGGCGGAGGCACAAAGGTGGAAATCAAA

>SEQ ID No. 18
2E8 Humanised VK Variant 3 DNA
810   GACGTGCTGATGACCCAGACCCCCCTGTCCCTGTCCGTGACCCTGGGCCAGCCTGCCTCCATCTCCTGCAAGTC
CTCCCAGTCCCTGCTGCACTCCGACGGCAAGACCTACCTGAACTGGCTGCAGCAGCGGCCTGGCCAGTCTCCTC
GGCGGCTGATCTACCTGGTGTCCAAGCTGGACTCCGGCGTGCCCGACAGATTCACCGGCTCTGGCTCCGGCACC
GACTTCACCCTGAAGATCTCCCGGGTGGAAGCCGAGGACGTGGGCATCTACTACTGCTGGCAGGGCACCCATCT
GTGGACCTTCGGCGGAGGCACAAAGGTGGAAATCAAA
815

>SEQ ID No. 19
2E8 Humanised VK Variant 4 DNA
GACGTGGTGATGACCCAGACCCCCCTGTCCCTGTCCGTGACCCTGGGCCAGCCTGCCTCCATCTCCTGCAAGTC
CTCCCAGTCCCTGCTGCACTCCGACGGCAAGACCTACCTGAACTGGCTGCAGCAGCGGCCTGGCCAGTCTCCTC
820   GGCGGCTGATCTACCTGGTGTCCAAGCTGGACTCCGGCGTGCCCGACAGATTCTCCGGCTCTGGCTCCGGCACC
GACTTCACCCTGAAGATCTCCCGGGTGGAAGCCGAGGACGTGGGCATCTACTACTGCTGGCAGGGCACCCATCT
GTGGACCTTCGGCGGAGGCACAAAGGTGGAAATCAAA

>SEQ ID No. 20
825   2E8 Humanised VH Variant 1 amino acid
EVHLVESGGGLVQPGGSLRLSCAASGFTFSRYGMSWVRQAPGKGLELVAMMKTKGGRTYYPDSVKGRFTISRDNAKNS
LYLQMSSLKAEDTAIYFCASDGYYWGQGTTVTVSS

>SEQ ID No. 21
830   2E8 Humanised VH Variant 2 amino acid
EVHLVESGGGLVQPGGSLRLSCAASGFTFSRYGMSWVRQAPGKGLELVAMMKTKGGRTYYPDSVKGRFTISRDNAKNS
LYLQMNSLRAEDTAIYFCASDGYYWGQGTTVTVSS

>SEQ ID No. 22
835   2E8 Humanised VH Variant 3 amino acid
EVHLVESGGGLVQPGGSLRLSCAASGFTFSRYGMSWVRQAPGKGLELVAMMKTKGGRTYYPDSVKGRFTISRDNAKNS
LYLQMNSLRAEDTAIYYCASDGYYWGQGTTVTVSS

>SEQ ID No. 23
840   2E8 Humanised VH Variant 4 amino acid
EVHLVESGGGLVQPGGSLRLSCAASGFTFSRYGMSWVRQAPGKGLELVAMMKTKGGRTYYPDSVKGRFTISRDNAKNS
LYLQMNSLRAEDTAVYYCASDGYYWGQGTTVTVSS

>SEQ ID No. 24
845   2E8 Humanised VH Variant 5 amino acid
EVHLVESGGGLVQPGGSLRLSCAASGFTFSRYGMSWVRQAPGKGLEWVAMMKTKGGRTYYPDSVKGRFTISRDNAKN
SLYLQMNSLRAEDTAIYYCASDGYYWGQGTTVTVSS

850 >SEQ ID No. 25
2E8 Humanised VK Variant 1 amino acid
DVLMTQTPLTLSVTLGQPASISCKSSQSLLHSDGKTYLNWLQQRPGQSPKRLIYLVSKLDSGVPDRFTGSGSGTDFTLKISR
VEAEDVGIYYCWQGTHLWTFGGGTKVEIK

855 >SEQ ID No. 26
2E8 Humanised VK Variant 2 amino acid
DVLMTQTPLTLSVTLGQPASISCKSSQSLLHSDGKTYLNWLQQRPGQSPRRLIYLVSKLDSGVPDRFTGSGSGTDFTLKISR
VEAEDVGIYYCWQGTHLWTFGGGTKVEIK

860 >SEQ ID No. 27
2E8 Humanised VK Variant 3 amino acid
DVLMTQTPLSLSVTLGQPASISCKSSQSLLHSDGKTYLNWLQQRPGQSPRRLIYLVSKLDSGVPDRFTGSGSGTDFTLKISR
VEAEDVGIYYCWQGTHLWTFGGGTKVEIK

865 >SEQ ID No. 28
2E8 Humanised VK Variant 4 amino acid
DVVMTQTPLSLSVTLGQPASISCKSSQSLLHSDGKTYLNWLQQRPGQSPRRLIYLVSKLDSGVPDRFSGSGSGTDFTLKIS
RVEAEDVGIYYCWQGTHLWTFGGGTKVEIK

870

CLAIMS:

1. An anti-CD52 antibody comprising at least one, two, three, four or five CDR sequences selected from the group consisting of

    (i)   CDRH1 comprising sequence RYGMS (SEQ ID NO:5)

    (ii)   CDRH2 comprising sequence MMKTKGGRTYYPDSVKG (SEQ ID NO: 6)

    (iii)   CDRH3 comprising sequence DGYY (SEQ ID NO:7)

    (iv)   CDRL1 comprising sequence KSSQSLLHSDGKTYLN (SEQ ID NO:8);

    (v)   CDRL2 comprising sequence LVSKLDS (SEQ ID NO:9) ; and

    (vi)   CDRL3 comprising sequence WQGTHLWT (SEQ ID NO:10).

2. An anti-CD52 antibody comprising one or more variable region sequences selected from the group consisting of SEQ ID NOS: 20-24 for the heavy chain variable region in combination with one or more sequences selected from the group consisting of SEQ ID NOS: 25-28 for the light chain variable region.

3. An anti-CD52 antibody comprising SEQ ID NO: 22 for the heavy chain variable region in combination with SEQ ID NO: 28 for the light chain variable region.

4. An anti-CD52 antibody of claims 1 to 3 which, when tested *in vitro* for induction of CD4+ helper T cell responses in 50 human blood samples with a distribution of HLA-DR allotypes from the human population, gives rise to <=4% of T cell responses.

5. The antibody of claims 1 to 4 wherein the variable region sequences are entirely derived from sequences in human antibody variable regions.

6. The antibody of claim 5 which is comprised of variable regions together with human constant regions.

7. The antibody of claim 6 where the human heavy chain constant region is either isotype IgG1, IgG2, IgG3 or IgG4, or a mutated IgG constant region, and the human light chain human constant region is isotype kappa.

8. The antibody of claim 6 where the human constant regions are IgG1 and kappa.

9. The antibody of claim 6 where the human constant regions are IgG4 and kappa.

10. The antibody of claims 1 to 5 where the antibody is a scFv or Fab.

11. The antibody of claims 1 to 10 which is a component of a multispecific protein which specifically binds to human CD52 and, additionally, binds or interacts with one or more other molecules.

12. A polynucleotide encoding an antibody of any of claims 1-10.

13. A vector comprising the polynucleotide of claim 12.

14. A vector of claim 13 wherein the vector is an expression vector.

15. A host cell comprising a vector of claims 13 or 14.

16. The host cell of claim 15 wherein the host cell is prokaryotic.

17. The host cell of claim 15, wherein the host cell is eukaryotic.

18. The host cell of claim 17 wherein the host cell is mammalian.

19. A composition comprising an anti-CD52 antibody of any of claims 1-11.

20. A composition comprising a polynucleotide of claim 12 or a vector of claims 13 or 14.

5   21. A method for treating a disease including CLL and other leukemias; autoimmune diseases including multiple sclerosis, rheumatoid arthritis, vasculitis, myositis, and diabetes; and organ transplant rejection and graft-vs-host disease comprising administering an effective amount of an antibody of any one of claims 1 to 11, a polynucleotide of any one of claims 12 to 14 or a composition of claim 19 or 20 to a
10   subject in need of such treatment.

22. The method of claim 21 which further comprises co-administering an effective amount of a chemotherapeutic agent.

15   23. The method of any of claims 21 or 22 which further comprises co-administering a pharmaceutical carrier.

24. A method for detecting the presence of human CD52 antigen in a sample using the antibody of any one of claims 1 to 11 for diagnosis of a human CD52-related disease.

20
25. A method for detecting the presence of human CD52 antigen in a sample using the polynucleotide or vectors of claims 12 to 14 for diagnosis of a human CD52-related disease.

25   26. An anti-CD52 antibody which, when tested *in vitro* for induction of CD4+ helper T cell responses in 50 human blood samples with a distribution of HLA-DR allotypes from the human population, gives rise to <=4% of T cell responses.