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(54) Title: IRTA2 ANTIBODIES AND METHODS OF USE

(57) Abstract: Antibodies that specifically bind the extracellular domain of IRTA2 are disclosed herein. In one embodiment, these antibodies do not specifically bind IRTA1, IRTA3., IRTA4, or IRTA5. In one example, the antibodies are humanized antibodies. The antibodies can be conjugated to effector molecules, including detectable labels, radionucleotides, toxins and chemotherapeutic agents. The antibodies that specifically bind IRTA2 are of use to detect B cell malignancies, such as hairy cell leukemia and non-Hodgkin's lymphoma. These antibodies that specifically bind IRTA2 are also of use to treat B cell malignancies that express IRTA2, such as hairy cell leukemia and non-Hodgkin's lymphoma.



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IRTA2 ANTIBODIES AND METHODS OF USE**PRIORITY CLAIM**

This claims the benefit of U.S. Provisional Application No. 60/615,406, filed
5 September 30, 2004, which is incorporated by reference herein in its entirety.

FIELD

This relates to the field of immunotherapy, specifically to antibodies that bind
immunoglobulin superfamily receptor translocation associated 2 (IRTA2) and their use for
10 the detection and treatment of B cell malignancies.

BACKGROUND

Abnormalities of chromosome 1q21 are common in B cell malignancies, including
B cell lymphoma and myeloma, but the genes targeted by these aberrations are largely
15 unknown. Chromosomal abnormalities involving band 1q21-q23 are among the most
frequent genetic lesions in both B cell non-Hodgkins lymphoma and multiple myeloma.
Among non-Hodgkin's lymphoma subtypes, translocation breakpoints at 1q21-q23,
including translocations and duplications, have been reported, often as the single
chromosomal abnormality in follicular and diffuse large B cell lymphoma (DLCL) in
20 marginal-zone B cell lymphoma and in Burkitt lymphoma.

By cloning the breakpoints of a t(1:14)(q21:q32) chromosomal translocation in a
myeloma cell line, two genes were identified, termed immunoglobulin superfamily receptor
translocation associated (IRTA)1 and IRTA2. IRTA2 is identical to sequences identified as
BXMAS1 (Nakayama et al., *Biochim. Biophys. Res. Commun.* 285:830-7, 2001) and FcRH5
25 (Davis et al., *Proc. Natl. Acad. Sci. USA* 98:9772-7, 2001). Both IRTA1 and IRTA2 are
members of a family of related genes, the IRTA.

IRTA2 is a cell surface receptor with homologies to the Fc receptor families. It is
normally expressed in mature B cells, and has a different distribution in peripheral lymphoid
organs than IRTA1: IRTA1 is expressed in marginal zone B cells, while IRTA2 is also
30 expressed in germinal center centrocytes and in immunoblasts. IRTA2 expression is
deregulated in multiple myeloma and Burkitt lymphoma cell lines with 1q21 abnormalities
(see Miller et al., *Blood* 99:2662-2669, 2002). The high frequency of involvement of 1q21
structural rearrangements in B cell malignancies suggests that IRTA1 and IRTA2 are critical
to the pathogenesis of these diseases (see published PCT Application No. WO 01/38490).

Immunotherapy is a potent new weapon against cancer. Immunotherapy involves evoking an immune response against cancer cells based on their production of target antigens. Immunotherapy based on cell-mediated immune responses involves generating a cell-mediated response to cells that produce particular antigenic determinants, while
5 immunotherapy based on humoral immune responses involves generating specific antibodies to cells that produce particular antigenic determinants. Recombinant toxins are chimeric proteins in which a cell targeting moiety is fused to a toxin (Pastan et al., *Science*, 254:1173-1177, 1991). If the cell targeting moiety is the Fv portion of an antibody, the molecule is termed a recombinant immunotoxin (Chaudhary et al. *Nature*, 339:394-397,
10 1989). The toxin moiety is genetically altered so that it cannot bind to the toxin receptor present on most normal cells. Recombinant immunotoxins selectively kill cells which are recognized by the antigen binding domain. There remains a need for recombinant toxins which can be used to treat cancer, including B cell malignancies.

15 SUMMARY

Monoclonal antibodies that specifically bind the extracellular domain of IRTA2 are disclosed herein. In one embodiment, these antibodies do not specifically bind IRTA1, IRTA3, IRTA4, or IRTA5. Antibodies that bind IRTA2 can be conjugated to effector
20 molecules, including detectable labels, radionucleotides, toxins and chemotherapeutic agents. These antibodies are of use to detect B cell malignancies, such as hairy cell leukemia and non-Hodgkin's lymphoma. These antibodies are also of use to treat B cell malignancies that express IRTA2, such as hairy cell leukemia and non-Hodgkin's lymphoma.

25 The foregoing and other features and advantages will become more apparent from the following detailed description of several embodiments, which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE FIGURES

30 **FIG. 1A** is a schematic representation of the recombinant IRTA proteins and the plasmids construction. A pair of expression plasmids was generated for each IRTA: one encodes full-length protein under cytomegalovirus (CMV)-promoter of pcDNA3, the other encodes the extracellular domain with human IgG1 Fc as a fusion protein in the same vector. IRTAs possess three to nine immunoglobulin (Ig)-like domains at the extracellular
35 domains shown by circles. They can be classified into four groups based on their homology

(Davis et al., *Proc Natl Acad Sci USA* 2001;98:9772-7; Davis et al., *Immunol Rev* 2002;190:123-36), which are shown in different patterns of shading of the circles. To construct the plasmids encoding full-length IRTAs, DNA fragments shown as parenthesis with the primer numbers (primers are listed in Table 2) were PCR-amplified from human spleen cDNA or Daudi cells cDNA. They were connected in tandem into pcDNA3 vector using restriction enzymes shown. To make plasmids for Fc-fusion proteins, DNA fragments that code for extracellular domains including their own leader sequences were amplified and inserted into pcDNA3 vector with NotI-XbaI cDNA fragments that code for human Fc. **FIG. 1B** is a digital image of an SDS-PAGE of IRTA-Fc fusion proteins. Two μ gs of each IRTA-Fc fusion protein was separated on a 4-20% SDS-PAGE gel under reducing conditions. Proteins were visualized with Coomassie-blue staining. Bands of Fc-fusion proteins were seen with expected sizes.

FIGS. 2A-B are a bar graph and a set of plots showing the cross-reactivity of the anti-IRTA2 MAbs to other IRTA family members. **FIG. 2A** is a bar graph of the results from an ELISA of anti-IRTA2 MAbs to various Fc-fusion proteins. Each Fc-fusion protein indicated on the top of the column was captured on ELISA plates using secondary antibody (goat anti-human IgG). After washing, 100 ng/50 μ l/well of MAbs were added, followed by HRP-labeled goat anti-mouse IgG and TMBZ substrate. The signals in ELISA were normalized for the amounts of each Fc-fusion protein on the plates using HRP-goat anti-human Fc. The controls are MAbs 9E10 (anti-myc tag, IgG1), BerH2 (anti-CD30, IgG1), T408 (anti-CD30, IgG2a), T420 (anti-CD30, IgG2b) and HRP-goat anti-human Fc (1/2400 dilution). **FIG. 2B** is a set of plots of a FACS analysis of selected anti-IRTA2 MAbs using 293T cells transfected with plasmids coding IRTAs or CD30. Two days after transfection, the cells were harvested and incubated with 200 ng/100 μ l of each MAb, followed by PE-conjugated goat anti-mouse IgG F(ab')₂. Each panel shows the histogram versus log fluorescence (solid line) with the negative control (shed peak) without 1st antibodies. In the positive control panels, the MAbs cross-reactive to other IRTAs were used. They are F26 (anti-IRTA1 and anti-IRTA2, IgG2b), F109 (anti-IRTA3, IgG2b), F47 (anti-IRTA4, IgG2a), and F69 (anti-IRTA5, IgG2a). BerH2 (anti-CD30, IgG1) was also used. "+" indicates more than 1.5-fold than that of the control; "++" indicates more than 10-fold mean fluorescence intensity than that of the negative control.

FIGS. 3A-B are digital images illustrating the use of the anti-IRTA2 MAbs for immunofluorescence and Western blotting. **FIG 3A** is a digital image showing

immunofluorescence by the use of anti-IRTA2 MAb F56. 293T cells that were transfected with IRTA2 or CD30 plasmids were detached, combined in a 1:1 ratio, and seeded into chamber slides. The next day, the cells were fixed with 3.7% formaldehyde and incubated with a mixture of anti-IRTA2 MAb F56 (IgG1) and anti-CD30 MAb Ki-1 (IgG3) at 10 µg/ml. As the secondary reagents, a mixture of Alexa 488-conjugated goat-anti-mouse IgG1 and Alexa 555-conjugated goat-anti-mouse IgG3 was used (1:1000 dilution). Nuclei in the field were stained with DAPI (1 µg/ml). CD30 and F56 (anti-IRTA2) staining were observed with 555 nm filter and 488 nm, respectively. DAPI nuclei staining was detected by 460 nm filter. The merged image was created by a computer.

10 **FIG. 3B** is a digital image of a Western blot analysis of anti-IRTA2 MAbs. Twenty ng of IRTA2-Fc and CD30-Fc fusion proteins were blotted on each strip after separation on a 4-20% SDS-PAGE gel under reducing conditions. The proteins were probed with 10 µg/ml of each MAb or anti-CD30 MAb BerH-2, followed by alkaline phosphatase anti-mouse IgG. In the left blot, the Fc-fusion proteins were visualized using alkaline phosphatase anti-human Fc. Only the MAbs that reacted to the antigen in the blot (F20 and F25) are shown.

FIGS. 4A-D are graphs, digital images, and a table showing the expression of IRTA2 on cell lines. **FIG. 4A** are plots of the results of FACS analysis of 19 human cell lines derived from hematological malignancies. Each cell was stained with anti-IRTA2 MAb F56 or MAb F119, or control mouse IgG1 or PE-labeled CD19. Some cell lines that are negative for CD19, were stained with other CD markers as indicated in each panel. Each panel shows the histogram versus log fluorescence (solid line) with the negative control (shaded peak) without primary antibodies. Eight out of 19 lines were stained with anti-IRTA2 MAbs, six of which were derived from B-NHL. **FIG. 4B** is a digital image of a RT-PCR analysis of the cell lines for transmembrane type IRTA2 mRNA expression. Primers in separate exons specific for the transmembrane type of IRTA2 (IRTA2c) and primers for β-actin as the control were used for 26 cycles of PCR reaction with cDNAs of each cell. The cDNAs from the cell lines positive for IRTA2 protein expression in FACS resulted in the expected 432-bp size of IRTA2 and cell lines negative in the FACS showed no band. The actin primers gave the same intensity of PCR product from cDNAs of all cell lines. **FIG. 4C** is a digital image of a Western blot analysis of selected cell lines. Total cell lysates of the indicated cell lines or purified IRTA2-Fc protein were separated in 4-20% gradient SDS-PAGE gel under reducing condition. The proteins were transferred to a PVDF membrane and probed with F25 anti-

IRTA2 MAb. Cell lines positive in the FACS showed the same 150-kDa size of bands as the recombinant IRTA2 expressed in the transfected cells. In contrast, cell lines negative in FACS showed no such bands. **FIG. 4D** is a table summarizing the IRTA2 expression verified by FACS, RT-PCR, and Western blotting. The IRTA2 expression detected by

5 each method was well correlated with the other methods.

FIG. 5A-B are plots showing the expression of IRTA2 on HCL cells. PBMCs from two HCL patients were separated by a density gradient using Ficoll-Paque and 3-color staining was performed with CD22-FITC/IRTA2-PE/CD20-PerCPCy5.5 mixture or

10 CD103-FITC/IRTA2-PE/CD20-PerCPCy5.5 mixture. Fluorescence associated with live cells was analyzed by FACS Calibure. **FIG. 5A** is a set of plots showing the IRTA2-positive cells detected in the HCL patients were positive for CD20, CD22, and CD103, which are the markers of HCL cells. As shown in the histograms, 27% and 39% of PBMCs of patient #1 and patient #2 expressed IRTA2, respectively, when compared to

15 background staining (shaded peaks). Most of IRTA⁻ cells (red region) were CD22⁻/CD20⁻ or CD103⁻/CD20⁻ (red dot-blot panels), whereas most IRTA⁺ cells (blue region) expressed both CD22 and CD20 or CD103 and CD20 (blue dot-blot panels). **FIG. 5B** is a set of plots showing CD20⁺/CD22⁺ or CD20⁺/CD103⁺ cells in PBMCs from the patient (HCL cells) expressed IRTA2. The cells stained either CD22-FITC/IRTA2-PE/CD20-

20 PerCPCy5.5 or CD103-FITC/IRTA2-PE/CD20-PerCPCy5.5 were gated by CD20⁺/CD22⁺ or CD20⁺/CD103⁺, respectively (circles). The gated cells were shown in black lines in the histograms with background staining (shaded peaks). The histograms showed significant IRTA2 expression on CD20⁺/CD22⁺ or CD20⁺/CD103⁺ cells. The IRTA2 staining is inhibited by the presence of IRTA2-Fc antigen (red peak) but not by

25 CD30-Fc (blue peak), indicating that IRTA2 staining is specific.

FIG. 6 is the amino acid sequence of the heavy chain of the variable regions of F119 (SEQ ID NO: 1), F56 (SEQ ID NO: 3) and F25 (SEQ ID NO: 5). The positions of the complementarity determining regions (CDRs) and the framework regions (FRs) are

30 indicated.

FIG. 7 is the amino acid sequence of the light chain of the variable regions of F119 (SEQ ID NO: 2), F56 (SEQ ID NO: 4) and F25 (SEQ ID NO: 6). The positions of the complementarity determining regions (CDRs) and the framework regions (FRs) are

35 indicated.

FIG. 8 is a diagram of the epitope locations of anti-IRTA2 monoclonal antibodies. The circles show extracellular domains 1-9 for IRTA2c, and corresponding domains for IRTA2a and IRTA2b. Antibodies that bind the indicated domain are listed.

5

FIG. 9A and B are graphs showing calibration curves using IRTA-Fc standard for ELISA #1 (Fig. 9A) and ELISA #2 (Fig. 9B).

FIG. 10 is a graph showing correlation of ELISA #1 and ELISA #2.

10

FIG. 11 is a graph showing the amount of soluble IRTA2 (ng/ml) in patients' sera as tested in the two ELISAs.

SEQUENCE LISTING

15

The nucleic and amino acid sequences listed in the accompanying sequence listing are shown using standard letter abbreviations for nucleotide bases, and one letter code for amino acids, as defined in 37 C.F.R. 1.822. Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included by any reference to the displayed strand.

20

SEQ ID NO: 1 is the amino acid sequence of the V_H of F119.

SEQ ID NO: 2 is the amino acid sequence of the V_L of F119.

SEQ ID NO: 3 is the amino acid sequence of the V_H of F56.

SEQ ID NO: 4 is the amino acid sequence of the V_L of F56.

SEQ ID NO: 5 is the amino acid sequence of the V_H of F25.

25

SEQ ID NO: 6 is the amino acid sequence of the V_L of F25.

SEQ ID NO: 7 is the nucleic acid sequence of a polynucleotide encoding the V_H of F119.

SEQ ID NO: 8 is the nucleic acid sequence of a polynucleotide encoding the V_L of F119.

30

SEQ ID NO: 9 is the nucleic acid sequence of a polynucleotide encoding the V_H of F56.

SEQ ID NO: 10 is the nucleic acid sequence of a polynucleotide encoding the V_L of F56.

SEQ ID NO: 11 is the nucleic acid sequence of a polynucleotide encoding the V_H of

35 F25.

SEQ ID NO: 12 is the nucleic acid sequence of a polynucleotide encoding the V_L of F25.

SEQ ID NO: 13 is the amino acid sequence of an IRTA2 polypeptide.

SEQ ID NO: 14 is the amino acid sequence of an IRTA1 polypeptide.

5 SEQ ID NO: 15 is the amino acid sequence of an IRTA3 polypeptide.

SEQ ID NO: 16 is the amino acid sequence of an IRTA4 polypeptide.

SEQ ID NO: 17 is the amino acid sequence of an IRTA5 polypeptide.

SEQ ID NO: 18 is the amino acid sequence of a *Pseudomonas* exotoxin (PE).

10 SEQ ID NOs: 19-20 are the amino acid sequences of *Pseudomonas* exotoxin (PE) variants.

SEQ ID NOs: 21-28 are the amino acid sequences of exemplary human framework regions.

SEQ ID NOs: 29-52 are the nucleic acid sequences of primers.

15 DETAILED DESCRIPTION

I. Abbreviations

ALL: acute lymphocytic leukemia

AML: acute myelogenous leukemia

20 **ATCC:** American Type Culture Collection

ATL: adult T cell leukemia

CDR: complementarity determining region

CLL: chronic lymphocytic leukemia

CML: chronic myelogenous leukemia

25 **dsFv:** disulfide stabilized fragment of a variable region

DLBCL: diffuse large B-cell lymphoma

DT: diphtheria toxin

ELISA: enzyme-linked immunosorbent assay

EM: effector molecule

30 **FACS:** fluorescence activated cell sorter

HCL: hairy cell leukemia

HD: Hodgkin's disease

HIV: human immunodeficiency virus

HRP: horseradish peroxidase

35 **IRTA:** immunoglobulin superfamily receptor translocation associated

IT: immunotoxin

kDa: kilodaltons

LCDR: light chain complementarity determining region

HCDR: heavy chain complementarity determining region

5 **Ig:** immunoglobulin

IRTA2: immunoglobulin superfamily receptor translocation associated 2

MAb: monoclonal antibody

MCL: mantle cell lymphoma

PE: *Pseudomonas* exotoxin

10 **scFv:** single chain fragment of a variable region

TMB: trimethoxybenzene

V_H: variable region of a heavy chain

V_L: variable region of a light chain

15 II. Terms

Unless otherwise noted, technical terms are used according to conventional usage.

Definitions of common terms in molecular biology may be found in Benjamin Lewin, *Genes V*, published by Oxford University Press, 1994 (ISBN 0-19-854287-9); Kendrew *et al.* (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8).

In order to facilitate review of the various embodiments of this disclosure, the following explanations of specific terms are provided:

25 **Administration:** The introduction of a composition into a subject by a chosen route. For example, if the chosen route is intravenous, the composition is administered by introducing the composition into a vein of the subject.

Amplification: Of a nucleic acid molecule (e.g., a DNA or RNA molecule) refers to use of a technique that increases the number of copies of a nucleic acid molecule in a specimen. An example of amplification is the polymerase chain reaction, in which a biological sample collected from a subject is contacted with a pair of oligonucleotide primers, under conditions that allow for the hybridization of the primers to a nucleic acid template in the sample. The primers are extended under suitable conditions, dissociated from the template, and then re-annealed, extended, and dissociated to amplify the number of

35 copies of the nucleic acid. The product of amplification may be characterized by

electrophoresis, restriction endonuclease cleavage patterns, oligonucleotide hybridization or ligation, and/or nucleic acid sequencing using standard techniques. Other examples of amplification include strand displacement amplification, as disclosed in U.S. Patent No. 5,744,311; transcription-free isothermal amplification, as disclosed in U.S. Patent No. 5,744,311; repair chain reaction amplification, as disclosed in WO 90/01069; ligase chain reaction amplification, as disclosed in EP-A-320 308; gap filling ligase chain reaction amplification, as disclosed in U.S. Patent No. 5,427,930; and NASBA™ RNA transcription-free amplification, as disclosed in U.S. Patent No. 6,025,134.

Animal: Living multi-cellular vertebrate organisms, a category that includes, for example, mammals and birds. The term mammal includes both human and non-human mammals. Similarly, the term “subject” includes both human and veterinary subjects.

Antibody: A polypeptide ligand comprising at least a light chain or heavy chain immunoglobulin variable region which specifically recognizes and binds an epitope of an antigen, such as IRTA2 or a fragment thereof. This includes intact immunoglobulins and the variants and portions of them well known in the art, such as Fab' fragments, F(ab)₂ fragments, single chain Fv proteins (“scFv”), and disulfide stabilized Fv proteins (“dsFv”). A scFv protein is a fusion protein in which a light chain variable region of an immunoglobulin and a heavy chain variable region of an immunoglobulin are bound by a linker, while in dsFvs, the chains have been mutated to introduce a disulfide bond to stabilize the association of the chains. The term also includes genetically engineered forms such as chimeric antibodies (e.g., humanized murine antibodies), heteroconjugate antibodies (e.g., bispecific antibodies). See also, *Pierce Catalog and Handbook*, 1994-1995 (Pierce Chemical Co., Rockford, IL); Kuby, J., *Immunology*, 3rd Ed., W.H. Freeman & Co., New York, 1997.

Typically, a naturally occurring immunoglobulin has heavy (H) chains and light (L) chains interconnected by disulfide bonds. There are two types of light chain, lambda (λ) and kappa (κ). There are five main heavy chain classes (or isotypes) which determine the functional activity of an antibody molecule: IgM, IgD, IgG, IgA and IgE.

Each heavy and light chain contains a constant region and a variable region, (the regions are also known as “domains”). In combination, the heavy and the light chain variable regions specifically bind the antigen. Light and heavy chain variable regions contain a “framework” region interrupted by three hypervariable regions, also called “complementarity-determining regions” or “CDRs”. The extent of the framework region and CDRs have been defined (see, Kabat et al., *Sequences of Proteins of Immunological Interest*, U.S. Department of Health and Human Services, 1991, which is hereby

incorporated by reference). The Kabat database is now maintained online. The sequences of the framework regions of different light or heavy chains are relatively conserved within a species. The framework region of an antibody, that is the combined framework regions of the constituent light and heavy chains, serves to position and align the CDRs in three-dimensional space.

The CDRs are primarily responsible for binding to an epitope of an antigen. The CDRs of each chain are typically referred to as CDR1, CDR2, and CDR3, numbered sequentially starting from the N-terminus, and are also typically identified by the chain in which the particular CDR is located. Thus, a V_H CDR3 is located in the variable domain of the heavy chain of the antibody in which it is found, whereas a V_L CDR1 is the CDR1 from the variable domain of the light chain of the antibody in which it is found.

References to " V_H " or "VH" refer to the variable region of an immunoglobulin heavy chain, including that of an Fv, scFv, dsFv or Fab. References to " V_L " or "VL" refer to the variable region of an immunoglobulin light chain, including that of an Fv, scFv, dsFv or Fab.

A "monoclonal antibody" is an antibody produced by a single clone of B-lymphocytes or by a cell into which the light and heavy chain genes of a single antibody have been transfected. Monoclonal antibodies are produced by methods known to those of skill in the art, for instance by making hybrid antibody-forming cells from a fusion of myeloma cells with immune spleen cells. These fused cells and their progeny are termed "hybridomas." Monoclonal antibodies include humanized monoclonal antibodies.

A "humanized" immunoglobulin is an immunoglobulin including a human framework region and one or more CDRs from a non-human (such as a mouse, rat, or synthetic) immunoglobulin. The non-human immunoglobulin providing the CDRs is termed a "donor," and the human immunoglobulin providing the framework is termed an "acceptor." In one embodiment, all the CDRs are from the donor immunoglobulin in a humanized immunoglobulin. Constant regions need not be present, but if they are, they must be substantially identical to human immunoglobulin constant regions, i.e., at least about 85-90%, such as about 95% or more identical. Hence, all parts of a humanized immunoglobulin, except possibly the CDRs, are substantially identical to corresponding parts of natural human immunoglobulin sequences. A "humanized antibody" is an antibody comprising a humanized light chain and a humanized heavy chain immunoglobulin. A humanized antibody binds to the same antigen as the donor antibody that provides the CDRs. The acceptor framework of a humanized immunoglobulin or antibody may have a limited number of substitutions by amino acids taken from the donor framework.

Humanized or other monoclonal antibodies can have additional conservative amino acid substitutions which have substantially no effect on antigen binding or other immunoglobulin functions. Humanized immunoglobulins can be constructed by means of genetic engineering (e.g., see U.S. Patent No. 5,585,089).

5 **Binding affinity:** Affinity of an antibody for an antigen. In one embodiment, affinity is calculated by a modification of the Scatchard method described by Frankel et al., *Mol. Immunol.*, 16:101-106, 1979. In another embodiment, binding affinity is measured by an antigen/antibody dissociation rate. In yet another embodiment, a high binding affinity is measured by a competition radioimmunoassay. In several examples, a high binding affinity
10 is at least about 1×10^{-8} M. In other embodiments, a high binding affinity is at least about 1.5×10^{-8} , at least about 2.0×10^{-8} , at least about 2.5×10^{-8} , at least about 3.0×10^{-8} , at least about 3.5×10^{-8} , at least about 4.0×10^{-8} , at least about 4.5×10^{-8} , or at least about 5.0×10^{-8} M.

Chemotherapeutic agents: Any chemical agent with therapeutic usefulness in the
15 treatment of diseases characterized by abnormal cell growth. Such diseases include tumors, neoplasms, and cancer as well as diseases characterized by hyperplastic growth such as psoriasis. In one embodiment, a chemotherapeutic agent is an agent of use in treating a lymphoma, leukemia, or another tumor. In one embodiment, a chemotherapeutic agent is a radioactive compound. One of skill in the art can readily identify a chemotherapeutic agent
20 of use (e.g. see Slapak and Kufe, *Principles of Cancer Therapy*, Chapter 86 in Harrison's Principles of Internal Medicine, 14th edition; Perry et al., *Chemotherapy*, Ch. 17 in Abeloff, Clinical Oncology 2nd ed., © 2000 Churchill Livingstone, Inc; Baltzer, L., Berkery, R. (eds): *Oncology Pocket Guide to Chemotherapy*, 2nd ed. St. Louis, Mosby-Year Book, 1995; Fischer, D.S., Knobf, M.F., Durivage, H.J. (eds): *The Cancer Chemotherapy Handbook*, 4th
25 ed. St. Louis, Mosby-Year Book, 1993). Combination chemotherapy is the administration of more than one agent to treat cancer. One example is the administration of an antibody that binds IRTA2 or a fragment thereof used in combination with a radioactive or chemical compound.

Chimeric antibody: An antibody which includes sequences derived from two
30 different antibodies, which typically are of different species. Most typically, chimeric antibodies include human and murine antibody domains, generally human constant regions and murine variable regions, murine CDRs and/or murine SDRs.

Conservative variants: "Conservative" amino acid substitutions are those substitutions that do not substantially affect or decrease an activity or antigenicity of IRTA2.
35 For example, an IRTA2 polypeptide can include at most about 1, at most about 2, at most

about 5, and most about 10, or at most about 15 conservative substitutions and specifically bind an antibody that binds the original IRTA polypeptide. The term conservative variation also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid, provided that antibodies raised to the substituted polypeptide also immunoreact with the unsubstituted polypeptide. Non-conservative substitutions are those that reduce an activity or antigenicity.

Complementarity Determining Region (CDR): Amino acid sequences which together define the binding affinity and specificity of the natural Fv region of a native Ig binding site. The light and heavy chains of an Ig each have three CDRs, designated L-CDR1, L-CDR2, L-CDR3 and H-CDR1, H-CDR2, H-CDR3, respectively. By definition, the CDRs of the light chain are bounded by the residues at positions 24 and 34 (L-CDR1), 50 and 56 (L-CDR2), 89 and 97 (L-CDR3); the CDRs of the heavy chain are bounded by the residues at positions 31 and 35b (H-CDR1), 50 and 65 (H-CDR2), 95 and 102 (H-CDR3), using the numbering convention delineated by Kabat et al., (1991) *Sequences of Proteins of Immunological Interest*, 5th Edition, U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Bethesda, MD (NIH Publication No. 91-3242).

Contacting: Placement in direct physical association. Includes both in solid and liquid form.

Cytotoxicity: The toxicity of a molecule, such as an immunotoxin, to the cells intended to be targeted, as opposed to the cells of the rest of an organism. In one embodiment, in contrast, the term "toxicity" refers to toxicity of an immunotoxin to cells other than those that are the cells intended to be targeted by the targeting moiety of the immunotoxin, and the term "animal toxicity" refers to toxicity of the immunotoxin to an animal by toxicity of the immunotoxin to cells other than those intended to be targeted by the immunotoxin.

Degenerate variant: A polynucleotide encoding an IRTA2 polypeptide that includes a sequence that is degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included as long as the amino acid sequence of the IRTA2 polypeptide encoded by the nucleotide sequence is unchanged.

Effector molecule: The portion of a chimeric molecule that is intended to have a desired effect on a cell to which the chimeric molecule is targeted. Effector molecule is also known as an effector moiety (EM), therapeutic agent, or diagnostic agent, or similar terms.

Therapeutic agents include such compounds as nucleic acids, proteins, peptides, amino acids or derivatives, glycoproteins, radioisotopes, lipids, carbohydrates, or recombinant viruses. Nucleic acid therapeutic and diagnostic moieties include antisense nucleic acids, derivatized oligonucleotides for covalent cross-linking with single or duplex
5 DNA, and triplex forming oligonucleotides. Alternatively, the molecule linked to a targeting moiety, such as an anti-IRTA2 antibody, may be an encapsulation system, such as a liposome or micelle that contains a therapeutic composition such as a drug, a nucleic acid (e.g., an antisense nucleic acid), or another therapeutic moiety that can be shielded from direct exposure to the circulatory system. Means of preparing liposomes attached to
10 antibodies are well known to those of skill in the art. See, for example, U.S. Patent No. 4,957,735; and Connor et al., *Pharm. Ther.* 28:341-365, 1985. Diagnostic agents or moieties include radioisotopes and other detectable labels. Detectable labels useful for such purposes are also well known in the art, and include radioactive isotopes such as ^{32}P , ^{125}I , and ^{131}I , fluorophores, chemiluminescent agents, and enzymes.

15 **Epitope:** An antigenic determinant. These are particular chemical groups or peptide sequences on a molecule that are antigenic, i.e. that elicit a specific immune response. An antibody specifically binds a particular antigenic epitope on a polypeptide.

Expressed: Translation of a nucleic acid into a protein. Proteins may be expressed and remain intracellular, become a component of the cell surface membrane, or be secreted
20 into the extracellular matrix or medium

Expression Control Sequences: Nucleic acid sequences that regulate the expression of a heterologous nucleic acid sequence to which it is operatively linked. Expression control sequences are operatively linked to a nucleic acid sequence when the expression control sequences control and regulate the transcription and, as appropriate,
25 translation of the nucleic acid sequence. Thus expression control sequences can include appropriate promoters, enhancers, transcription terminators, a start codon (i.e., ATG) in front of a protein-encoding gene, splicing signal for introns, maintenance of the correct reading frame of that gene to permit proper translation of mRNA, and stop codons. The term "control sequences" is intended to include, at a minimum, components whose presence
30 can influence expression, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences. Expression control sequences can include a promoter.

A promoter is a minimal sequence sufficient to direct transcription. Also included are those promoter elements which are sufficient to render promoter-dependent gene
35 expression controllable for cell-type specific, tissue-specific, or inducible by external signals

or agents; such elements may be located in the 5' or 3' regions of the gene. Both constitutive and inducible promoters are included (see e.g., Bitter et al., *Methods in Enzymology* 153:516-544, 1987). For example, when cloning in bacterial systems, inducible promoters such as pL of bacteriophage lambda, plac, ptrp, ptac (ptrp-lac hybrid promoter) and the like
5 may be used. In one embodiment, when cloning in mammalian cell systems, promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the retrovirus long terminal repeat; the adenovirus late promoter; the vaccinia virus 7.5K promoter) can be used. Promoters produced by recombinant DNA or synthetic techniques may also be used to provide for transcription of the nucleic acid
10 sequences.

Framework Region: Amino acid sequences interposed between CDRs. Includes variable light and variable heavy framework regions. The framework regions serve to hold the CDRs in an appropriate orientation for antigen binding.

Hairy Cell Leukemia: A form of chronic leukemia marked by splenomegaly and
15 by an abundance of abnormal large mononuclear cells covered by hair-like villi in the bone marrow, spleen, liver, and peripheral blood. Hairy cell leukemia is also called leukemic reticuloendotheliosis.

Host cells: Cells in which a vector can be propagated and its DNA expressed. The cell may be prokaryotic or eukaryotic. The term also includes any progeny of the subject
20 host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. However, such progeny are included when the term "host cell" is used.

Immune response: A response of a cell of the immune system, such as a B cell, T cell, or monocyte, to a stimulus. In one embodiment, the response is specific for a particular
25 antigen (an "antigen-specific response"). In one embodiment, an immune response is a T cell response, such as a CD4+ response or a CD8+ response. In another embodiment, the response is a B cell response, and results in the production of specific antibodies.

Immunoconjugate: A covalent linkage of an effector molecule to an antibody. The effector molecule can be a detectable label or an immunotoxin. Specific, non-limiting
30 examples of toxins include, but are not limited to, abrin, ricin, *Pseudomonas* exotoxin (PE, such as PE35, PE37, PE38, and PE40), diphtheria toxin (DT), botulinum toxin, or modified toxins thereof, or other toxic agents that directly or indirectly inhibit cell growth or kill cells. For example, PE and DT are highly toxic compounds that typically bring about death through liver toxicity. PE and DT, however, can be modified into a form for use as an
35 immunotoxin by removing the native targeting component of the toxin (e.g., domain Ia of

PE and the B chain of DT) and replacing it with a different targeting moiety, such as an antibody. A “chimeric molecule” is a targeting moiety, such as a ligand or an antibody, conjugated (coupled) to an effector molecule. The term “conjugated” or “linked” refers to making two polypeptides into one contiguous polypeptide molecule. In one embodiment, an antibody is joined to an effector molecule (EM). In another embodiment, an antibody joined to an effector molecule is further joined to a lipid or other molecule to a protein or peptide to increase its half-life in the body. The linkage can be either by chemical or recombinant means. In one embodiment, the linkage is chemical, wherein a reaction between the antibody moiety and the effector molecule has produced a covalent bond formed between the two molecules to form one molecule. A peptide linker (short peptide sequence) can optionally be included between the antibody and the effector molecule. Because immunoconjugates were originally prepared from two molecules with separate functionalities, such as an antibody and an effector molecule, they are also sometimes referred to as “chimeric molecules.” The term “chimeric molecule,” as used herein, therefore refers to a targeting moiety, such as a ligand or an antibody, conjugated (coupled) to an effector molecule.

Immunogenic peptide: A peptide which comprises an allele-specific motif or other sequence, such as an N-terminal repeat, such that the peptide will bind an MHC molecule and induce a cytotoxic T lymphocyte (“CTL”) response, or a B cell response (e.g. antibody production) against the antigen from which the immunogenic peptide is derived.

In one embodiment, immunogenic peptides are identified using sequence motifs or other methods, such as neural net or polynomial determinations, known in the art. Typically, algorithms are used to determine the “binding threshold” of peptides to select those with scores that give them a high probability of binding at a certain affinity and will be immunogenic. The algorithms are based either on the effects on MHC binding of a particular amino acid at a particular position, the effects on antibody binding of a particular amino acid at a particular position, or the effects on binding of a particular substitution in a motif-containing peptide. Within the context of an immunogenic peptide, a “conserved residue” is one which appears in a significantly higher frequency than would be expected by random distribution at a particular position in a peptide. In one embodiment, a conserved residue is one where the MHC structure may provide a contact point with the immunogenic peptide. In one specific non-limiting example, an immunogenic polypeptide includes a region of IRTA2, or a fragment thereof, wherein the polypeptide that is expressed on the cell surface of a host cell that expresses the full-length IRTA2 polypeptide.

Immunogenic composition: A composition comprising an IRTA2 polypeptide that induces a measurable CTL response against cells expressing IRTA2 polypeptide, or induces a measurable B cell response (e.g. production of antibodies) against an IRTA2 polypeptide. It further refers to isolated nucleic acids encoding an IRTA2 polypeptide that can be used to express the IRTA2 polypeptide (and thus be used to elicit an immune response against this polypeptide). For *in vitro* use, the immunogenic composition may consist of the isolated protein or peptide. For *in vivo* use, the immunogenic composition will typically comprise the protein or peptide in pharmaceutically acceptable carriers, and/or other agents. Any particular peptide, such as an IRTA2 polypeptide, or nucleic acid encoding the polypeptide, can be readily tested for its ability to induce a CTL or B cell response by art-recognized assays. Immunogenic compositions can include adjuvants, which are well known to one of skill in the art.

Immunologically reactive conditions: Includes reference to conditions which allow an antibody raised against a particular epitope to bind to that epitope to a detectably greater degree than, and/or to the substantial exclusion of, binding to substantially all other epitopes. Immunologically reactive conditions are dependent upon the format of the antibody binding reaction and typically are those utilized in immunoassay protocols or those conditions encountered *in vivo*. See Harlow & Lane, *supra*, for a description of immunoassay formats and conditions. The immunologically reactive conditions employed in the methods are "physiological conditions" which include reference to conditions (e.g., temperature, osmolarity, pH) that are typical inside a living mammal or a mammalian cell. While it is recognized that some organs are subject to extreme conditions, the intra-organismal and intracellular environment normally lies around pH 7 (i.e., from pH 6.0 to pH 8.0, more typically pH 6.5 to 7.5), contains water as the predominant solvent, and exists at a temperature above 0°C and below 50°C. Osmolarity is within the range that is supportive of cell viability and proliferation.

Inhibiting or treating a disease: Inhibiting the full development of a disease or condition, for example, in a subject who is at risk for a disease such as a tumor (for example, a leukemia or a lymphoma). "Treatment" refers to a therapeutic intervention that ameliorates a sign or symptom of a disease or pathological condition after it has begun to develop. As used herein, the term "ameliorating," with reference to a disease or pathological condition, refers to any observable beneficial effect of the treatment. The beneficial effect can be evidenced, for example, by a delayed onset of clinical symptoms of the disease in a susceptible subject, a reduction in severity of some or all clinical symptoms of the disease, a slower progression of the disease, a reduction in the number of metastases,

an improvement in the overall health or well-being of the subject, or by other parameters well known in the art that are specific to the particular disease. A "prophylactic" treatment is a treatment administered to a subject who does not exhibit signs of a disease or exhibits only early signs for the purpose of decreasing the risk of developing pathology.

5 **Immunoglobulin superfamily receptor translocation associated (IRTA):** A receptor in B cells which binds immunoglobulin and is important in B cell development. There are five types of IRTAs, termed IRTA1, IRTA2, IRTA3, IRTA4 and IRTA5. De-regulation of wild-type IRTA2 perturbs B cell immunological responses and is involved in B cell malignancy. The amino acid sequence of these IRTAs, and nucleic acids encoding
10 IRTA polypeptides are disclosed in published PCT Application No. WO 01/38490, which is incorporated herein by reference.

The cytoplasmic domains of the IRTAs contain consensus immunoreceptor tyrosine-based activation motifs (ITAMs) and/or immunoreceptor tyrosine-based inhibitory motifs (ITIMs). Expression of IRTAs was investigated in human tissues at the RNA level
15 by Northern blot, reverse transcriptase-polymerase chain reaction (RT-PCR) and by *in situ* hybridization; messenger RNAs of IRTAs have been shown to be abundant in lymphoid tissues and each member is differentially expressed by mature B lineage cells in human tonsil (for example, see Hatzivassiliou et al., *Immunity* 14:277-89, 2001).

20 **Isolated:** An "isolated" biological component (such as a nucleic acid or protein or organelle) has been substantially separated or purified away from other biological components in the cell of the organism in which the component naturally occurs, i.e., other chromosomal and extra-chromosomal DNA and RNA, proteins and organelles. Nucleic acids and proteins that have been "isolated" include nucleic acids and proteins purified by standard purification methods. The term also embraces nucleic acids and proteins prepared
25 by recombinant expression in a host cell as well as chemically synthesized nucleic acids.

Label: A detectable compound or composition that is conjugated directly or indirectly to another molecule, such as an antibody or a protein, to facilitate detection of that molecule. Specific, non-limiting examples of labels include fluorescent tags, enzymatic linkages, and radioactive isotopes.

30 **Ligand:** Any molecule which specifically binds an IRTA2 protein and includes, *inter alia*, antibodies that specifically bind an IRTA2 protein. In alternative embodiments, the ligand is a protein or a small molecule (one with a molecular weight less than 6 kiloDaltons).

Linker peptide: A peptide within an antibody binding fragment (e.g., Fv fragment)
35 which serves to indirectly bond the variable heavy chain to the variable light chain.

“Linker” can also refer to a peptide serving to link a targeting moiety, such as a scFv, to an effector molecule, such as a cytotoxin or a detectable label.

The terms “conjugating,” “joining,” “bonding” or “linking” refer to making two polypeptides into one contiguous polypeptide molecule, or to covalently attaching a
5 radionuclide or other molecule to a polypeptide, such as an scFv. In the specific context, the terms include reference to joining a ligand, such as an antibody moiety, to an effector molecule (“EM”). The linkage can be either by chemical or recombinant means. “Chemical means” refers to a reaction between the antibody moiety and the effector molecule such that there is a covalent bond formed between the two molecules to form one molecule.

10 **Lymphocytes:** A type of white blood cell that is involved in the immune defenses of the body. There are two main types of lymphocytes: B cells and T cells.

Mammal: This term includes both human and non-human mammals. Similarly, the term “subject” includes both human and veterinary subjects.

Major Histocompatibility Complex or MHC: Generic designation meant to
15 encompass the histocompatibility antigen systems described in different species, including the human leukocyte antigens (“HLA”). The term “motif” refers to the pattern of residues in a peptide of defined length, usually about 8 to about 11 amino acids, which is recognized by a particular MHC allele. The peptide motifs are typically different for each MHC allele and differ in the pattern of the highly conserved residues and negative binding residues.

20 **Neoplasia and Tumor:** The process of abnormal and uncontrolled growth of cells. Neoplasia is one example of a proliferative disorder.

The product of neoplasia is a neoplasm (a tumor), which is an abnormal growth of tissue that results from excessive cell division. A tumor that does not metastasize is referred to as “benign.” A tumor that invades the surrounding tissue and/or can metastasize is
25 referred to as “malignant.” Examples of hematological tumors include leukemias, including acute leukemias (such as acute lymphocytic leukemia, acute myelocytic leukemia, acute myelogenous leukemia and myeloblastic, promyelocytic, myelomonocytic, monocytic and erythroleukemia), chronic leukemias (such as chronic myelocytic (granulocytic) leukemia, chronic myelogenous leukemia, and chronic lymphocytic leukemia), polycythemia vera,
30 lymphoma, Hodgkin's disease, non-Hodgkin's lymphoma (indolent and high grade forms), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, myelodysplastic syndrome, hairy cell leukemia and myelodysplasia.

Examples of solid tumors, such as sarcomas and carcinomas, include fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, and other sarcomas,
35 synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon

- carcinoma, lymphoid malignancy, pancreatic cancer, breast cancer, lung cancers, ovarian cancer, prostate cancer, hepatocellular carcinoma, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, medullary carcinoma, bronchogenic carcinoma,
- 5 renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, Wilms' tumor, cervical cancer, testicular tumor, bladder carcinoma, and CNS tumors (such as a glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma and retinoblastoma).
- 10 **Non-Hodgkin's Lymphoma:** A heterogeneous group of malignant lymphomas (a tumor of the lymphoid tissue), the only common feature being an absence of the giant Reed-Sternberg cells characteristic of Hodgkin's disease. These neoplasias arise from the lymphoid components of the immune system, and present a clinical picture broadly similar to that of Hodgkin's disease except the disease is initially more widespread, with the most
- 15 common manifestation being painless enlargement of one or more peripheral lymph nodes. There have been numerous classifications of the non-Hodgkin's lymphomas; the most recent system is the Revised European American Lymphoma (REAL) Classification.
- Nucleic acid:** A polymer composed of nucleotide units (ribonucleotides, deoxyribonucleotides, related naturally occurring structural variants, and synthetic non-
- 20 naturally occurring analogs thereof) linked via phosphodiester bonds, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof. Thus, the term includes nucleotide polymers in which the nucleotides and the linkages between them include non-naturally occurring synthetic analogs, such as, for example and without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl
- 25 phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs), and the like. Such polynucleotides can be synthesized, for example, using an automated DNA synthesizer. The term "oligonucleotide" typically refers to short polynucleotides, generally no greater than about 50 nucleotides. It will be understood that when a nucleotide sequence is represented by a DNA sequence (i.e., A, T, G, C), this also includes an RNA sequence
- 30 (i.e., A, U, G, C) in which "U" replaces "T."

Conventional notation is used herein to describe nucleotide sequences: the left-hand end of a single-stranded nucleotide sequence is the 5'-end; the left-hand direction of a double-stranded nucleotide sequence is referred to as the 5'-direction. The direction of 5' to 3' addition of nucleotides to nascent RNA transcripts is referred to as the transcription

direction. The DNA strand having the same sequence as an mRNA is referred to as the “coding strand;” sequences on the DNA strand having the same sequence as an mRNA transcribed from that DNA and which are located 5' to the 5'-end of the RNA transcript are referred to as “upstream sequences;” sequences on the DNA strand having the same
5 sequence as the RNA and which are 3' to the 3' end of the coding RNA transcript are referred to as “downstream sequences.”

“cDNA” refers to a DNA that is complementary or identical to an mRNA, in either single stranded or double stranded form.

“Encoding” refers to the inherent property of specific sequences of nucleotides in a
10 polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (i.e., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA produced by that gene produces the protein in a cell
15 or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and non-coding strand, used as the template for transcription, of a gene or cDNA can be referred to as encoding the protein or other product of that gene or cDNA. Unless otherwise specified, a “nucleotide sequence encoding an amino acid sequence” includes all nucleotide sequences
20 that are degenerate versions of each other and that encode the same amino acid sequence. Nucleotide sequences that encode proteins and RNA may include introns.

“Recombinant nucleic acid” refers to a nucleic acid having nucleotide sequences that are not naturally joined together. This includes nucleic acid vectors comprising an amplified or assembled nucleic acid which can be used to transform a suitable host cell. A
25 host cell that comprises the recombinant nucleic acid is referred to as a “recombinant host cell.” The gene is then expressed in the recombinant host cell to produce, e.g., a “recombinant polypeptide.” A recombinant nucleic acid may serve a non-coding function (e.g., promoter, origin of replication, ribosome-binding site, etc.) as well.

A first sequence is an “antisense” with respect to a second sequence if a
30 polynucleotide whose sequence is the first sequence specifically hybridizes with a polynucleotide whose sequence is the second sequence.

Terms used to describe sequence relationships between two or more nucleotide sequences or amino acid sequences include “reference sequence,” “selected from,”
“comparison window,” “identical,” “percentage of sequence identity,” “substantially
35 identical,” “complementary,” and “substantially complementary.”

For sequence comparison of nucleic acid sequences, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters are used. Methods of alignment of sequences for comparison are well known in the art. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482, 1981, by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443, 1970, by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444, 1988, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection (see, e.g., *Current Protocols in Molecular Biology* (Ausubel et al., eds 1995 supplement)).

One example of a useful algorithm is PILEUP. PILEUP uses a simplification of the progressive alignment method of Feng & Doolittle, *J. Mol. Evol.* 35:351-360, 1987. The method used is similar to the method described by Higgins & Sharp, *CABIOS* 5:151-153, 1989. Using PILEUP, a reference sequence is compared to other test sequences to determine the percent sequence identity relationship using the following parameters: default gap weight (3.00), default gap length weight (0.10), and weighted end gaps. PILEUP can be obtained from the GCG sequence analysis software package, e.g., version 7.0 (Devereaux et al., *Nuc. Acids Res.* 12:387-395, 1984).

Another example of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and the BLAST 2.0 algorithm, which are described in Altschul et al., *J. Mol. Biol.* 215:403-410, 1990 and Altschul et al., *Nucleic Acids Res.* 25:3389-3402, 1997. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). The BLASTN program (for nucleotide sequences) uses as defaults a word length (W) of 11, alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands. The BLASTP program (for amino acid sequences) uses as defaults a word length (W) of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915, 1989).

Oligonucleotide: A linear polynucleotide sequence of up to about 100 nucleotide bases in length.

Operably linked: A first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter, such as the IRTA2 promoter, is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein-coding regions, in the same reading frame.

ORF (open reading frame): A series of nucleotide triplets (codons) coding for amino acids without any termination codons. These sequences are usually translatable into a peptide.

Peptide: A chain of amino acids of between 3 and 30 amino acids in length. In one embodiment, a peptide is from about 10 to about 25 amino acids in length. In yet another embodiment, a peptide is from about 11 to about 20 amino acids in length. In yet another embodiment, a peptide is about 12 amino acids in length.

An "IRTA2 peptide" is a series of contiguous amino acid residues from an IRTA2 protein, such as a fragment of IRTA2 protein of about 10 to about 25 amino acids in length, such as about 11 to about 20 amino acid in length, such as about 12 consecutive amino acids of an IRTA2 protein. In one example, with respect to immunogenic compositions comprising an IRTA2 peptide, the term further refers to variations of these peptides in which there are conservative substitutions of amino acids, so long as the variations do not alter by more than about 20% (such as no more than about 1%, about 5%, or about 10%) the ability of the peptide to produce a B cell response, or, when bound to a Major Histocompatibility Complex Class I molecule, to activate cytotoxic T lymphocytes against cells expressing wild-type IRTA2 protein. Induction of CTLs using synthetic peptides and CTL cytotoxicity assays are taught in, e.g., U.S. Patent No. 5,662,907.

"Soluble" forms of IRTA2 are secreted and can bind to the antibodies disclosed herein. Soluble IRTA2 may be, for example, a product resulting from alternative splicing of the IRTA2 gene, or a soluble extracellular domain that has been shed from cell surface-expressed full-length IRTA2.

Peptide modifications: IRTA2 polypeptides include synthetic embodiments of peptides described herein. In addition, analogs (non-peptide organic molecules), derivatives (chemically functionalized peptide molecules obtained starting with the disclosed peptide sequences) and variants (homologs) of these proteins can be utilized in the methods described herein. Each polypeptide is comprised of a sequence of amino acids, which may be either L- and/or D- amino acids, naturally occurring and otherwise.

Peptides may be modified by a variety of chemical techniques to produce derivatives having essentially the same activity as the unmodified peptides, and optionally having other desirable properties. For example, carboxylic acid groups of the protein, whether carboxyl-terminal or side chain, may be provided in the form of a salt of a pharmaceutically-acceptable cation or esterified to form a C₁-C₁₆ ester, or converted to an amide of formula NR₁R₂ wherein R₁ and R₂ are each independently H or C₁-C₁₆ alkyl, or combined to form a heterocyclic ring, such as a 5- or 6- membered ring. Amino groups of the peptide, whether amino-terminal or side chain, may be in the form of a pharmaceutically-acceptable acid addition salt, such as the HCl, HBr, acetic, benzoic, toluene sulfonic, maleic, tartaric and other organic salts, or may be modified to C₁-C₁₆ alkyl or dialkyl amino or further converted to an amide.

Hydroxyl groups of the peptide side chains may be converted to C₁-C₁₆ alkoxy or to a C₁-C₁₆ ester using well-recognized techniques. Phenyl and phenolic rings of the peptide side chains may be substituted with one or more halogen atoms, such as fluorine, chlorine, bromine or iodine, or with C₁-C₁₆ alkyl, C₁-C₁₆ alkoxy, carboxylic acids and esters thereof, or amides of such carboxylic acids. Methylene groups of the peptide side chains can be extended to homologous C₂-C₄ alkylenes. Thiols can be protected with any one of a number of well-recognized protecting groups, such as acetamide groups. Those skilled in the art will also recognize methods for introducing cyclic structures into the IRTA2 peptides to select and provide conformational constraints to the structure that result in enhanced stability.

Peptidomimetic and organomimetic embodiments are envisioned, whereby the three-dimensional arrangement of the chemical constituents of such peptido- and organomimetics mimic the three-dimensional arrangement of the peptide backbone and component amino acid side chains, resulting in such peptido- and organomimetics of an IRTA2 polypeptide having measurable or enhanced ability to generate an immune response. For computer modeling applications, a pharmacophore is an idealized, three-dimensional definition of the structural requirements for biological activity. Peptido- and organomimetics can be designed to fit each pharmacophore with current computer modeling software (using computer assisted drug design or CADD). See Walters, "Computer-Assisted Modeling of Drugs", in Klegerman & Groves, eds., 1993, *Pharmaceutical Biotechnology*, Interpharm Press, Buffalo Grove, IL, pp. 165-174 and *Principles of Pharmacology* Munson (ed.) 1995, Ch. 102, for descriptions of techniques used in CADD. Also included are mimetics prepared using such techniques.

Pharmaceutical agent: A chemical compound or composition capable of inducing a desired therapeutic or prophylactic effect when properly administered to a subject or a cell.

Pharmaceutically acceptable carriers: The pharmaceutically acceptable carriers of use are conventional. *Remington's Pharmaceutical Sciences*, by E. W. Martin, Mack Publishing Co., Easton, PA, 15th Edition, 1975, describes compositions and formulations suitable for pharmaceutical delivery of the fusion proteins herein disclosed.

In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. For solid compositions (e.g., powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

Polynucleotide: The term polynucleotide or nucleic acid sequence refers to a polymeric form of nucleotide at least 10 bases in length. A recombinant polynucleotide includes a polynucleotide that is not immediately contiguous with both of the coding sequences with which it is immediately contiguous (one on the 5' end and one on the 3' end) in the naturally occurring genome of the organism from which it is derived. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g., a cDNA) independent of other sequences. The nucleotides can be ribonucleotides, deoxyribonucleotides, or modified forms of either nucleotide. The term includes single- and double- stranded forms of DNA. An *IRTA2* polynucleotide is a nucleic acid encoding an *IRTA2* polypeptide.

Polypeptide: Any chain of amino acids, regardless of length or post-translational modification (e.g., glycosylation or phosphorylation). In one embodiment, the polypeptide is *IRTA2* polypeptide. A "residue" refers to an amino acid or amino acid mimetic incorporated in a polypeptide by an amide bond or amide bond mimetic. A polypeptide has an amino terminal (N-terminal) end and a carboxy terminal end.

Probes and primers: A probe comprises an isolated nucleic acid attached to a detectable label or reporter molecule. Primers are short nucleic acids, and can be DNA

oligonucleotides 15 nucleotides or more in length. Primers may be annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, and then extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR) or other nucleic-acid amplification methods known in the art. One of skill in the art will appreciate that the specificity of a particular probe or primer increases with its length. Thus, for example, a primer comprising 20 consecutive nucleotides will anneal to a target with a higher specificity than a corresponding primer of only 15 nucleotides. Thus, in order to obtain greater specificity, probes and primers may be selected that comprise 20, 25, 30, 35, 40, 50 or more consecutive nucleotides.

Promoter: A promoter is an array of nucleic acid control sequences that directs transcription of a nucleic acid. A promoter includes necessary nucleic acid sequences near the start site of transcription, such as, in the case of a polymerase II type promoter, a TATA element. A promoter also optionally includes distal enhancer or repressor elements which can be located as much as several thousand base pairs from the start site of transcription. Both constitutive and inducible promoters are included (see e.g., Bitter et al., *Methods in Enzymology* 153:516-544, 1987).

Specific, non-limiting examples of promoters include promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the retrovirus long terminal repeat; the adenovirus late promoter; the vaccinia virus 7.5K promoter) may be used. Promoters produced by recombinant DNA or synthetic techniques may also be used. A polynucleotide can be inserted into an expression vector that contains a promoter sequence which facilitates the efficient transcription of the inserted genetic sequence of the host. The expression vector typically contains an origin of replication, a promoter, as well as specific nucleic acid sequences that allow phenotypic selection of the transformed cells.

Protein purification: The IRTA2 polypeptides disclosed herein can be purified by any of the means known in the art. See, e.g., *Guide to Protein Purification*, ed. Deutscher, *Meth. Enzymol.* 185, Academic Press, San Diego, 1990; and Scopes, *Protein Purification: Principles and Practice*, Springer Verlag, New York, 1982. Substantial purification denotes purification from other proteins or cellular components. A substantially purified protein is at least 60%, 70%, 80%, 90%, 95% or 98% pure. Thus, in one specific, non-limiting example, a substantially purified protein is 90% free of other proteins or cellular components.

Purified: The term purified does not require absolute purity; rather, it is intended as a relative term. Thus, for example, a purified peptide preparation is one in which the peptide or protein is more enriched than the peptide or protein is in its natural environment within a cell. In one embodiment, a preparation is purified such that the protein or peptide
5 represents at least 50% of the total peptide or protein content of the preparation.

Recombinant: A recombinant nucleic acid is one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of
10 isolated segments of nucleic acids, e.g., by genetic engineering techniques.

Selectively hybridize: Hybridization under moderately or highly stringent conditions that excludes non-related nucleotide sequences.

In nucleic acid hybridization reactions, the conditions used to achieve a particular level of stringency will vary, depending on the nature of the nucleic acids being hybridized.
15 For example, the length, degree of complementarity, nucleotide sequence composition (e.g., GC versus AT content), and nucleic acid type (e.g., RNA versus DNA) of the hybridizing regions of the nucleic acids can be considered in selecting hybridization conditions. An additional consideration is whether one of the nucleic acids is immobilized, for example, on a filter.

20 A specific, non-limiting example of progressively higher stringency conditions is as follows: 2 x SSC/0.1% SDS at about room temperature (hybridization conditions); 0.2 x SSC/0.1% SDS at about room temperature (low stringency conditions); 0.2 x SSC/0.1% SDS at about 42°C (moderate stringency conditions); and 0.1 x SSC at about 68°C (high stringency conditions). One of skill in the art can readily determine variations on these
25 conditions (e.g., *Molecular Cloning: A Laboratory Manual*, 2nd ed., Vol. 1-3, ed. Sambrook et al., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989). Washing can be carried out using only one of these conditions, e.g., high stringency conditions, or each of the conditions can be used, e.g., for 10-15 minutes each, in the order listed above, repeating any or all of the steps listed. However, as mentioned above, optimal conditions will vary,
30 depending on the particular hybridization reaction involved, and can be determined empirically.

Sequence identity: The similarity between amino acid sequences is expressed in terms of the similarity between the sequences, otherwise referred to as sequence identity. Sequence identity is frequently measured in terms of percentage identity (or similarity or
35 homology); the higher the percentage, the more similar the two sequences are. Homologs or

variants of an IRTA2 polypeptide will possess a relatively high degree of sequence identity when aligned using standard methods.

Methods of alignment of sequences for comparison are well known in the art.

Various programs and alignment algorithms are described in: Smith and Waterman, *Adv.*

5 *Appl. Math.* 2:482, 1981; Needleman and Wunsch, *J. Mol. Biol.* 48:443, 1970; Pearson and Lipman, *Proc. Natl. Acad. Sci. U.S.A.* 85:2444, 1988; Higgins and Sharp, *Gene* 73:237, 1988; Higgins and Sharp, *CABIOS* 5:151, 1989; Corpet et al., *Nucleic Acids Research* 16:10881, 1988; and Pearson and Lipman, *Proc. Natl. Acad. Sci. U.S.A.* 85:2444, 1988. Altschul et al., *Nature Genet.* 6:119, 1994, presents a detailed consideration of sequence alignment methods
10 and homology calculations.

The NCBI Basic Local Alignment Search Tool (BLAST) (Altschul et al., *J. Mol. Biol.* 215:403, 1990) is available from several sources, including the National Center for Biotechnology Information (NCBI, Bethesda, MD) and on the internet, for use in connection with the sequence analysis programs blastp, blastn, blastx, tblastn and tblastx. A description
15 of how to determine sequence identity using this program is available on the NCBI website on the internet.

Homologs and variants of a V_L or a V_H of an antibody that specifically binds an IRTA2 polypeptide are typically characterized by possession of at least about 75%, for example at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%
20 sequence identity counted over the full length alignment with the amino acid sequence of IRTA2 using the NCBI Blast 2.0, gapped blastp set to default parameters. For comparisons of amino acid sequences of greater than about 30 amino acids, the Blast 2 sequences function is employed using the default BLOSUM62 matrix set to default parameters, (gap existence cost of 11, and a per residue gap cost of 1). When aligning short peptides (fewer than around 30
25 amino acids), the alignment should be performed using the Blast 2 sequences function, employing the PAM30 matrix set to default parameters (open gap 9, extension gap 1 penalties). Proteins with even greater similarity to the reference sequences will show increasing percentage identities when assessed by this method, such as at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity. When less
30 than the entire sequence is being compared for sequence identity, homologs and variants will typically possess at least 80% sequence identity over short windows of 10-20 amino acids, and may possess sequence identities of at least 85% or at least 90% or 95% depending on their similarity to the reference sequence. Methods for determining sequence identity over such short windows are available at the NCBI website on the internet. One of skill in the art will
35 appreciate that these sequence identity ranges are provided for guidance only; it is entirely

possible that strongly significant homologs could be obtained that fall outside of the ranges provided.

Specific binding agent: An agent that binds substantially only to a defined target. Thus an IRTA2 specific binding agent is an agent that binds substantially to an IRTA2 polypeptide. In one embodiment, the specific binding agent is a monoclonal or polyclonal antibody that specifically binds the IRTA2 polypeptide.

The term "specifically binds" refers, with respect to an antigen such as IRTA2, to the preferential association of an antibody or other ligand, in whole or part, with a cell or tissue bearing that antigen and not to cells or tissues lacking that antigen. It is, of course, recognized that a certain degree of non-specific interaction may occur between a molecule and a non-target cell or tissue. Nevertheless, specific binding may be distinguished as mediated through specific recognition of the antigen. Although selectively reactive antibodies bind antigen, they may do so with low affinity. On the other hand, specific binding results in a much stronger association between the antibody (or other ligand) and cells bearing the antigen than between the bound antibody (or other ligand) and cells lacking the antigen. Specific binding typically results in greater than 2-fold, such as greater than 5-fold, greater than 10-fold, or greater than 100-fold increase in amount of bound antibody or other ligand (per unit time) to a cell or tissue bearing the IRTA2 polypeptide as compared to a cell or tissue lacking the polypeptide. Specific binding to a protein under such conditions requires an antibody that is selected for its specificity for a particular protein. A variety of immunoassay formats are appropriate for selecting antibodies or other ligands specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically immunoreactive with a protein. See Harlow & Lane, *Antibodies, A Laboratory Manual*, Cold Spring Harbor Publications, New York (1988), for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity.

Subject: Living multi-cellular vertebrate organisms, a category that includes both human and veterinary subjects, including human and non-human mammals.

T Cell: A white blood cell critical to the immune response. T cells include, but are not limited to, CD4⁺ T cells and CD8⁺ T cells. A CD4⁺ T lymphocyte is an immune cell that carries a marker on its surface known as "cluster of differentiation 4" (CD4). These cells, also known as helper T cells, help orchestrate the immune response, including antibody responses as well as killer T cell responses. CD8⁺ T cells carry the "cluster of differentiation 8" (CD8) marker. In one embodiment, CD8 T cells are cytotoxic T lymphocytes. In another embodiment, a CD8 cell is a suppressor T cell.

Targeting moiety: A portion of a chimeric molecule intended to provide the molecule with the ability to bind specifically to the IRTA2 polypeptide. A “ligand” is a targeting molecule specific for the IRTA2 polypeptide and is generally synonymous with “targeting moiety.” An antibody is one version of a ligand.

5 **Therapeutically effective amount:** A quantity of a specific substance sufficient to achieve a desired effect in a subject being treated. For instance, this can be the amount necessary to inhibit or suppress growth of a tumor. In one embodiment, a therapeutically effective amount is the amount necessary to eliminate a tumor. When administered to a subject, a dosage will generally be used that will achieve target tissue concentrations (for
10 example, in tumors) that has been shown to achieve a desired *in vitro* effect.

Toxin: A molecule that is cytotoxic for a cell. Toxins include abrin, ricin, *Pseudomonas* exotoxin (PE), diphtheria toxin (DT), botulinum toxin, saporin, restrictocin or gelonin, or modified toxins thereof. For example, PE and DT are highly toxic compounds that typically bring about death through liver toxicity. PE and DT, however, can be
15 modified into a form for use as an immunotoxin by removing the native targeting component of the toxin (e.g., domain Ia of PE or the B chain of DT) and replacing it with a different targeting moiety, such as an antibody.

Transduced: A transduced cell is a cell into which has been introduced a nucleic acid molecule by molecular biology techniques. As used herein, the term transduction
20 encompasses all techniques by which a nucleic acid molecule might be introduced into such a cell, including transfection with viral vectors, transformation with plasmid vectors, and introduction of naked DNA by electroporation, lipofection, and particle gun acceleration.

Vector: A nucleic acid molecule as introduced into a host cell, thereby producing a transformed host cell. A vector may include nucleic acid sequences that permit it to
25 replicate in a host cell, such as an origin of replication. A vector may also include one or more selectable marker genes and other genetic elements known in the art.

Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this
30 disclosure belongs. The singular terms “a,” “an,” and “the” include plural referents unless context clearly indicates otherwise. Similarly, the word “or” is intended to include “and” unless the context clearly indicates otherwise. It is further to be understood that all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for description. Although
35 methods and materials similar or equivalent to those described herein can be used in the

practice or testing of this disclosure, suitable methods and materials are described below. The term “comprises” means “includes.” All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including explanations of terms, will control. In addition,
 5 the materials, methods, and examples are illustrative only and not intended to be limiting.

Monoclonal Antibodies that Bind IRTA2

Antibodies are disclosed herein that bind IRTA2. The immunoglobulin (Ig) superfamily receptor translocation associated 2 (IRTA2) gene is a gene targeted by one of
 10 the 1q21 abnormalities that occurs in B cell malignancies (Hatzivassiliou et al., *Immunity* 14:277-89, 2001). This gene has also been identified as BXMAS1 (Nakayama et al., *Biochem Biophys Res Commun* 285:830-7, 2001, herein incorporated by reference) and Fc receptor homolog 5 (FcRH5) (Miller et al., *Blood* 99:2662-9, 2002, herein incorporated by reference).

15 A series of genes homologous to IRTA2/BXMAS1/FcRH5 have been identified in the same locus of the human genome. A total of five IRTA family members have been identified (for example, see Davis et al., *Immunol Rev* 190:123-36, 2002). These genes, termed immunoglobulin superfamily translocation associated (IRTA) genes code for individual members of the Ig receptor family with three to nine Ig-like extracellular
 20 domains.

Monoclonal antibodies are disclosed herein that specifically bind the extracellular domain of IRTA2. In one example, IRTA2 has an amino acid sequence set forth as:

25 MLLWVILLVLAPVSGQFARTPRPIIFLQPPWTTVFQGERVTLTC
 KGFRFYSPQKTKWYHRYLGKEILRETPDNILEVQESGEYRCQAQGSPLSSPVHLDFFSS
 ASLILQAPLSVFEGDSVVLRCRAKAEVTLNNTIYKNDNVLAFLNKRDFHIPHACLKD
 30 NGAYRCTGYKESCCPVSSNTVKIQVQEPFTRPVLRASSFQPISGNPVTLTCETQLSLE
 RSDVPLRFRFFRDDQTLGLGWSLSPNFQITAMWSKDSGFYWCKAATMPHSVISDSPRS
 WIQVQIPASHPVLTLSPKALNFEGTKVTLHCETQEDSLRTLYRFYHEGVPLRHKSVR
 35 CERGASISFSLTTENSGNYYCTADNGLGAKPSKAVSLSVTPVSHPVNLNLSSPEDLIIF
 EGAKVTLHCEAQRGSLPILYQFHEDAALERRSANSAGGVAISFSLTAEHSGNYYCTA
 40 DNGFGPQRSKAVSLITVPVSHPVLTLSAEALTFEGATVTLHCEVQRGSPQILYQFY
 HEDMPLWSSSTPSVGRVSFSFSLTEGHSGNYYCTADNFGPQRSEVVSFLFVTPVSRP

ILTLRVPRAQAVVGDLLELHCEAPRGSPPILYWIFYHEDVTLGSSSAPSGGEASFNLSL
 TAEHSGNYSCEANGLVAQHSDTISLSVIVPVSRIILTFRAPRAQAVVGDLLELHCEA
 5 LRGSSPILYWIFYHEDVTLGKISAPSGGGASFNLSLTTEHSGIYSCEADNGLEAQRSEM
 VTLKVAGEWALPTSSTSEN (SEQ ID NO: 13)

10 Additional examples of IRTA2 are disclosed in published PCT Application No. WO 01/38490, which is incorporated herein by reference.

In one embodiment, the antibodies that bind IRTA2 do not specifically bind IRTA1, IRTA3, IRTA4 or IRTA5, which have the following amino acid sequences:

15 IRTA1:
 mllwasllaf apvcgqsaaa hkpvisvhpp wttffkgerv tltcngfqfy atekttwyhr
 hywgekltlp pgntlevres glyrcqargs prsnprvllf ssdslilqap ysvfegdtlv
 lrchrrrkek ltavkytwng nilsisnksw dllipqassn nngnyrcigy gdendvfrsn
 fkiikiqelf phpelkatds qptegnsnl scetqlpper sdtplhfnff rdgevilsdw
 20 stypelqlpt vwrensgsyw cgaetvrni hkhspslqih vqripvsgvl letqpsggga
 vegemlvllvc svaegtgdtt fswhredmge slgrktqrs1 raelelpair qshaggyct
 adnsygpvqs mvlntvret pgnrdglvaa gatggllsal llavallfhc wrrrksvgvf
 lgdetr1ppa pgpgesshsi cpaqvelqsl yvdvhpkkgd lvyseiqtq lgeeeeants
 rtiledkdvs vvysevktqh pdnsagkiss kdes (SEQ ID NO: 14)

25 IRTA3:
 mllwllllil tpgreqsgva pkavlllnpp wstafkgekv alicssishs laaggdywyh
 deklkikhhd kiqitepgny qcktrgssls davehvefspd wlilqalhpv fegdnvilrc
 qgkdnknthq kvyykdgkql pnsynlekit vnsvsrdnsk yhctayrkfy ildievtskp
 30 lniqvqelfl hpvlrassst piegspmtlt cetqlspqrp dvqlqfslfr dsqtlglgws
 rsprlqipam wtedsgsywc evetvthsik krslrsqirv qrvpvsnnl eirptggqli
 egenmvlics vaqsggtvtf shwkegrvrs lgrktqrsll aelhvlvke sdagryycaa
 dnvhsplst wirvtvripv shpvltrfap rahtvvgdll elhceslrgrs ppilyrfyhe
 dvtlgnssap sgggasfnls ltaehsgnys cdadnglgaq hshgvs1rvt vpvsrpvltl
 35 rapgaqavvg dllelhcesl rgsfpilywf yheddtlgni sahsgggasf nls1ttehsg
 nysceadngl gaqhskvvtl nvtgtsrnr1 gltaagitgl vlsilv1aaa aallhyarar
 rkpqglsatg tsshspsecq epsssrpsri dpqepthskp lapmelepmy snvpngdsnp
 iysqiwsiqh tkensancpm mhqeheeltv lyse1kkthp ddsageassr graheeddee
 nyenvprvll asdh (SEQ ID NO: 15)

40 IRTA4:
 mllwsllvif davteqadsl tlvapssvfe gdsivlkcqg eqnwkiqkma yhkdnkelsv
 fkkfsdfl1q savlsdsgny fcstkgqlfl wdkt1snivki kvqelfqrpv ltassfqp1e
 ggpvs1kcet r1spqrldvq lqfcffrenq vlsgswsssp elqisavwse dtgsywckae
 45 tvthrirkqs lqsqihvqri p1snvsleir apggqvtegg kl1llcsvgag gtgnvtfswy
 reatgtsmgk ktqrs1sael eipavkesda gkyycradng hvpiqskvvn ipvripv1rp
 v1tlr1spgaq aavgdllelh cealrgsppi lyqfyhedvt lgnssapsgg gasfnls1ta
 ehsgnyscea nnglgaqcse avpvsisgpd gyrrdlmtag vlwglfgvlg ftgvall1ya
 lf1hkisgess atneprgasr pnpqeft1yss ptpdmeelqp vyv1nvgsvdv dvvysqv1wsm
 50 qqpessanir tllenkdsqv iyssvkks (SEQ ID NO: 16)

IRTA5:

mlprlllllic aplcepaelf liaspshpte gspvtltckm pflqssdaqf qfcffrdtra
 lgpgwssspk lqiaamwked tgsywceagt maskvlrsrr sqinvhrvpv advsletqpp
 5 ggqvmegdrl vlicsvamgt gditflwykg avglnlqskt qrshtaeyei psvresdaeq
 yyvvaengyg pspsglvst vripvsrpil mlrapraqaa vedvlelhce alrgspilly
 wfyhedittg srsapsggga sfnlslteeh sgnysceann glgaqrseav tlnftvptga
 rsnhltsuvi eglstltpa tvallfcygl krkigrsars dplrsllpspl pgeftylnsp
 tpgqlqpiye nvnvsgdev yslayynqpe qesvaaetlg thmedkvsld iysrlrkani
 10 tdvdyedam (SEQ ID NO: 17).

The sequence of these proteins is well known in the art, and are also provided in WO 01/39490, which is incorporated by reference herein. Thus, in one embodiment, the antibodies disclosed herein can be used to differentiate the expression of IRTA2 from
 15 IRTA1, IRTA3, IRTA4, and/or IRTA5. In one example, the antibody specifically binds IRTA2, but does not specifically bind IRTA1, IRTA3, IRTA4, and/or IRTA5.

Disclosed herein is the amino acid sequence of several murine monoclonal antibodies. These monoclonal antibodies each include a variable heavy (V_H) and a variable light (V_L) chain and specifically bind IRTA2. For example, the antibodies can bind IRTA2
 20 with an affinity constant of at least $10^7 M^{-1}$, such as at least $10^8 M^{-1}$ at least $5 \times 10^8 M^{-1}$ or at least $10^9 M^{-1}$.

In one embodiment, the antibody is F119, and includes a V_H polypeptide including an amino acid set forth as SEQ ID NO: 1 and a V_L polypeptide including an amino acid sequence set forth as SEQ ID NO: 2. In another embodiment, the monoclonal antibody is
 25 F56, and includes a V_H polypeptide including an amino acid set forth as SEQ ID NO: 3 and a V_L polypeptide including an amino acid sequence set forth a SEQ ID NO: 4. In a further embodiment, the monoclonal antibody is F25, and includes a V_H polypeptide including an amino acid set forth as SEQ ID NO: 5 and a V_L polypeptide including an amino acid sequence set forth a SEQ ID NO: 6. The amino acid sequences of the V_H and V_L of F119,
 30 F56 and F25 are also set forth in Fig. 6. Functional fragments of these antibodies are also encompassed by this disclosure (see below).

In several embodiments the antibody includes a V_H polypeptide having an amino acid sequence at least about 90% identical, such as at least about 95%, at least about 98%, or at least about 99% identical to the amino acid sequence set forth as SEQ ID NO: 1 and a V_L
 35 polypeptide having an amino acid sequence at least about 90% identical, such as at least about 95%, at least about 98%, or at least about 99% identical to the amino acid sequence set forth as SEQ ID NO: 2. In another embodiment, the monoclonal antibody includes a V_H polypeptide having an amino acid sequence at least about 90% identical, such as at least about 95%, at least about 98%, or at least about 99% identical to the amino acid sequence

set forth as SEQ ID NO: 3 and a V_L polypeptide having an amino acid sequence at least about 90% identical, such as at least about 95%, at least about 98%, or at least about 99% identical, to the amino acid sequence set forth as set forth a SEQ ID NO: 4. In a further embodiment, the monoclonal antibody includes a V_H polypeptide having an amino acid
 5 sequence at least about 90% identical, such as at least about 95%, at least about 98%, or at least about 99% identical, to the amino acid sequence set forth as SEQ ID NO: 5 and a V_L polypeptide including an amino acid sequence at least about 90% identical, such as at least about 95%, at least about 98%, or at least about 99% identical to the amino acid sequence set forth as SEQ ID NO: 6.

10 In another embodiment, the antibody is F1, which is produced by a hybridoma deposited in accordance with the Budapest Treaty as American Type Culture Collection (ATCC) Deposit No. XXXX, deposited in accordance with the Budapest Treaty on September 22, 2005 or is a humanized form thereof. In another embodiment, the antibody is F44, which is produced by a hybridoma deposited as American Type Culture Collection
 15 (ATCC) Deposit No. YYYY, deposited in accordance with the Budapest Treaty on September 22, 2005, or is a humanized form thereof. In another embodiment, the antibody is F54, which is produced by a hybridoma deposited with American Type Culture Collection (ATCC) Deposit No. ZZZZ, deposited in accordance with the Budapest Treaty on September 22, 2005 or is a humanized form thereof. Hybridoma cells and their progeny
 20 that secrete the monoclonal antibodies F1, F44 and F54 are also encompassed by this disclosure.

25 Variants of these antibodies are also provided, such as those that have V_H or V_L domains that have similar or identical CDRs to antibodies F54, F44 or F1 that specifically bind IRTA2 or an antigenic epitope thereof. The epitopes of IRTA2 that these monoclonal antibodies bind to are disclosed in Table 4. F25 binds to epitope 2, which is located at amino acids 1 to 109 of SEQ ID NO:13, namely,

MLLWVILLVLAPVSGQFARTPRPIIFLQPPWTTVFQGERVTLTCKGFRFYSPQKTKWYHRYLGKEILR
 ETPDNILEVQESGEYRCQAQGSPLSSPVHLDFSSASLILQAP (amino acids 1 to 109 of

30 SEQ ID NO: 1. F54 binds to epitope 1, which is located at amino acids 1 to 190 of SEQ ID NO:13, namely

MLLWVILLVLAPVSGQFARTPRPIIFLQPPWTTVFQGERVTLTCKGFRFYSPQKTKWYHRYLGKEILR
 ETPDNILEVQESGEYRCQAQGSPLSSPVHLDFSSASLILQAPLSVFEGDSVVLRCRAKAEVTLNNTIY
 KNDNVLAFLNKRITDFHI PHACLDNGAYRCTGYKESCCPVSSNTVKIQVQEPFTR (amino acids 1
 to 190 of SEQ ID NO:13) .

F44 and F119 bind to epitope 3, which is located at amino acids 191 to 286 of SEQ ID NO:13, namely

PVLRASSFQPISGNPVTLTCETQLSLERSDVPLRFRFRDDQTLGLGWSLSPNFQITAMWSKDSGFYW
CKAATMPHSVISDSPRSWIQVQIPASH (amino acids 191 to 286 of SEQ ID NO: 1).

5

F56 binds to epitope 11, which is located at amino acids 287 to 565 of SEQ ID NO:13, namely

PVLTLSPEKALNFEGTKVTLHCETQEDSLRTLRYFYHEGVPLRHKSVRCERGASISFSLTTENSGNYY
CTADNGLGAKPSKAVSLSVTPVSHPVNLSSPEDLIFEGAKVTLHCEAQRGSLPILYQFHHEDAAL
10 RRSANSAGGVAISFSLTAEHSGNYYCTADNGFGPQRSKAVSLSTITVPVSHPVLTLSAEALTFEGATV
TLHCEVQRGSPQILYQFYHEDMPLWSSSTPSVGRVSFSFSLTEGHSGNYYCTADNGFGPQRSEVVSLF
VTVPVSR (amino acids 287 to 565 of SEQ ID NO: 13)

F1 binds to epitope 5, which is located at amino acids 566 to 746 of SEQ ID NO:13, namely

15 PILTLRVPRQAQAVVGDLLLELHCEAPRGSPILYWFYHEDVTLGSSSAPSGGEASFNLSTAEHSGNYS
CEANGLVAQHSDTISLSVIVPVSRLTFRAPQAQAVVGDLLLELHCEALRGSPILYWFYHEDVTLG
KISAPSGGGASFNLSTTEHSGIYSCEADNGLEAQRSEMVTLKVA (amino acids 566 to
746 of SEQ ID NO: 13)

20 These epitopes, and the extracellular domains where they are found are also shown in Fig. 8.

In one embodiment, an antibody that binds IRTA2 specifically binds to amino acid residues 1 to 109 of the amino acid sequence set forth as SEQ ID NO:13. In another embodiment, the antibody specifically binds to amino acid residues 1 to 190 of the amino acid sequence set forth as SEQ ID NO:13. In yet another embodiment, the antibody
25 specifically binds to amino acid residues 191 to 286 of the amino acid sequence set forth as SEQ ID NO:13. In yet another embodiment, the antibody specifically binds to amino acid residues 287 to 565 of the amino acid sequence set forth as SEQ ID NO:13. In yet another embodiment, the antibody specifically binds to amino acid residues 566 to 746 of the amino acid sequence set forth as SEQ ID NO:13.

The location of the CDRs is further shown in Fig. 6. These are set forth as follows:

Antibody	chain	Sequence
F119	V _H -CDR1	Amino acid 31 to 35 of SEQ ID NO: 1
F119	V _H CDR2	Amino acid 50 to 65 of SEQ ID NO: 1
F119	V _H -CDR3	Amino acid 95 to 105 of SEQ ID NO: 1*
F119	V _L -CDR1	Amino acid 24-35 of SEQ ID NO: 2*
F119	V _L -CCR2	Amino acid 51-57 of SEQ ID NO: 2
F119	V _L -CDR3	Amino acid 90-98 of SEQ ID NO: 2
F56	V _H -CDR1	Amino acid 31 to 35 of SEQ ID NO: 3
F56	V _H CDR2	Amino acid 50 to 65 of SEQ ID NO: 3
F56	V _H -CDR3	Amino acid 95 to 102 of SEQ ID NO: 3*
F56	V _L -CDR1	Amino acid 24-34 of SEQ ID NO: 4
F56	V _L -CCR2	Amino acid 50-56 of SEQ ID NO: 4
F56	V _L -CDR3	Amino acid 89-97 of SEQ ID NO: 4
F25	V _H -CDR1	Amino acid 31 to 35 of SEQ ID NO: 5
F25	V _H CDR2	Amino acid 50 to 66 of SEQ ID NO: 5
F25	V _H -CDR3	Amino acid 96 to 101 of SEQ ID NO: 5*
F25	V _L -CDR1	Amino acid 24-34 of SEQ ID NO: 6
F25	V _L -CCR2	Amino acid 50-56 of SEQ ID NO: 6
F25	V _L -CDR3	Amino acid 89-97 of SEQ ID NO: 6

* numbering slightly different from the numbers shown in Fig. 6 due to presence of additional residues in the alignment (indicated by letters A-F in Fig. 6).

5

The production of chimeric antibodies, which include a framework region from one antibody and the CDRs from a different antibody, is well known in the art. For example, humanized antibodies can be routinely produced. The antibody or antibody fragment can be a humanized immunoglobulin having complementarity determining regions (CDRs) from a donor monoclonal antibody that binds IRTA2 (such as those from F1, F44 or F54, produced by the above-described hybridomas deposited with ATCC, or F25, F56 or F119, see the above table) and immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chain frameworks. Generally, the humanized immunoglobulin specifically binds to IRTA2 with an affinity constant of at least 10^7 M^{-1} , such as at least 10^8 M^{-1} at least $5 \times 10^8 \text{ M}^{-1}$ or at least 10^9 M^{-1} .

15

Humanized monoclonal antibodies can be produced by transferring donor complementarity determining regions (CDRs) from heavy and light variable chains of the donor mouse immunoglobulin (such as F1, F25, F44, F54, F56 or F119) into a human variable domain, and then substituting human residues in the framework regions when
5 required to retain affinity. The use of antibody components derived from humanized monoclonal antibodies obviates potential problems associated with the immunogenicity of the constant regions of the donor antibody. Techniques for producing humanized monoclonal antibodies are described, for example, by Jones et al., *Nature* 321:522, 1986; Riechmann et al., *Nature* 332:323, 1988; Verhoeyen et al., *Science* 239:1534, 1988; Carter
10 et al., *Proc. Nat'l Acad. Sci. U.S.A.* 89:4285, 1992; Sandhu, *Crit. Rev. Biotech.* 12:437, 1992; and Singer et al., *J. Immunol.* 150:2844, 1993. The antibody may be of any isotype, but in several embodiments the antibody is an IgG, including but not limited to, IgG₁, IgG₂, IgG₃ and IgG₄.

In one embodiment, the sequence of the humanized immunoglobulin heavy chain variable region framework can be at least about 65% identical to the sequence of the donor
15 immunoglobulin heavy chain variable region framework. Thus, the sequence of the humanized immunoglobulin heavy chain variable region framework can be at least about 75%, at least about 85%, at least about 99% or at least about 95%, identical to the sequence of the donor immunoglobulin heavy chain variable region framework. Human framework
20 regions, and mutations that can be made in a humanized antibody framework regions, are known in the art (see, for example, in U.S. Patent No. 5,585,089, which is incorporated herein by reference).

Exemplary human antibodies are LEN and 21/28 CL. The sequences of the heavy and light chain frameworks are known in the art. Exemplary light chain frameworks of
25 human mAb LEN have the following sequences:

FR1: DIVMTQS PDSLAVSLGERATINC (SEQ ID NO: 21)

FR2: WYQQKPGQPPLLIY (SEQ ID NO: 22)

FR3: GVPDRPFGSGSGTDFTLTISSLQAEDVAVYYC (SEQ ID NO: 23)

30 FR4: FGQGQTKLEIK (SEQ ID NO: 24)

Exemplary heavy chain frameworks of human mAb 21/28' CL have the following sequences:

35 FR1: QVQLVQSGAEVKKPQASVKVSCASQYTFT (SEQ ID NO: 25)

FR2: WVRQAPGQRLEWMG (SEQ ID NO: 26)

FR3: RVTITRDTSASTAYMELSSLRSEDVAVYYCAR (SEQ ID NO: 27)

FR4: WGQGTLVTVSS (SEQ ID NO: 28).

Antibodies, such as murine monoclonal antibodies, chimeric antibodies, and
5 humanized antibodies, include full length molecules as well as fragments thereof, such as Fab, F(ab')₂, and Fv which include a heavy chain and light chain variable region and are capable of binding the epitopic determinant. These antibody fragments retain some ability to selectively bind with their antigen or receptor. These fragments include:

(1) Fab, the fragment which contains a monovalent antigen-binding fragment of
10 an antibody molecule, can be produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain;

(2) Fab', the fragment of an antibody molecule can be obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain; two Fab' fragments are obtained per antibody molecule;

15 (3) (Fab')₂, the fragment of the antibody that can be obtained by treating whole antibody with the enzyme pepsin without subsequent reduction; F(ab')₂ is a dimer of two Fab' fragments held together by two disulfide bonds;

(4) Fv, a genetically engineered fragment containing the variable region of the light chain and the variable region of the heavy chain expressed as two chains; and

20 (5) Single chain antibody (such as scFv), defined as a genetically engineered molecule containing the variable region of the light chain, the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule.

Methods of making these fragments are known in the art (see for example, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York,
25 1988). In several examples, the variable region included in the immunotoxin is an F1, F54, F44, F119, F56 or F25 Fv, which includes the variable region of the light chain and the variable region of the heavy chain expressed as individual polypeptides. In one group of embodiments, the antibodies have V_L and V_H regions having the amino acid sequences shown above (for example, see SEQ ID NO: 1 and SEQ ID NO: 2; SEQ ID NO: 3 and SEQ
30 ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, or the sequences for V_L and V_H regions for antibodies F1, F44, or F54 (see above). Fv antibodies are typically about 25 kDa and contain a complete antigen-binding site with three CDRs per each heavy chain and each light chain. To produce these antibodies, the V_H and the V_L can be expressed from two individual nucleic acid constructs in a host cell. If the V_H and the V_L are expressed non-
35 contiguously, the chains of the Fv antibody are typically held together by noncovalent

interactions. However, these chains tend to dissociate upon dilution, so methods have been developed to crosslink the chains through glutaraldehyde, intermolecular disulfides, or a peptide linker. Thus, in one example, the Fv can be a disulfide stabilized Fv (dsFv), wherein the heavy chain variable region and the light chain variable region are chemically
5 linked by disulfide bonds.

In an additional example, the Fv fragments comprise V_H and V_L chains connected by a peptide linker. These single-chain antigen binding proteins (sFv) are prepared by constructing a structural gene comprising DNA sequences encoding the V_H and V_L domains connected by an oligonucleotide. The structural gene is inserted into an expression vector,
10 which is subsequently introduced into a host cell such as *E. coli*. The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing sFvs are known in the art (see Whitlow et al., *Methods: a Companion to Methods in Enzymology*, Vol. 2, page 97, 1991; Bird et al., *Science* 242:423, 1988; U.S. Patent No. 4,946,778; Pack et al., *Bio/Technology* 11:1271, 1993; and Sandhu,
15 *supra*).

Antibody fragments can be prepared by proteolytic hydrolysis of the antibody or by expression in *E. coli* of DNA encoding the fragment. Antibody fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods. For example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to
20 provide a 5S fragment denoted F(ab')₂. This fragment can be further cleaved using a thiol reducing agent; and optionally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab' monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab' fragments and an Fc fragment directly (see U.S. Patent No. 4,036,945 and U.S. Patent No. 4,331,647, and
25 references contained therein; Nisonhoff et al., *Arch. Biochem. Biophys.* 89:230, 1960; Porter, *Biochem. J.* 73:119, 1959; Edelman et al., *Methods in Enzymology*, Vol. 1, page 422, Academic Press, 1967; and Coligan et al. at sections 2.8.1-2.8.10 and 2.10.1-2.10.4).

Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic,
30 chemical, or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

One of skill will realize that conservative variants of the antibodies can be produced. Such conservative variants employed in antibody fragments, such as dsFv fragments or in scFv fragments, will retain critical amino acid residues necessary for correct
35 folding and stabilizing between the V_H and the V_L regions, and will retain the charge

characteristics of the residues in order to preserve the low pI and low toxicity of the molecules. Amino acid substitutions (such as at most one, at most two, at most three, at most four, or at most five amino acid substitutions) can be made in the V_H and the V_L regions to increase yield. Conservative amino acid substitution tables providing
5 functionally similar amino acids are well known to one of ordinary skill in the art. The following six groups are examples of amino acids that are considered to be conservative substitutions for one another:

- 1) Alanine (A), Serine (S), Threonine (T);
- 2) Aspartic acid (D), Glutamic acid (E);
- 10 3) Asparagine (N), Glutamine (Q);
- 4) Arginine (R), Lysine (K);
- 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and
- 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

Thus, one of skill in the art can readily review SEQ ID NOs: 1-6, or the sequences of the F1,
15 F44 and/or F54 antibody, locate one or more of the amino acids in the brief table above, identify a conservative substitution, and produce the conservative variant using well-known molecular techniques.

Effector molecules, such as therapeutic, diagnostic, or detection moieties can be linked to an antibody that specifically binds IRTA2, using any number of means known to
20 those of skill in the art. Both covalent and noncovalent attachment means may be used. The procedure for attaching an effector molecule to an antibody varies according to the chemical structure of the effector. Polypeptides typically contain a variety of functional groups; e.g., carboxylic acid (COOH), free amine (-NH₂) or sulfhydryl (-SH) groups, which are available for reaction with a suitable functional group on an antibody to result in the
25 binding of the effector molecule. Alternatively, the antibody is derivatized to expose or attach additional reactive functional groups. The derivatization may involve attachment of any of a number of linker molecules such as those available from Pierce Chemical Company, Rockford, IL. The linker can be any molecule used to join the antibody to the effector molecule. The linker is capable of forming covalent bonds to both the antibody and
30 to the effector molecule. Suitable linkers are well known to those of skill in the art and include, but are not limited to, straight or branched-chain carbon linkers, heterocyclic carbon linkers, or peptide linkers. Where the antibody and the effector molecule are polypeptides, the linkers may be joined to the constituent amino acids through their side groups (e.g., through a disulfide linkage to cysteine) or to the alpha carbon amino and
35 carboxyl groups of the terminal amino acids.

In some circumstances, it is desirable to free the effector molecule from the antibody when the immunoconjugate has reached its target site. Therefore, in these circumstances, immunoconjugates will comprise linkages that are cleavable in the vicinity of the target site. Cleavage of the linker to release the effector molecule from the antibody
5 may be prompted by enzymatic activity or conditions to which the immunoconjugate is subjected either inside the target cell or in the vicinity of the target site. When the target site is a tumor, a linker which is cleavable under conditions present at the tumor site (e.g. when exposed to tumor-associated enzymes or acidic pH) may be used.

In view of the large number of methods that have been reported for attaching a
10 variety of radiodiagnostic compounds, radiotherapeutic compounds, label (e.g. enzymes or fluorescent molecules) drugs, toxins, and other agents to antibodies one skilled in the art will be able to determine a suitable method for attaching a given agent to an antibody or other polypeptide.

Therapeutic agents include various drugs such as vinblastine, daunomycin and the
15 like, and effector molecules such as cytotoxins such as native or modified *Pseudomonas* exotoxin or Diphtheria toxin, encapsulating agents, (e.g., liposomes) which themselves contain pharmacological compositions, target moieties and ligands. The choice of a particular therapeutic agent depends on the particular target molecule or cell and the biological effect desired to be evoked. Thus, for example, the therapeutic agent may be an
20 effector molecule that is cytotoxin which is used to bring about the death of a particular target cell. Conversely, where it is merely desired to invoke a non-lethal biological response, a therapeutic agent can be conjugated to a non-lethal pharmacological agent or a liposome containing a non-lethal pharmacological agent.

Toxins can be employed with antibodies that specifically bind IRTA2 and
25 fragments of these antibodies, such as a svFv or a dsFv, to yield chimeric molecules, which are of use as immunotoxins. Exemplary toxins include *Pseudomonas* exotoxin (PE), ricin, abrin, diphtheria toxin and subunits thereof, ribotoxin, ribonuclease, saporin, and calicheamicin, as well as botulinum toxins A through F. These toxins are well known in the art and many are readily available from commercial sources (for example, Sigma Chemical
30 Company, St. Louis, MO).

Diphtheria toxin is isolated from *Corynebacterium diphtheriae*. Typically, diphtheria toxin for use in immunotoxins is mutated to reduce or to eliminate non-specific toxicity. A mutant known as CRM107, which has full enzymatic activity but markedly reduced non-specific toxicity, has been known since the 1970's (Laird and Groman, *J. Virol.*
35 19:220, 1976), and has been used in human clinical trials. See, U.S. Patent No. 5,792,458

and U.S. Patent No. 5,208,021. As used herein, the term “diphtheria toxin” refers as appropriate to native diphtheria toxin or to diphtheria toxin that retains enzymatic activity but which has been modified to reduce non-specific toxicity.

Ricin is the lectin RCA60 from *Ricinus communis* (Castor bean). The term “ricin” also references toxic variants thereof. For example, see, U.S. Patent No. 5,079,163 and U.S. Patent No. 4,689,401. *Ricinus communis* agglutinin (RCA) occurs in two forms designated RCA₆₀ and RCA₁₂₀ according to their molecular weights of approximately 65 and 120 kD, respectively (Nicholson & Blaustein, *J. Biochim. Biophys. Acta* 266:543, 1972). The A chain is responsible for inactivating protein synthesis and killing cells. The B chain binds ricin to cell-surface galactose residues and facilitates transport of the A chain into the cytosol (Olsnes et al., *Nature* 249:627-631, 1974 and U.S. Patent No. 3,060,165).

Ribonucleases have also been conjugated to targeting molecules for use as immunotoxins (see Suzuki et al., *Nat Biotech* 17:265-70, 1999). Exemplary ribotoxins such as α -sarcin and restrictocin are discussed in, e.g., Rathore et al., *Gene* 190:31-5, 1997; and Goyal and Batra, *Biochem* 345 Pt 2:247-54, 2000. Calicheamicins were first isolated from *Micromonospora echinospora* and are members of the enediyne antitumor antibiotic family that cause double strand breaks in DNA that lead to apoptosis (see, e.g., Lee et al., *J. Antibiot* 42:1070-87, 1989). The drug is the toxic moiety of an immunotoxin in clinical trials (see, e.g., Gillespie et al., *Ann Oncol* 11:735-41, 2000).

Abrin includes toxic lectins from *Abrus precatorius*. The toxic principles, abrin a, b, c, and d, have a molecular weight of from about 63 and 67 kD and are composed of two disulfide-linked polypeptide chains A and B. The A chain inhibits protein synthesis; the B chain (abrin-b) binds to D-galactose residues (see, Funatsu et al., *Agr. Biol. Chem.* 52:1095, 1988; and Olsnes, *Methods Enzymol.* 50:330-335, 1978).

In one embodiment, the toxin is *Pseudomonas* exotoxin (PE). Native *Pseudomonas* exotoxin A (“PE”) is an extremely active monomeric protein (molecular weight 66 kD), secreted by *Pseudomonas aeruginosa*, which inhibits protein synthesis in eukaryotic cells. The native PE sequence and the sequence of modified PE are provided in U.S. Patent No. 5,602,095, incorporated herein by reference. In one embodiment, native PE has a sequence set forth as:

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AEEAFDLWNE CAKACVLDLK DGVRSSRMSV DPAIADTNGQ GVLHYSMVLE GGNDALKLAI
DNALSITSDG LTIRLEGGVE PNKPVRYSYT RQARGSWSLN WLVPIGHEKP SNIKVFIHEL
NAGNQLSHMS PIYTIEMGDE LLAKLARDAT FFVRAHESNE MQPTLAISHA GVSVVMAQTQ
PRREKRWSEW ASGKVLCLLD PLDGVYNYLA QQRCNLDDTW EGKIYRVLAG NPAKHDLDIK
PTVISHRLHF PEGGSLAALT AHQACHLPLE TFTRHRQPRG WEQLEQCGYP VQRLVALYLA

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5 ARLSWNQVDQ VIRNALASPG SGGDLGEAIR EQPEQARLAL TLAAAESERF VRQGTGNDEA
 GAANADVSL TCPVAAGECA GPADSGDALL ERNYPTGAEF LGDGGDVSFS TRGTQNWTV
 RLLQAHROLE ERGYVFGYH GTFLEAAQSI VFGGVRARSQ DLDAIWRGFY IAGDPALAYG
 YAQDQEPDAR GRIRNGALLR VYVPRSSLPG FYRTSLTLAA PEAAGEVERL IGHPLPLRLD
 AITGPEEEGG RLETILGWPL AERTVVIPIA IPTDPRNVGG DLDPSSIPIK EQAISALPDY
 ASQPGKPPRE DLK (SEQ ID NO: 18)

The method of action of PE is inactivation of the ADP-ribosylation of elongation
 factor 2 (EF-2). The exotoxin contains three structural domains that act in concert to cause
 10 cytotoxicity. Domain Ia (amino acids 1-252) mediates cell binding. Domain II (amino
 acids 253-364) is responsible for translocation into the cytosol and domain III (amino acids
 400-613) mediates ADP ribosylation of elongation factor 2. The function of domain Ib
 (amino acids 365-399) remains undefined, although a large part of it, amino acids 365-380,
 can be deleted without loss of cytotoxicity. See Siegall et al., *J. Biol. Chem.* 264:14256-
 15 14261, 1989.

The term "*Pseudomonas* exotoxin" ("PE") as used herein refers as appropriate to a
 full-length native (naturally occurring) PE or to a PE that has been modified. Such
 modifications may include, but are not limited to, elimination of domain Ia, various amino
 acid deletions in domains Ib, II and III, single amino acid substitutions and the addition of
 20 one or more sequences at the carboxyl terminus, such as KDEL (SEQ ID NO: 19) and
 REDL (SEQ ID NO: 20), see Siegall et al., *supra*. In several examples, the cytotoxic
 fragment of PE retains at least 50%, preferably 75%, more preferably at least 90%, and most
 preferably 95% of the cytotoxicity of native PE. In one embodiment, the cytotoxic fragment
 is more toxic than native PE.

25 Thus, the PE used in the immunotoxins disclosed herein includes the native
 sequence, cytotoxic fragments of the native sequence, and conservatively modified variants
 of native PE and its cytotoxic fragments. Cytotoxic fragments of PE include those which
 are cytotoxic with or without subsequent proteolytic or other processing in the target cell
 (e.g., as a protein or pre-protein). Cytotoxic fragments of PE known in the art include PE40,
 30 PE38, and PE35.

In several embodiments, the PE has been modified to reduce or eliminate non-
 specific cell binding, typically by deleting domain Ia, as taught in U.S. Patent No.
 4,892,827, although this can also be achieved, for example, by mutating certain residues of
 domain Ia. U.S. Patent No. 5,512,658, for instance, discloses that a mutated PE in which
 35 Domain Ia is present but in which the basic residues of domain Ia at positions 57, 246, 247,
 and 249 are replaced with acidic residues (glutamic acid, or "E") exhibits greatly diminished
 non-specific cytotoxicity. This mutant form of PE is sometimes referred to as PE4E.

PE40 is a truncated derivative of PE (see, Pai et al., *Proc. Nat'l Acad. Sci. USA* 88:3358-62, 1991; and Kondo et al., *J. Biol. Chem.* 263:9470-9475, 1988). PE35 is a 35 kD carboxyl-terminal fragment of PE in which amino acid residues 1-279 have been deleted and the molecule commences with a met at position 280 followed by amino acids 281-364 and 381-613 of native PE. PE35 and PE40 are disclosed, for example, in U.S. Patent No. 5,602,095 and U.S. Patent No. 4,892,827.

In some embodiments, the cytotoxic fragment PE38 is employed. PE38 is a truncated PE pro-protein composed of amino acids 253-364 and 381-613 of SEQ ID NO: 16 which is activated to its cytotoxic form upon processing within a cell (see e.g., U.S. Patent No. 5,608,039, and Pastan et al., *Biochim. Biophys. Acta* 1333:C1-C6, 1997).

While in some embodiments, the PE is PE4E, PE40, or PE38, any form of PE in which non-specific cytotoxicity has been eliminated or reduced to levels in which significant toxicity to non-targeted cells does not occur can be used in the immunotoxins disclosed herein so long as it remains capable of translocation and EF-2 ribosylation in a targeted cell.

Conservatively modified variants of PE or cytotoxic fragments thereof have at least 80% sequence similarity, preferably at least 85% sequence similarity, more preferably at least 90% sequence similarity, and most preferably at least 95% sequence similarity at the amino acid level, with the PE of interest, such as PE38.

Nucleic acids encoding the amino acid sequences of the antibodies and immunotoxins are provided herein. For example an exemplary nucleic acid sequence encoding the F119 V_H is provided as

GATGTGCAGCTGGTGGAGTCTGGGGGAGGCTTAGTGCAGCCTGGAGGGTCCCGGAACTCTCCTGTACAGCCTC
TGGATTCACTTTCAGTAGCTTTGGAATGCACTGGGTTCGTCAGGCTCCAGAGAAGGGGCTGGAGTGGGTCGCAT
ACATTAGTAGTGGCAGTAATAACATCTACTTTGCGGACACAGTGAAGGGCCGATTACCATCTCCAGAGACAAT
CCCAAGAACACCCTGTTCTCTGCAAATGACCAGTCTAAGGTCTGAGGACACGGCCATGTATTACTGTGCAAGATC
GGAATACTACGGTAGTAGCCATATGGACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCA (SEQ ID

NO: 7), an exemplary nucleic acid sequence encoding F119 V_L is provided as

AGTATTGTGATGACCCAGACTCCCAAATTCCTGCTTGTATCAGCAGGAGACAGGGTTACCATAACCTGCAAGGC
CAGTCAGAGTGTGAGTAATGATGTAGCTTGGTTCCAACAGAAGCCAGGGCAGTCTCCTAAACTGCTGATATACT
ATGCATCCAATCACTACACTGGAGTCCCTGATCGCTTCACTGGCAGTGGATATGGGACGGATTCACTTTACCA
TCAGCACTGTGCAGGCTGAAGACCTGGCAGTTTATTTCTGTGTCAGCAGGATTATAGCTCTCCTCCGACGTTCCGGTG
GAGGCACCAAGCTGGAAATCAAACGGGCTGATGCTGCA (SEQ ID NO: 8, an exemplary nucleic acid
sequence encoding the F56 V_H is provided as

GATGTGCACCTGGTGGAGTCTGGGGGAGGCTTAGTGCAGCCTGGAGGGTCCCGGAACTCTCCTGTGCAGCCTC
TGGATTCACTTTCAGTATCTTTGGATTGCACTGGGTTCGTCAGGCTCCAGAGAAGGGGCTGGAGTGGGTCGCATA
CATTAGTGGTGACAGTAATACCATCTACTATGCAGACACAGTGAAGGGCCGATTACCATCTCCAGAGACAATC
CCAAGAACACCCTGTTCTCTGCAAATGACCAGTCTAAGGTCTGAGGACACGGCCATGTATTACTGTGCAAGAAAT

AGCTACTATGCTCTGGACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCA (SEQ ID NO: 9), an exemplary nucleic acid sequence encoding F56 V_L is provided as

GAAAATGTGTTACCCAGTCTCCAGCAATCATGTCTGTATCTCCAGGTGAAAAGGTCACCATGACCTGCAGGGC
CAGCTCAAGTGTGAGTTCCAGTTACTTGCACTGGTACCAGCAGAGGTCAGGTGCCTCCCCAAAATCTGGATTTA
5 TAGCACATCCAACCTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGACTGGGACCTCTTACTCTCTCAC
AATCAGCAGTGTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAGCAGTACAGTGGTTACCCGTGGACGTTTCG
GTGGAGGCACCAAGTTGGAAATCAAACGGGCTGATGCTGCA (SEQ ID NO: 10), an exemplary nucleic
acid sequence encoding the F25 V_H is provided as

GAGGTTCAACTGCAGCAGTCTGGACCTGAGCTGGTGAAGCCTGGGACTTCAGTGAAGATGTCCTGCAAGGCTTC
10 TGGTTACTCATTTACTGGCTACTTTATGAACTGGATGATGCAGAACCATGGAAAGAGCCTTGAGTGGATTGGAC
GTATTAATCTTTACAATGGTGATACTTTCTACAACCAGAAGTTCAAGGACAAGGCCACATTGACTGTGGACAAA
TCCTCTAACACAGTCCACATGGAGTTCCGGAGCCTGGCATCTGAGGACTCTGCAGTCTATTATTGTGCAAGTAGT
CTGTACTACTTTGACTACTGGGGCCAAGGCACCACTTTCACAGTCTCCTCA (SEQ ID NO: 11), an

exemplary nucleic acid sequence encoding F25 V_L is provided as

GACATTGTGATGACCCAGTCTCACAATTCATGTCCACATCAGTAGGAGACAGGGTCAATATTACCTGTAAGGC
15 CAGTCAGGATGTGGGTACTAATGTAGCCTGGTATCAACAGAAACCAGGGCAATCTCCTAAACTACTGATTTACT
GGGCATCCACCCGGCAGACTGGAGTCCCTGATCGCTTCACAGGCAGTGGATCTGGGACAGATTTCACTCTCACC
ATTAGCAATGTGCACTGAAGACTTGTCAGATTATTTCTGTCAGCAATATAGCAGCTATCCTCTCACGTTCCGGT
GCTGGGACCAAGCTGGAGCTGAAACGGGCTGATGCTGCA (SEQ ID NO: 12). Other nucleic acids

20 encoding antibodies including one or more CDRs from F25, F56, and/or F119 can readily be
produced by one of skill in the art, using the amino acid sequences provided herein, and the
genetic code. Nucleic acids encoding the amino acid sequences of the antibodies produced
by F1, F44 and F54 can be readily determined by one of skill in the art. In addition, one of
skill can readily construct a variety of clones containing functionally equivalent nucleic
25 acids, such as nucleic acids which differ in sequence but which encode the same effector
molecule ("EM") or antibody sequence. Thus, nucleic acids encoding antibodies,
conjugates and fusion proteins are provided herein.

Nucleic acid sequences encoding the antibodies and/or immunotoxins can be
prepared by any suitable method including, for example, cloning of appropriate sequences
30 or by direct chemical synthesis by methods such as the phosphotriester method of Narang et
al., *Meth. Enzymol.* 68:90-99, 1979; the phosphodiester method of Brown et al., *Meth.*
Enzymol. 68:109-151, 1979; the diethylphosphoramidite method of Beaucage et al., *Tetra.*
Lett. 22:1859-1862, 1981; the solid phase phosphoramidite triester method described by
Beaucage & Caruthers, *Tetra. Letts.* 22(20):1859-1862, 1981, e.g., using an automated
35 synthesizer as described in, for example, Needham-VanDevanter et al., *Nucl. Acids Res.*
12:6159-6168, 1984; and, the solid support method of U.S. Patent No. 4,458,066. Chemical
synthesis produces a single stranded oligonucleotide. This can be converted into double
stranded DNA by hybridization with a complementary sequence, or by polymerization with

a DNA polymerase using the single strand as a template. One of skill would recognize that while chemical synthesis of DNA is generally limited to sequences of about 100 bases, longer sequences may be obtained by the ligation of shorter sequences.

Exemplary nucleic acids encoding sequences encoding an antibody that specifically
5 binds IRTA2, such as an immunotoxin, can be prepared by cloning techniques. Examples of appropriate cloning and sequencing techniques, and instructions sufficient to direct persons of skill through many cloning exercises are found in Sambrook et al., *supra*, Berger and Kimmel (eds.), *supra*, and Ausubel, *supra*. Product information from manufacturers of biological reagents and experimental equipment also provide useful information. Such
10 manufacturers include the SIGMA Chemical Company (Saint Louis, MO), R&D Systems (Minneapolis, MN), Pharmacia Amersham (Piscataway, NJ), CLONTECH Laboratories, Inc. (Palo Alto, CA), Chem Genes Corp., Aldrich Chemical Company (Milwaukee, WI), Glen Research, Inc., GIBCO BRL Life Technologies, Inc. (Gaithersburg, MD), Fluka Chemica-Biochemika Analytika (Fluka Chemie AG, Buchs, Switzerland), Invitrogen (San
15 Diego, CA), and Applied Biosystems (Foster City, CA), as well as many other commercial sources known to one of skill.

Nucleic acids can also be prepared by amplification methods. Amplification methods include polymerase chain reaction (PCR), the ligase chain reaction (LCR), the transcription-based amplification system (TAS), the self-sustained sequence replication
20 system (3SR). A wide variety of cloning methods, host cells, and *in vitro* amplification methodologies are well known to persons of skill.

In one example, an antibody of use is prepared by inserting the cDNA which encodes a variable region from an antibody that specifically binds IRTA2 into a vector which comprises the cDNA encoding an effector molecule (EM). The insertion is made so
25 that the variable region and the EM are read in frame so that one continuous polypeptide is produced. Thus, the encoded polypeptide contains a functional Fv region and a functional EM region. In one embodiment, cDNA encoding a cytotoxin is ligated to a scFv so that the cytotoxin is located at the carboxyl terminus of the scFv. In one example, cDNA encoding a *Pseudomonas* exotoxin ("PE"), mutated to eliminate or to reduce non-specific binding, is
30 ligated to a scFv so that the toxin is located at the amino terminus of the scFv. In another example, PE38 is located at the amino terminus of the scFv. In a further example, cDNA encoding a cytotoxin is ligated to a heavy chain variable region of an antibody that specifically binds IRTA2, so that the cytotoxin is located at the carboxyl terminus of the heavy chain variable region. The heavy chain-variable region can subsequently be ligated to
35 a light chain variable region of the antibody that specifically binds IRTA2 using disulfide

bonds. In a yet another example, cDNA encoding a cytotoxin is ligated to a light chain variable region of an antibody that binds IRTa2, so that the cytotoxin is located at the carboxyl terminus of the light chain variable region. The light chain-variable region can subsequently be ligated to a heavy chain variable region of the antibody that specifically binds IRTA2 using disulfide bonds.

Once the nucleic acids encoding the immunotoxin, antibody, or fragment thereof are isolated and cloned, the protein can be expressed in a recombinantly engineered cell such as bacteria, plant, yeast, insect and mammalian cells. One or more DNA sequences encoding the immunotoxin, antibody, or fragment thereof can be expressed *in vitro* by DNA transfer into a suitable host cell. The cell may be prokaryotic or eukaryotic. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. Methods of stable transfer, meaning that the foreign DNA is continuously maintained in the host, are known in the art.

Polynucleotide sequences encoding the immunotoxin, antibody, or fragment thereof, can be operatively linked to expression control sequences. An expression control sequence operatively linked to a coding sequence is ligated such that expression of the coding sequence is achieved under conditions compatible with the expression control sequences. The expression control sequences include, but are not limited to appropriate promoters, enhancers, transcription terminators, a start codon (i.e., ATG) in front of a protein-encoding gene, splicing signal for introns, maintenance of the correct reading frame of that gene to permit proper translation of mRNA, and stop codons.

The polynucleotide sequences encoding the immunotoxin, antibody, or fragment thereof can be inserted into an expression vector including, but not limited to a plasmid, virus or other vehicle that can be manipulated to allow insertion or incorporation of sequences and can be expressed in either prokaryotes or eukaryotes. Hosts can include microbial, yeast, insect and mammalian organisms. Methods of expressing DNA sequences having eukaryotic or viral sequences in prokaryotes are well known in the art. Biologically functional viral and plasmid DNA vectors capable of expression and replication in a host are known in the art.

Transformation of a host cell with recombinant DNA may be carried out by conventional techniques as are well known to those skilled in the art. Where the host is prokaryotic, such as *E. coli*, competent cells which are capable of DNA uptake can be prepared from cells harvested after exponential growth phase and subsequently treated by the CaCl_2 method using procedures well known in the art. Alternatively, MgCl_2 or RbCl

can be used. Transformation can also be performed after forming a protoplast of the host cell if desired, or by electroporation.

When the host is a eukaryote, such methods of transfection of DNA as calcium phosphate coprecipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors may be used. Eukaryotic cells can also be cotransformed with polynucleotide sequences encoding the immunotoxin, antibody, or fragment thereof, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to use a eukaryotic viral vector, such as simian virus 40 (SV40) or bovine papilloma virus, to transiently infect or transform eukaryotic cells and express the protein (see for example, *Eukaryotic Viral Vectors*, Cold Spring Harbor Laboratory, Gluzman ed., 1982). One of skill in the art can readily use an expression systems such as plasmids and vectors of use in producing proteins in cells including higher eukaryotic cells such as the COS, CHO, HeLa and myeloma cell lines.

Isolation and purification of recombinantly expressed polypeptide can be carried out by conventional means including preparative chromatography and immunological separations. Once expressed, the recombinant immunotoxins can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, and the like (see, generally, R. Scopes, *Protein Purification*, Springer-Verlag, N.Y., 1982). Substantially pure compositions of at least about 90 to 95% homogeneity are disclosed herein, and 98 to 99% or more homogeneity can be used for pharmaceutical purposes. Once purified, partially or to homogeneity as desired, if to be used therapeutically, the polypeptides should be substantially free of endotoxin.

Methods for expression of single chain antibodies and/or refolding to an appropriate active form, including single chain antibodies, from bacteria such as *E. coli* have been described and are well-known and are applicable to the antibodies disclosed herein. See, Buchner et al., *Anal. Biochem.* 205:263-270, 1992; Pluckthun, *Biotechnology* 9:545, 1991; Huse et al., *Science* 246:1275, 1989 and Ward et al., *Nature* 341:544, 1989, all incorporated by reference herein.

Often, functional heterologous proteins from *E. coli* or other bacteria are isolated from inclusion bodies and require solubilization using strong denaturants, and subsequent refolding. During the solubilization step, as is well known in the art, a reducing agent must be present to separate disulfide bonds. An exemplary buffer with a reducing agent is: 0.1 M Tris pH 8, 6 M guanidine, 2 mM EDTA, 0.3 M DTE (dithioerythritol). Reoxidation of the disulfide bonds can occur in the presence of low molecular weight thiol reagents in reduced

and oxidized form, as described in Saxena et al., *Biochemistry* 9: 5015-5021, 1970, incorporated by reference herein, and especially as described by Buchner et al., *supra*.

Renaturation is typically accomplished by dilution (e.g., 100-fold) of the denatured and reduced protein into refolding buffer. An exemplary buffer is 0.1 M Tris, pH 8.0, 0.5 M
5 L-arginine, 8 mM oxidized glutathione (GSSG), and 2 mM EDTA.

As a modification to the two chain antibody purification protocol, the heavy and light chain regions are separately solubilized and reduced and then combined in the refolding solution. An exemplary yield is obtained when these two proteins are mixed in a molar ratio such that a 5 fold molar excess of one protein over the other is not exceeded. It
10 is desirable to add excess oxidized glutathione or other oxidizing low molecular weight compounds to the refolding solution after the redox-shuffling is completed.

In addition to recombinant methods, the immunotoxins, EM, and antibodies disclosed herein can also be constructed in whole or in part using standard peptide synthesis. Solid phase synthesis of the polypeptides of less than about 50 amino acids in length can be
15 accomplished by attaching the C-terminal amino acid of the sequence to an insoluble support followed by sequential addition of the remaining amino acids in the sequence. Techniques for solid phase synthesis are described by Barany & Merrifield, *The Peptides: Analysis, Synthesis, Biology. Vol. 2: Special Methods in Peptide Synthesis, Part A.* pp. 3-284; Merrifield et al., *J. Am. Chem. Soc.* 85:2149-2156, 1963, and Stewart et al., *Solid*
20 *Phase Peptide Synthesis, 2nd ed.*, Pierce Chem. Co., Rockford, Ill., 1984. Proteins of greater length may be synthesized by condensation of the amino and carboxyl termini of shorter fragments. Methods of forming peptide bonds by activation of a carboxyl terminal end (e.g., by the use of the coupling reagent N, N'-dicyclohexylcarbodiimide) are well known in the art.

25

Pharmaceutical Compositions and Therapeutic Methods

Compositions are provided that include one or more of the antibodies that specifically bind IRTA2 (for example, an immunotoxin) that are disclosed herein in a carrier. The compositions can be prepared in unit dosage forms for administration to a
30 subject. The amount and timing of administration are at the discretion of the treating physician to achieve the desired purposes. The antibody can be formulated for systemic or local (such as intra-tumor) administration. In one example, the antibody that specifically binds IRTA2 is formulated for parenteral administration, such as intravenous administration. Any of the antibodies disclosed herein, variants thereof and humanized forms thereof can be
35 used in these methods.

The compositions for administration can include a solution of the antibody that specifically binds IRTA2 dissolved in a pharmaceutically acceptable carrier, such as an aqueous carrier. A variety of aqueous carriers can be used, e.g., buffered saline and the like. These solutions are sterile and generally free of undesirable matter. These compositions
5 may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of antibody in these formulations
10 can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration selected and the subject's needs.

A typical pharmaceutical composition for intravenous administration includes about 0.1 to 10 mg of antibody per subject per day. Dosages from 0.1 up to about 100 mg per
15 subject per day may be used, particularly if the agent is administered to a secluded site and not into the circulatory or lymph system, such as into a body cavity or into a lumen of an organ. Actual methods for preparing administrable compositions will be known or apparent to those skilled in the art and are described in more detail in such publications as *Remington's Pharmaceutical Science, 19th ed.*, Mack Publishing Company, Easton, PA
20 (1995).

Antibodies may be provided in lyophilized form and rehydrated with sterile water before administration, although they are also provided in sterile solutions of known concentration. The antibody solution is then added to an infusion bag containing 0.9% Sodium Chloride, USP, and typically administered at a dosage of from 0.5 to 15 mg/kg of
25 body weight. Considerable experience is available in the art in the administration of antibody drugs, which have been marketed in the U.S. since the approval of Rituxan® in 1997. Antibody drugs can be administered by slow infusion, rather than in an intravenous push or bolus. In one example, a higher loading dose is administered, with subsequent, maintenance doses being administered at a lower level. For example, an initial loading dose
30 of 4 mg/kg may be infused over a period of some 90 minutes, followed by weekly maintenance doses for 4-8 weeks of 2 mg/kg infused over a 30 minute period if the previous dose was well tolerated.

The antibody that specifically binds IRTA2 can be administered to slow or inhibit the growth of cells, such as tumor cells. In one embodiment, a method is provided for
35 inhibiting the growth of a B cell of a non-Hodgkin's lymphoma or a hairy cell leukemia.

The method includes contacting the cell with an effective amount of at least one antibody disclosed herein conjugated to an effector molecule.

Methods for measuring cell growth are well known in the art (see, for example, Lewis *et al.* (1996) *Cancer Res.* 56:1457-65; Tian *et al.* (2001) *Nutrition and Cancer* 40:180-184). The method may be performed *in vivo* or *in vitro*.

A method is also provided for treating a hairy cell leukemia or a B cell lymphoma in a subject, including administering to the subject a therapeutically effective amount of at least one antibody disclosed herein conjugated to an effector molecule. In these applications, a therapeutically effective amount of an antibody-effector molecule conjugate, such as an immunotoxin, is administered to a subject in an amount sufficient to inhibit growth of antigen-expressing cells. Suitable subjects include those with a tumor, such as a B cell malignancy (such as a lymphoma or leukemia). These malignancies include B cell lymphoma, multiple myeloma, Burkitt's lymphoma, marginal zone lymphoma, diffuse large cell lymphoma, non-Hodgkin's lymphoma, and hairy cell leukemia and follicular lymphoma. Thus, suitable subjects include subjects that have hairy cell leukemia or non-Hodgkin's lymphoma.

Amounts effective for this use will depend upon the severity of the disease and the general state of the patient's health. A therapeutically effective amount of the antibody is that which provides either subjective relief of a symptom(s) or an objectively identifiable improvement as noted by the clinician or other qualified observer. These compositions can be administered in conjunction with another chemotherapeutic agent, either simultaneously or sequentially.

Single or multiple administrations of the compositions are administered depending on the dosage and frequency as required and tolerated by the patient. In any event, the composition should provide a sufficient quantity of at least one of the antibodies disclosed herein, such as an immunotoxin, to effectively treat the patient. The dosage can be administered once but may be applied periodically until either a therapeutic result is achieved or until side effects warrant discontinuation of therapy. In one example, a dose of the antibody, such as an immunotoxin, is infused for thirty minutes every other day. In this example, about one to about ten doses can be administered, such as three or six doses can be administered every other day. In a further example, a continuous infusion is administered for about five to about ten days. The subject can be treated with the immunotoxin at regular intervals, such as monthly, until a desired therapeutic result is achieved. Generally, the dose is sufficient to treat or ameliorate symptoms or signs of disease without producing unacceptable toxicity to the patient.

Controlled release parenteral formulations can be made as implants, oily injections, or as particulate systems. For a broad overview of protein delivery systems see, Banga, A.J., *Therapeutic Peptides and Proteins: Formulation, Processing, and Delivery Systems*, Technomic Publishing Company, Inc., Lancaster, PA, (1995) incorporated herein by
5 reference. Particulate systems include microspheres, microparticles, microcapsules, nanocapsules, nanospheres, and nanoparticles. Microcapsules contain the therapeutic protein, such as a cytotoxin or a drug, as a central core. In microspheres the therapeutic is dispersed throughout the particle. Particles, microspheres, and microcapsules smaller than
10 about 1 μm are generally referred to as nanoparticles, nanospheres, and nanocapsules, respectively. Capillaries have a diameter of approximately 5 μm so that only nanoparticles are administered intravenously. Microparticles are typically around 100 μm in diameter and are administered subcutaneously or intramuscularly. See, e.g., Kreuter, J., *Colloidal Drug Delivery Systems*, J. Kreuter, ed., Marcel Dekker, Inc., New York, NY, pp. 219-342 (1994); and Tice & Tabibi, *Treatise on Controlled Drug Delivery*, A. Kydonieus, ed., Marcel
15 Dekker, Inc. New York, NY, pp. 315-339, (1992) both of which are incorporated herein by reference.

Polymers can be used for ion-controlled release of the antibody compositions disclosed herein. Various degradable and nondegradable polymeric matrices for use in controlled drug delivery are known in the art (Langer, *Accounts Chem. Res.* 26:537-542,
20 1993). For example, the block copolymer, polaxamer 407, exists as a viscous yet mobile liquid at low temperatures but forms a semisolid gel at body temperature. It has been shown to be an effective vehicle for formulation and sustained delivery of recombinant interleukin-2 and urease (Johnston et al., *Pharm. Res.* 9:425-434, 1992; and Pec et al., *J. Parent. Sci. Tech.* 44(2):58-65, 1990). Alternatively, hydroxyapatite has been used as a microcarrier for
25 controlled release of proteins (Ijntema et al., *Int. J. Pharm.* 112:215-224, 1994). In yet another aspect, liposomes are used for controlled release as well as drug targeting of the lipid-capsulated drug (Betageri et al., *Liposome Drug Delivery Systems*, Technomic Publishing Co., Inc., Lancaster, PA (1993)). Numerous additional systems for controlled delivery of therapeutic proteins are known (see U.S. Patent No. 5,055,303; U.S. Patent No.
30 5,188,837; U.S. Patent No. 4,235,871; U.S. Patent No. 4,501,728; U.S. Patent No. 4,837,028; U.S. Patent No. 4,957,735; U.S. Patent No. 5,019,369; U.S. Patent No. 5,055,303; U.S. Patent No. 5,514,670; U.S. Patent No. 5,413,797; U.S. Patent No. 5,268,164; U.S. Patent No. 5,004,697; U.S. Patent No. 4,902,505; U.S. Patent No. 5,506,206; U.S. Patent No. 5,271,961; U.S. Patent No. 5,254,342 and U.S. Patent No.
35 5,534,496).

Among various uses of the antibodies disclosed herein are disease conditions caused by specific human cells that may be eliminated by the toxic action of an immunotoxin, such as the treatment of malignant cells, including hairy cell leukemia and non-Hodgkin's lymphoma, expressing IRTA2.

5

Diagnostic Methods and Kits

It is disclosed herein that IRTA2 is expressed in hairy leukemia cells and by non-Hodgkin's lymphoma cells. Thus, expression of IRTA2 can be used to diagnose hairy cell leukemia or non-Hodgkin's lymphoma, or can be used to stage these cancers. Thus, methods are provided for detecting B cell non-Hodgkin's lymphoma or hairy cell leukemia in a subject. The method includes contacting a biological sample from the subject with at least one antibody that specifically binds IRTA2, and detecting the presence of bound antibody. In one example, soluble IRTA2 is detected.

A method is also provided herein for detecting IRTA2 in a biological sample. The method includes contacting the sample with one or more of an antibody that specifically binds IRTA2, such as F1, F25, F44, F54 or F119 (or a variant thereof) to form an antibody-IRTA2 complex. The presence or absence of the complex is detected. In one example, the method detects the presence of soluble IRTA2. It should be noted that the method can also include the use of any antibody disclosed in Table 4 or listed in Fig. 8, or a humanized form thereof, or a variant thereof, wherein the antibody binds an epitope of IRTA2.

The sample can be any sample, including, but not limited to, tissue from biopsies, autopsies and pathology specimens. Biological samples also include sections of tissues, such as frozen sections taken for histological purposes. Biological samples further include body fluids, such as blood, serum, spinal fluid or urine. A biological sample is typically obtained from a mammal, such as a rat, mouse, cow, dog, guinea pig, rabbit, or primate. In one embodiment, the primate is macaque, chimpanzee, or a human. In a further embodiment, the subject has a B cell malignancy, such as hairy cell leukemia or non-Hodgkin's lymphoma or is suspected of having such a B cell malignancy.

In one embodiment, a method is provided for detecting IRTA2 in a biological sample, such as a blood, serum, or plasma sample. Kits for detecting a polypeptide will typically comprise an antibody that specifically binds IRTA2, as disclosed herein.

In some embodiments, an antibody fragment, such as an Fv fragment is included in the kit. For *in vivo* uses, the antibody can be a scFv fragment. In a further embodiment, the antibody is labeled (such as with a fluorescent, radioactive, or an enzymatic label).

In one embodiment, a kit includes instructional materials disclosing means of use of an antibody that specifically binds IRTA2. The instructional materials may be written, in an electronic form (e.g. computer diskette or compact disk) or may be visual (e.g. video files). The kits may also include additional components to facilitate the particular application for which the kit is designed. Thus, for example, the kit may additionally contain means of detecting a label (such as enzyme substrates for enzymatic labels, filter sets to detect fluorescent labels, appropriate secondary labels such as a secondary antibody, or the like). The kits may additionally include buffers and other reagents routinely used for the practice of a particular method. Such kits and appropriate contents are well known to those of skill in the art.

In one embodiment, the diagnostic kit comprises an immunoassay. Although the details of the immunoassays may vary with the particular format employed, the method of detecting a IRTA2 in a biological sample generally includes the steps of contacting the biological sample with an antibody which specifically reacts, under immunologically reactive conditions, to an IRTA2 polypeptide. The antibody is allowed to specifically bind under immunologically reactive conditions to form an immune complex, and the presence of the immune complex (bound antibody) is detected directly or indirectly.

Methods of determining the presence or absence of a cell surface marker are well known in the art. For example, the antibodies can be conjugated to other compounds including, but not limited to, enzymes, magnetic beads, colloidal magnetic beads, haptens, fluorochromes, metal compounds, radioactive compounds or drugs. The antibodies can also be utilized in immunoassays such as but not limited to radioimmunoassays (RIAs), enzyme linked immunosorbant assays (ELISA), or immunohistochemical assays. The antibodies can also be used for fluorescence activated cell sorting (FACS). A FACS employs a plurality of color channels, low angle and obtuse light-scattering detection channels, and impedance channels, among other more sophisticated levels of detection, to separate or sort cells (see U.S. Patent No. 5, 061,620).

The antibodies of use in the methods provided include any of the antibodies described above. These antibodies include, but are not limited to, an F1, F44, or F54 antibody (produced by a hybridoma or progeny thereof deposited as ATCC Deposit No. XXXX; ATCC Deposit No. YYYY, or ATCC Deposit No. ZZZZ) or a humanized form thereof. The antibodies of use in the methods disclosed herein also include an antibody that includes both a light and heavy chain with CDR1, CDR2 and CDR3, wherein L-CDR1 comprises the amino acid sequence set forth as amino acids 31 to 35 of SEQ ID NO:5, L-CDR2 comprises the amino acid sequence set forth as amino acids 50 to 66 of SEQ ID

NO:5, and L-CDR3 comprises the amino acid sequence set forth as amino acids 96 to 101
SEQ ID NO:5, and wherein H-CDR1 comprises the amino acid sequence set forth as amino
acids 24 to 34 of SEQ ID NO:6, H-CDR2 comprises the amino acid sequence set forth as
amino acids 50 to 56 of SEQ ID NO:6, and H-CDR3 comprises the amino acid sequence set
5 forth as amino acids 89-97 of SEQ ID NO:6. The antibodies of use in the methods disclosed
herein also include an antibody that includes both a light and heavy chain with CDR1,
CDR2 and CDR3, wherein L-CDR1 comprises the amino acid sequence set forth as amino
acids 31 to 35 of SEQ ID NO:1, L-CDR2 comprises the amino acid sequence set forth as
amino acids 50 to 65 of SEQ ID NO:1, and L-CDR3 comprises the amino acid sequence set
10 forth as amino acids 95 to 105 of SEQ ID NO:1, and wherein H-CDR1 comprises the amino
acid sequence set forth as amino acids 24 to 35 of SEQ ID NO:2, H-CDR2 comprises the
amino acid sequence set forth as amino acids 51 to 57 of SEQ ID NO:2, and H-CDR3
comprises the amino acid sequence set forth as amino acids 90 to 98 of SEQ ID NO:2. The
antibodies of use in the methods disclosed herein also include an antibody that includes
15 antibody comprises both a light and heavy chain with CDR1, CDR2 and CDR3, wherein L-
CDR1 comprises the amino acid sequence set forth as amino acids 31 to 35 of SEQ ID
NO:3, L-CDR2 comprises the amino acid sequence set forth as amino acids 50 to 65 of SEQ
ID NO:3, and L-CDR3 comprises the amino acid sequence set forth as amino acids 95 to
102 of SEQ ID NO:3, and wherein H-CDR1 comprises the amino acid sequence set forth as
20 amino acids 24 to 34 of SEQ ID NO:4, H-CDR2 comprises the amino acid sequence set
forth as amino acids 50 to 56 of SEQ ID NO:4 and H-CDR3 comprises the amino acid
sequence set forth as amino acids 89 to 97 of SEQ ID NO:4. One or more of these
antibodies may be used in the methods provided herein.

In one embodiment, the method is a sandwich ELISA. A sandwich ELISA requires
25 two antibodies that bind to epitopes that do not overlap on the antigen. This can be
accomplished with either two monoclonal antibodies that recognize discrete sites on the
antigen of interest, such as IRTA2. To utilize this assay, one antibody (the "capture"
antibody) is bound to a solid phase, such as but not limited to the bottom of a well on a
microtiter plate, such as a 24, 48 or 96 well plate of polyvinylchloride (PVC). A sample,
30 possibly including the antigen of interest (IRTA2 or a soluble form thereof) is then added
and allowed to complex with the bound antibody. Unbound material is then removed with a
wash, and a labeled second antibody (the "detection" antibody) is allowed to bind to the
antigen, thus completing the "sandwich." The assay can be quantitated by measuring the
amount of labeled second antibody bound to the matrix, through the use of a substrate, such
35 as a colorimetric substrate. Exemplary non-limiting enzymes are those that convert a

colorless substrate to a colored product, such as p-nitrophenylphosphate (pNPP), which is converted to the yellow p-nitrophenol by alkaline phosphatase. Substrates used with peroxidase include 2,2'-azo-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), o-phenylenediamine (OPD) and 3,3',5,5'-tetramethylbenzidine base (TMB), which yield
5 green, orange and blue colors, respectively.

Without being bound by theory, it is believed that an advantage of this method is that the antigen does not need to be purified prior to use, and that these assays are very specific. It should be noted that monoclonal antibody combinations must be paired, indicating that each monoclonal antibody that binds the antigen of interest (IRTA2)
10 specifically binds a distinct epitopes on the antigen (IRTA2) in order to maximize sensitivity.

Protocols for sandwich ELSIAs are well known in the art. One exemplary non-limiting protocol includes binding a purified unlabeled antibody to the bottom of each well on a plate by adding approximately 50 μ L of antibody solution to each well (20 μ g/mL in
15 PBS). Generally, PVC will bind approximately 100ng/well (300 ng/cm²); at least 1 μ g/well can be utilized. The plates are incubated for a sufficient time to allow the antibody to bind, such as overnight at 4 °C. The wells are then washed with a buffer, such as phosphate buffered saline (PBS) to remove unbound antibody. Any remaining sites for protein binding on the microtiter plate are saturated by incubating with blocking buffer. For
20 example, for assays that do not utilize horseradish peroxidase, the wells are 3% bovine serum albumin (BSA)/PBS with 0.02% sodium azide, and incubated for 2 hours to overnight in a humid atmosphere at room temperature. The sample of interest is then added, such as in a volume of about 50 μ L. If dilution is required, dilutions can be made in the blocking buffer (3% BSA/PBS). Incubate for at least 2 hours at room temperature in a
25 humid atmosphere. The wells are then washed, and the labeled second antibody is added. Generally, for accurate quantitation, the second antibody diluted in blocking buffer and is used in excess. The wells are incubated for about 2 hours (or more) and the unbound labeled second antibody is removed by washing with buffer, such as PBS. A commercially available substrate, such as those listed above is then added to the wells and incubated
30 according the manufacturer's instructions. Optical densities at target wavelengths can be measured on a commercially available ELISA plate reader.

In one embodiment, the method includes contacting a first antibody adhered to a solid support with a sample to form an antibody-IRTA2 complex; washing the solid surface to remove any unbound material and contacting the complex with a second antibody. The
35 method includes contacting a second antibody that binds a different epitope of IRTA2 to the

sample, washing to remove any unbound second antibody, and detecting the presence of bound second antibody. In one specific, non-limiting example, the antibody is labeled with an enzyme. In another specific non-limiting example, the second antibody is labeled with avidin or biotin. The binding of the second antibody is then detected using biotinylated or
5 avidinated enzyme, respectively. In one example, the enzyme is horseradish peroxidase. In one embodiment, F1 and F25 are utilized in the assay. For example, F1 can be attached to a solid substrate. In another embodiment, F44 and F54 are utilized in the assay. For example, F44 can be attached to a solid substrate.

In one embodiment, the method includes using a solid surface comprising a first
10 antibody produced by a hybridoma or a progeny thereof deposited as ATCC Deposit No. XXX, or a humanized form of this antibody. The sample is incubated with the solid surface, and after washing, the solid surface is incubated with a second antibody including both a light and heavy chain with CDR1, CDR2 and CDR3, wherein L-CDR1 comprises the amino acid sequence set forth as amino acids 31 to 35 of SEQ ID NO:5, L-CDR2 comprises the
15 amino acid sequence set forth as amino acids 50 to 66 of SEQ ID NO:5, and L-CDR3 comprises the amino acid sequence set forth as amino acids 96 to 101 SEQ ID NO:5, and wherein H-CDR1 comprises the amino acid sequence set forth as amino acids 24 to 34 of SEQ ID NO:6, H-CDR2 comprises the amino acid sequence set forth as amino acids 50 to
20 acids 56 of SEQ ID NO:6, and H-CDR3 comprises the amino acid sequence set forth as amino acids 89-97 of SEQ ID NO:6. In one example, the second antibody is labeled with biotin. Detection of bound IRTA2 is performed by incubating enzyme bound streptavidin such as horseradish peroxidase (HRP) streptavidin, with the solid surface. After washing, a substrate is incubated with the solid surface to detect the presence of bound second antibody, thereby detecting soluble IRTA2.

In another embodiment, the method includes using a solid surface comprising an antibody produced by a hybridoma or a progeny thereof deposited as ATCC Deposit No. YYYY or a humanized form thereof. . The sample is incubated with the solid surface, and after washing, the solid surface is incubated with a second antibody. The second antibody is produced by a hybridoma or a progeny thereof deposited as ATCC) Deposit No. ZZZZ, or
30 is a humanized form of this antibody. In one example, the second antibody is labeled with biotin. Detection of bound IRTA2 is performed by incubating enzyme bound streptavidin with the solid surface. Detection of bound IRTA2 is performed by incubating enzyme bound streptavidin such as horseradish peroxidase (HRP) streptavidin, with the solid surface. After washing a substrate is incubated with the solid surface to detect the presence of bound
35 second antibody, thereby detecting soluble IRTA2.

The disclosure is illustrated by the following non-limiting Examples.

EXAMPLES

Example 1

Materials and Methods

Cells: Nineteen human hematologic cell lines were used in this study (Table 1).

Table 1 Human hematological cell lines used in this study

	Name	Origin	EBV status ^a	Reference or ATCC number ^b
10	JD38	B-NHL ^c (undifferentiated)	—	24
	Karpas 1106	B-NHL (mediastinal lymphoblastic)	—	25
	OCI-Ly2	B-NHL (diffuse large cell type)	—	26
15	OCI-Ly7	B-NHL (diffuse large cell type)	—	26
	OCI-Ly10	B-NHL (immunoblastic)	—	26
	SU-DHL-5	B-NHL (diffuse large cell, noncleaved cell type)	—	27
20	SU-DHL-6	B-NHL (diffuse, mixed small and large cell type)	—	27
	CA46	Burkitt's lymphoma	—	28
	Daudi	Burkitt's lymphoma	+	CCL-213
N	AMALWA	Burkitt's lymphoma	+	CRL-1432
	NC-37	Burkitt's lymphoma	+	CCL-214
25	Raji	Burkitt's lymphoma	+	CCL-86
	Ramos	Burkitt's lymphoma	—	CRL-1596
	CCRF-SB	Lymphoblastic leukemia	+	CCL-120
K	arpas 299	Anaplastic large cell lymphoma	—	29
	L540	Hodgkin's disease	—	30
30	MOLT-4	T-cell lymphoma	—	CRL-1582
	RPMI 6666	Hodgkin's disease	+	CCL-113
	U-937	Histiocytic lymphoma	—	CRL-1593.2

^aEBV, Epstein-Barr virus

35 ^bATCC, American Type Culture Collection, Manassas, VA 20208, <http://www.atcc.org>

^cB-NHL, B-cell non-Hodgkin's lymphoma

CA46, CCRF-SB, JD38, MOLT-4, NAMALWA, NC-37, Raji, Ramos, RPMI 6666, SU-DHL-5, SU-DHL-6 and U-937 cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS; HyClone, Logan, UT). Daudi cells were grown in RPMI 1640 medium supplemented with 20% FBS. Karpas 299 and L540 cells were grown in Iscove's modified Dulbecco's medium (IMDM) supplemented with 10% FBS. OCI-Ly2, OCI-Ly7, OCI-Ly10 and Karpas 1106 cells were maintained in IMDM supplemented with 15% normal human serum. 293T cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% FBS. Sp2/0-neo myeloma cells (Nagata et al., *J. Immunol Methods* 280:59-72, 2003) were used for the fusion partner for the hybridoma formation and maintained in IMDM with 15% FBS. Blood cells were obtained from patients with HCL via an approved protocol.

Primers. The DNA oligo primers are listed in Table 2. The primers were synthesized by Lofstrand Laboratories (Gaithersburg, MD).

Table 2 Oligonucleotide primers used in this study

No.	Name	Sequence ^a
20	1	IR2/-fr1F 5'-CGC GGATCC AAAGCCACCATG CTGCTGTGGGTGATATTACTGG-3' SEQ ID NO: 29
	2	IR2-fr1R 5'-CCCT GAATTC TCTGTAGTCAGTGAGAAGCTGATGG-3' SEQ ID NO: 30
25	3	IR2-fr2F 5'-GAAACCCAGGAAGATTCTCTGCGCACT-3' SEQ ID NO: 31
30	4	IR2-fr2R 5'-ATAGTTTA GCGGCCGC CTGTGGCAAAAGGGCCACTTC-3' SEQ ID NO: 32
	5	IR2-fr3F 5'-GACACAATATCACTCAGTGTATAGTTCC-3' SEQ ID NO: 33
35	6	IR2-fr3R 5'-ATAGTTTA GCGGCCGC TCATCTGTGAGGAGCTGAGGAAGCC-3' SEQ ID NO: 34
40	7	IR1-F 5'-CGC GATATC AAAGCCACCATG CTGCTGTGGGCGTCCTTG-3' SEQ ID NO: 35
	8	IR1-R 5'-ATAGTTTA GCGGCCGC TTAACTTTCTTCATCCTTAGAGCTGATC-3' SEQ ID NO: 36
45	9	IR1-FcR 5'-ATAGTTTA GCGGCCGC CATCTCTGTTGCCTGGGGTCTC-3' SEQ ID NO: 37

10	IR3-fr1F	5'-CGG <u>GATATC</u> AAAGCCACCATGCTTCTGTGGCTGCTGCTGC-3'	SEQ ID NO: 38	
5	11	IR3-fr1R	5'-ATAGTTTA <u>GCGGCCGC</u> CGCCTGTTCTGTTCTGGAAGTTCC-3'	SEQ ID NO: 39
	12	IR3-fr2F	5'-GTCTCGCCCCGTCTCACCCTCAGG-3'	SEQ ID NO: 40
10	13	IR3-fr2R	5'-ATAGTTTA <u>GCGGCCGC</u> CTAGTGGTCTGAGGCCAGTAATACACG-3'	SEQ ID NO: 41
15	14	IR4-fr1F	5'-CGC <u>GGATCC</u> AAAGCCACCATGCTGCTGTGGTCATTGCTGG-3'	SEQ ID NO: 42
	15	IR4-fr1R	5'-ATAGTTTA <u>GCGGCCGC</u> CAGCTGTCATGAGGTCTCTTCTATAGC-3'	SEQ ID NO: 43
20	16	IR4-fr2F	5'-GACTCACAGGATCAGAAAACAGAGCCTCC-3'	SEQ ID NO: 44
	17	IR4-fr2R	5'-ATAGTTTAGCGGCCGCTTATGATTTCTTCACAGAAGAGTAG-3'	SEQ ID NO: 45
25	18	IR5-F	5'-CGGAATTCAAAGCCACCATGCTGCCGAGGCTGTTGCTGT-3'	SEQ ID NO: 46
	19	IR5-R	5'-ATAGTTTAGCGGCCGCTTACATAGCATCTTCATAGTCCACATCTG-3'	SEQ ID NO: 47
30	20	IR5-FcR	5'-ATAGTTTAGCGGCCGCTGAGGTAAGATGATTGCTTCTGGC-3'	SEQ ID NO: 48
35	21	Actin-F	5'-GCATGGGTCAGAAGGAT-3'	SEQ ID NO: 49
	22	Actin-R	5'-CCAATGGTGATGACCTG-3'	SEQ ID NO: 50
40	23	2c-F	5'-AGCCCTTCAGACTCGGACTC-3'	SEQ ID NO: 51
45	24	2c-R	5'-TGGGGCAGCCTAAATCTT-3'	SEQ ID NO: 52

^aRecognition sites of restriction enzymes are squared. They are GGATCC (BamHI), GAATTC (EcoRI), GCGGCCGC (NotI), and GATATC (EcoRV). Initiation codons (ATG) are underlined.

50 **Plasmids.** Five different IRTA cDNAs (IRTA1, IRTA2, IRTA3, IRTA4 and IRTA5) were cloned and a pair of expression vectors was constructed for each cDNA. One encodes the full-length protein under cytomegalovirus (CMV) promoter and the other

encodes the extracellular domain genetically fused with human IgG1 Fc portion. The GenBank accession numbers of reference sequences of IRTA1 to 5 are AF343659, AF343662, AF459027, AF459633 and AF459634, respectively.

The IRTA DNAs were amplified by PCR from cDNA of human spleen (Clontech, Palo Alto, CA) or from Daudi cells (prepared from total RNA using random hexamer and Superscript III Reverse Transcriptase; Invitrogen, Carlsbad, CA). The primers used for PCR are listed in Table 2. The cDNA fragments were digested with the restriction enzymes listed in the table and ligated into the multicloning site of the pcDNA3 plasmid (Invitrogen). For IRTA2, IRTA3, and IRTA4, two or three DNA fragments were cloned into the vector and connected in tandem in the same vector. Finally, full-length cDNAs of 5 different IRTAs were cloned under a CMV-promoter in pcDNA3.

For Fc-fusion proteins, a Not I-Xba I fragment of pRB-2k1-CD30 (Nagata et al., *J. Immunol Methods* 280:59-72, 2003), which contains the hinge and Fc portion of IgG1 with three introns, was subcloned into pcDNA3. To prevent its possible interaction with IRTAs, the first 8 amino acids of the constant region 2 of the Fc (amino acids 231-238) were changed from APELLGGP (SEQ ID NO: X) to APPVAGP (SEQ ID NO: X) by a PCR-based method. These mutations (E233P, L234V, L235V and deletion of G237) reduce the affinity of IgG1 for FcγRI to a non-detectable level (Chappel et al., *Proc Natl Acad Sci USA* 88:9036-40, 1991). DNA fragments that encode the extracellular domains of the IRTAs, including the signal peptides, were amplified by PCR, and then inserted upstream of the human Fc in pcDNA3. The plasmids for the expression of human CD30 (pHRm30c) (Diehl et al., *J Cancer Res Clin Oncol* 101:11-124, 1981) or the extracellular domain of the CD30-Fc fusion protein (pRB-2k1-CD30) (Nagata et al., *Clin Cancer Res* 8:2345-55, 2002) were used as controls in some experiments.

Transfection of 293T Cells with the Plasmids. In a typical experiment, 5×10^5 of 293T cells were seeded in a 10-cm dish (BD Biosciences, Bedford, MA) 24 hours prior to the transfection. Four µg of plasmid DNA was transfected by Lipofectamine and Plus reagent (Invitrogen) according to the manufacturer's instruction. After 48-72 hour transfection, the cells transfected with the plasmids encoding full-length IRTAs or CD30 were used for fluorescence activated cell sorter (FACS) analysis, cell-enzyme-linked immunosorbent assay (cell-ELISA), Western blot or immunofluorescence.

Preparation of the Fc-fusion Proteins. The 293T cells transfected with plasmids encoding Fc-fusion proteins secreted the Fc-fusion proteins in the supernatants. From day 2 to day 8 after transfection, the Fc-fusion proteins in the media were harvested daily. The concentration of the Fc-fusion proteins was measured by a human IgG-specific

sandwich ELISA using purified CD30-Fc as the standard. Several of the Fc-fusion proteins were purified with protein A Sepharose 4 Fast Flow (Amersham Biosciences, Piscataway, NJ).

Production of MAbs. A 6-week-old female Balb/c mouse was DNA-immunized 5 times with the IRTA2 plasmid as previously described (Nagata et al., *J Immunol Methods* 280:59-72, 2003). The mouse was boosted with 293T cells transfected with the same plasmid, and 3 days later the spleen cells were fused with SP2/0-neo myeloma cells as described previously (Nagata et al., *J Immunol Methods* 280:59-72, 2003). The hybridomas were screened for secretion of specific MAbs in an ELISA using IRTA2-Fc antigen. After 10 multiple rounds of cell cloning by limiting dilution, the established hybridomas were grown to a high density to harvest the MAbs in the culture supernatants. The isotype of the MAbs was determined by mouse MAb isotyping reagents (ISO2; Sigma-Aldrich, St. Louis, MO). Ig concentrations in the culture supernatants were determined by a sandwich ELISA as previously described (Nagata et al., *Hybridoma* 10:369-78, 1991).

ELISA. In a typical indirect ELISA for the screening of hybridomas, microtiter plates (MaxiSorp; Nalge Nunc, Rochester, NY) were coated with 100 ng/50 μ l/well of goat anti-human IgG in phosphate buffered saline (PBS) for 2 hours at room temperature. Next, 50 ng/100 μ l/well of IRTA2-Fc in blocking buffer (25% DMEM, 5% FBS, 25 mM HEPES, 0.5% bovine serum albumin, 0.1% sodium azide in PBS) was added to each well and 20 incubated for 2 hours at room temperature. After washing with PBS containing 0.05% Tween 20, 50 μ l of the hybridoma supernatants were added and incubated for 2 hours at room temperature. After washing, the bound MAbs were detected by a 2-hour incubation with horseradish peroxidase (HRP)-labeled goat anti-mouse IgG (Jackson ImmunoResearch, Grove, PA) followed by tethamethylbenzidine (TMB) substrate kit (Pierce, Rockford, IL).

25 In some experiments, other IRTA-Fc fusion proteins were used instead of IRTA2-Fc.

Affinity Determination. Affinities (dissociation constant, K_d) of the anti-IRTA2 MAbs to IRTA2-Fc were determined by ELISA as previously described (Friguet et al., *J Immunol Methods* 77:305-19, 1985). Briefly, each MAb was incubated with various concentrations of IRTA2 -Fc at room temperature for 20 hours to reach equilibrium; then 30 the amount of MAb that remains unbound at equilibrium was measured by the indirect ELISA as described above.

Cell-ELISA. 293T cells that had been transfected with the IRTA2 expression plasmid or a CD30 expression plasmid 48 hours prior to use were seeded into 96 well plates (2 X 10⁴/well). After overnight culture, the cells were fixed with 3.7% formalin in the 35 culture medium at room temperature overnight. Incubation with a dilution of MAbs,

secondary antibody and substrate was performed as described in the ELISA protocol except that 1% FBS in PBS was used for washing.

FACS Analysis. Various lymphoma cell lines and transfected 293T cells were used in the FACS analysis. Typically, 4×10^5 cells were incubated with 250 ng of MAb in
5 100 μ l of PBS containing 5% FBS and 0.1% sodium azide. After incubation for 1 hour at 4°C, the cells were washed twice with the same buffer and incubated with 1:100 dilution of R-phycoerythrin (R-PE)-labeled goat anti-mouse IgG F(ab')₂ (BioSource, Camarillo, CA) for 1 hour. After washing twice, the cells were suspended in 1 ml buffer, and the fluorescence associated with the live cells was measured using a FACS Caliber Flow
10 Cytometer (Becton Dickinson, Franklin Lakes, NJ).

For blood samples of patients with HCL or normal donors, whole peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Paque (Amersham Biosciences) and stained with mixtures of F56-R-PE (200 ng/ml, specially made by Molecular Probes, Eugene, OR, CD20 PerCP-Cy5.5, and CD22-FITC or CD103-FITC (Becton Dickinson).
15 In some experiments, IRTA2-Fc or CD30-Fc was added at 20 μ g/ml with the antibodies as the competitors. Compensations of fluorescence were set prior to each analysis using calibrated beads (CaliBRITE beads, Becton Dickinson).

Immunofluorescence Antibody. 293T cells were transfected with an IRTA2 or a CD30 plasmid; 48 hours after transfection, the cells were detached, counted, and
20 combined in a 1:1 ratio. A total 5×10^4 cells were seeded in each well of a 4-well chamber slide (Nalge Nunc). After 24 hours, the cells were washed with PBS and fixed in 3.7% formaldehyde for 5 minutes. After washing with PBS twice and blocking with 5% normal goat serum, the cells were incubated with a mixture of anti-IRTA2 MAb F56 (IgG1) and anti-CD30 MAb Ki-1 (IgG3) at 10 μ g/ml each. After washing, a mixture of
25 Alexa 488-conjugated goat-anti-mouse IgG1 (1:1000 dilution; Molecular Probes) and Alexa 555-conjugated goat-anti-mouse IgG3 (1:1000 dilution; Molecular Probes) was added to the cells. The primary antibodies, F56 and Ki-1, on the slides were probed by the different secondary reagents with different wave lengths of the emission based on the different subclass. Nuclei were stained with 4',6-Diamidino-2-phenylindole (DAPI)
30 (1 μ g/ml; Roche Applied Science, Indianapolis, IN). Negative controls were performed without antibodies.

Western Blotting. Typically, 40 μ g of cell lysates or 20 ng of Fc-fusion proteins for each well or strip were separated onto 4-20% sodium dodecyl sulfate (SDS) polyacrylamide gels (Bio-Rad, Hercules, CA) under reducing condition. Proteins were
35 transferred to a polyvinylidene difluoride membrane (0.2 μ m; Immuno-Blot; Bio-Rad) in

transfer buffer (25 mM Tris-HCl, 192 mM glycine, 30% (v/v) methanol, pH 8.3) at 4°C for 1 hour at 250 mA. After blocking with 5% skim milk in PBS, the membrane was incubated with 2.5-10 µg/ml of MAbs. The bound MAbs were detected with alkaline phosphatase-labeled goat anti-mouse IgG (BioSource) and 5-bromo-4-chloro-3-indolyl phosphate/p-nitroblue tetrazolium chloride substrate (BCIP/NBT; Pierce).

RT-PCR. The RNA expression of IRTA2 in the cell lines was examined by a RT-PCR method. Total RNA was prepared from each cell line using Trizol LS reagent (Invitrogen). cDNA was made from 4 µg of the total RNA by Superscript III Reverse Transcriptase (Invitrogen) with random hexamer priming according to the manufacture's instructions. PCRs were performed using cDNA from 100 ng RNA and 2 sets of primers for β-actin and IRTA2 (c isoform specific) listed in Table 1 with 26 cycles of amplification (95°C, 15 seconds; 55°C, 30 seconds; 72°C, 1 minute).

Example 2

Cloning of IRTA cDNAs and Preparation of Fc-fusion Proteins

Because of the high homology of IRTA2 to other family members, it was important to prepare all possible IRTA proteins to evaluate cross-reactivity of the anti-IRTA2 antibodies. All cDNAs of these IRTAs whose mRNA expression had been reported were amplified by RT-PCR and cloned into a pair of vectors: one for the expression of the full-length of the genes under a CMV-promoter and the other for the expression of the extracellular domains as fusion proteins using the Fc portion of human IgG1. Fig. 1A summarizes the structure of each IRTA and the plasmid construction. All full-length proteins were expressed on 293T cells transiently transfected with each plasmid as shown by cross-reactive MAbs (see below). All the Fc-fusion proteins were secreted into the culture supernatants of 293T cells transiently transfected with each plasmid although the production level varied (0.4-10 µg/ml). The purified Fc-fusion proteins migrated in SDS-PAGE with the expected sizes (Fig. 1B).

Example 3

Production of MAbs

A Balb/c mouse was DNA-immunized with the full-length IRTA2 expression plasmid and boosted with 293T cells transfected with the same plasmid (Nagata et al., *J Immunol Methods* 280:59-72, 2003). The fusion experiment resulted in 36 stable hybridomas secreting MAbs that reacted to the IRTA2 extracellular domain-Fc but not to CD30-Fc (Table 4). Four (11%) IgG1s, 24 (67%) IgG2as, and 8 (22%) IgG2bs were

obtained. All the MAbs reacted to IRTA2-Fc with high affinities (1.1-18.7 nM of Kds, 5.4 nM in average). In addition, all the MAbs reacted to IRTA2 expressed on 293T cells transfected with IRTA2 expression plasmid without background reactivity to 293T cells transfected with a CD30 plasmid. It was concluded that all the MAbs recognize the native conformation of IRTA2 on the plasma membrane with high affinity. Three MAbs (F25, F56 and F119) were initially chosen for further study based on their high affinities, different subclass, no cross-reactivity to other IRTA family members, and reactivity in immunocytochemistry and Western blotting (see below). The characteristics of the three MAbs are summarized in Table 3.

Table 3 Characteristics of anti-IRTA2 monoclonal antibodies used in this study^a

Name	IgG Subclass ^b	Reactivity in FACS (log MFI) ^c		Reactivity to IRTA2-Fc		Cross reactivity to other IRTAs ^d	Reactivity to denatured antigen	
		to IRTA2 /293T	to CD30 /293T	ELISA titer ^e (ml/μg)	Affinity ^f (Kd, nM)		Cell-ELISA titer ^g (ml/μg)	Western blotting ^h
F25	2a	2.59	0.74	320	1.2	-	0.6	+
F56	1	2.56	0.66	860	4.4	-	250	-
F119	2b	2.54	0.85	850	5.4	-	0.3	-

^aMAbs were screened based on the reactivity to IRTA2 in an ELISA. Thirty-six MAbs were selected and all of them specifically reacted to 293T cells transfected with IRTA2. The three MAbs shown were used for the analysis of cell lines and HCL cells.

^bAll MAbs possess κ light chain.

^cGeometric mean fluorescence intensity in a FACS analysis using 293T cells transfected with IRTA2 or CD30 expression plasmids. Transfection efficiencies were almost 100%. Some histograms of similar experiments are shown in Fig. 2B.

^dSummary of reactivity to IRTAs-Fc and 293T cells with IRTAs shown in Fig. 2A and B.

^eThe reciprocal of the MAb concentration needed to attain an absorbance of 0.2 in ELISA when a positive control (F56, 20 ng/ml) had an absorbance value of 1.0 in the same plate. These values are equal to the titers of 1 μg/ml of MAb solution. All MAbs did not react to a negative control (CD30-Fc).

^fDefined by an ELISA measurement of free MAb concentration in the MAb-IRTA2-Fc mixtures after making equilibrium (20 hr, 25°C).

^g293T cells transfected with the IRTA2 plasmid were used as the cell ELISA antigen after fixation of 3.7% formalin overnight. The values are the reciprocal of the MAb concentration needed to attain an absorbance of 0.2 in ELISA when a positive control (an antiserum, 1/4000) had an absorbance value of 0.5 in the same plate. These are equal to the titers of 1 μg/ml of MAb solution. All the MAbs did not react to a negative control (<0.2, CD30-transfected 293T).

^hReactivity to IRTA2-Fc and IRTA2-transfected cells in Western blotting. Parts of the results are shown in Fig. 3. +, positive; -, negative.

Table 4. Anti-IRTA2 Monoclonal Antibodies

Mab (use)	Kd(nM)	topo- epitope	IgG class	Reactivity to IRTA2 domains (Fc-fusions)													Reactivity to other IRTAs (Fc-fusion)					Reactivity to cells (FACS)			
				ED1-3	ED1-2	ED1	ED2	ED3	ED4-6	ED4-9	RfC-2a	ED1-8	1-8- RfC	full-RfC	ED9	IRTA1	IRTA2	IRTA3	IRTA4	IRTA5	IRTA2/2 93T	XG2	SUDHL+ OCI-Ly1		
F25	1.2	2	G2a	+	+	+	-	-	-	-	+	+	+	+	-	-	+	-	-	+	-	+	+		
F20	1.5	2	G2b	+	+	+	-	-	-	-	+	+	+	+	-	-	+	-	-	+	-	+	+		
F91	1.9	2	G2b	+	+	+	-	-	-	-	+	+	+	+	-	-	+	-	-	+	-	+	+		
F99	1.6	2	G2b	+	+	+	-	-	-	-	+	+	+	+	-	-	+	-	-	+	-	+	+		
F28	2.2	2	G2a	+	+	+	-	-	-	-	+	+	+	+	-	-	+	-	-	+	-	+	+		
F23	3.6	1	G2a	+	+	-	-	-	-	-	+	+	+	+	-	-	+	-	-	+	-	+	+		
F34	3.0	1	G2a	+	+	-	-	-	-	-	+	+	+	+	-	-	+	-	-	+	-	+	+		
F62	2.8	1	G2a	+	+	-	-	-	-	-	+	+	+	+	-	-	+	-	-	+	-	+	+		
F54	6.1	1	G2a	+	+	-	-	-	-	-	+	+	+	+	-	-	+	-	-	+	-	+	+		
F65	4.1	1	G2a	+	+	-	-	-	-	-	+	+	+	+	-	-	+	-	-	+	-	+	+		
F59	6.0	6	G1	+	-	-	-	+	-	-	+	+	+	+	-	-	+	-	-	+	-	+	+		
F15	7.6	6	G1	+	-	-	-	-	-	-	+	+	+	+	-	-	+	-	-	+	-	+	+		
F44	4.6	3	G2a	+	-	-	-	+	-	-	+	+	+	+	-	-	+	-	-	+	-	+	+		
F119	5.4	3	G2b	+	-	-	-	+	-	-	+	+	+	+	-	-	+	-	-	+	-	+	+		
F5	4.8	3	G2a	+	-	-	-	+	-	-	+	+	+	+	-	-	+	-	-	+	-	+	+		
F126	8.4	8	G2a	-	-	-	-	-	+	+	+	+	+	+	-	-	+	-	-	+	-	+	+		
F108	1.9	8	G2b	-	-	-	-	-	+	+	+	+	+	+	-	-	+	-	-	+	-	+	+		
F56	4.4	11	G1	-	-	-	-	-	+	+	+	+	+	+	-	-	+	-	-	+	-	+	+		
F51	3.7	11	G2a	-	-	-	-	-	+	+	+	+	+	+	-	-	+	-	-	+	-	+	+		
F26	2.7	11	G2b	-	-	-	-	-	+	+	+	+	+	+	-	-	+	-	-	+	-	+	+		
F69	4.1	10	G2a	-	-	-	-	-	+	+	+	+	+	+	-	-	+	-	-	+	-	+	+		
F117	3.5	10	G2a	-	-	-	-	-	+	+	+	+	+	+	-	-	+	-	-	+	-	+	+		
F66	7.5	10	G2a	-	-	-	-	-	+	+	+	+	+	+	-	-	+	-	-	+	-	+	+		
F100	3.6	9	G2b	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	+	-	+	+	+		
F46	6.6	9	G2a	-	-	-	-	-	-	+	+	+	+	+	-	-	+	+	+	-	+	+	+		
F112	4.4	-	G2a	-	-	-	-	-	-	+	+	+	+	+	-	-	+	+	+	-	+	+	+		
F47	4.9	-	G2a	-	-	-	-	-	-	+	+	+	+	+	-	-	+	+	+	-	+	-	+		
F109	18.7	-	G2b	-	-	-	-	-	-	+	+	+	+	+	-	-	+	+	+	+	+	-	+		
F127	13.0	-	G2a	-	-	-	-	-	-	+	+	+	+	+	-	-	+	+	+	+	+	-	+		
F124	10.6	7	G2a	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	-	+	-	+		
F1	5.7	5	G1	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	-	+	-	+		
F97	7.8	5	G2a	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	-	+	-	+		
F103	8.2	5	G2a	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	-	+	-	+		
F115	7.9	4	G2a	-	-	-	-	-	-	+	-	-	-	+	-	-	+	-	-	-	+	+	-		
F48	6.7	4	G2a	-	-	-	-	-	-	+	-	-	-	+	-	-	+	-	-	-	+	-	-		
F74	4.4	4	G2a	-	-	-	-	-	-	+	-	-	-	+	-	-	+	-	-	-	+	-	-		

Example 4**Cross-reactivity of the Anti-IRTA2 MABs**

To examine the cross-reactivity of the MABs to other IRTAs, the reactivity of each MAB to other IRTA-Fc fusion proteins was tested in an ELISA and to native IRTAs expressed on transfected 293T cells. Overall results indicate that 25 out of 36 MABs specifically reacted with IRTA2 whereas 11 MABs showed cross-reactivity to other IRTAs to various extents. As shown in Fig. 2, the binding of MABs, F25, F56, and F119 was specific to IRTA2 in both assays.

Example 5**Reactivity of the MABs to Denatured IRTA2**

The reactivity of the MABs was examined in a cell-ELISA, by immunocytochemistry and by Western blotting. In these methods, most of the epitopes on IRTA2 protein were probably denatured.

First, the reactivity of the MABs to formalin-fixed antigen on transfected 293T cells in a cell ELISA was tested. About two-thirds of MABs (21 out of 36) lost their reactivity to formalin-fixed IRTA2 (titers were less than 1), but 15 MABs bound to the formalin-fixed antigen to some extent. MAB F56 specifically reacted to IRTA2 with a good titer (Table 3), and was used in further studies.

An immunofluorescence study was performed using MAb F56 for the detection of IRTA2 in the transfected cells (Fig. 3A). Two dishes of 293T cells were separately transfected with IRTA2 or CD30 plasmids and were mixed at a 1:1 ratio and reseeded. The cells were fixed in formaldehyde and double-stained with a mixture of anti-IRTA2
5 MAb F56 (IgG1) and anti-CD30 MAb Ki-1 (IgG3). Four views with different filters of the same field are shown (Fig. 3A). The cells expressing IRTA2 and stained with F56 showed a green fluorescence signal on the plasma membrane (anti-IRTA2 F56). In contrast, different cells expressing CD30 and stained with anti-CD30 showed a red signal on the plasma membrane (CD30). The nuclei of the cell were visualized with DAPI. The
10 result indicates that MAb F56 can be used in immunofluorescence staining of cells after fixation with formalin to detect recombinant IRTA2 protein expressed on the plasma membrane.

Next, the reactivity of the MAbs to SDS-denatured antigen was examined in Western blotting. A mixture of IRTA2-Fc and CD30-Fc fusion proteins were blotted on
15 the membrane and incubated with each MAb. Only with MAbs F25 and F20 was a 160-kDa band of IRTA2-Fc visualized (Fig. 3B). These MAbs did not react with CD30-Fc on the same blots. The CD30-Fc band around 110-kDa was stained with an anti-human Fc antibody and an anti-CD30 MAb (Ber-H2), but not with F20 or F25. Therefore, the reactivity of F20 and F25 on the Western blot is specific. None of the other 34 MAbs
20 were able to react with IRTA2 in Western blotting, indicating they can detect native but not SDS-denatured IRTA2.

Example 6

Expression of IRTA2 on Human Cell Lines

25 IRTA2 expression was surveyed on a variety of human cancer cell lines by FACS using MAbs F56 and F119 (Fig. 4A). The characteristics of these cell lines are listed in Table 1. IRTA2 was detected on 7 B-cell lymphoma lines; 6 were derived from a different type of non-Hodgkin's lymphoma (NHL). Only 1 of 6 Burkitt's lymphoma cell lines was positive and this weakly expressed IRTA2 (Fig. 4A). All cells were stained
30 with the appropriate CD markers used as the positive controls and were not stained with mouse IgG1 as the negative control. To verify that the staining was specific, a 100-fold excess of IRTA2-Fc or CD30-Fc was used as the competitors of the staining. IRTA2-Fc was able to block staining of SU-DHL-5 and OCI-LY7 cells, whereas CD30-Fc was ineffective.

The expression of IRTA2 mRNA in cells was examined by RT-PCR (Fig. 4B). A set of primers (2cF and 2cR [Table 2]) for IRTA2 located on separate exons was designed to give a 432-bp PCR fragment. This primer set should detect only the spliced mRNA for the full-length of the transmembrane type of IRTA2 (IRTA2c) that was used as the antigen. As shown in Fig. 4B, the IRTA2 primers generated DNA fragments with expected size from the RNA of the cells positive for IRTA2 expression in FACS. In contrast, no IRTA2 cDNA was amplified from the RNA of FACS-negative cells, except for the trace amounts of the PCR product in Ramos cells. The actin primers generated similar amounts of the DNA fragment from all cells.

Next, IRTA2 protein expression in several cell lines was examined by Western blotting using MAb F25 (Fig. 4C). The IRTA2 protein was detected in the three B-cell NHL (B-NHL) cell lines tested (SU-DHL-5, SU-DHL-6, and JD38) as bands around 150-kDa, which is the same size as the recombinant IRTA2 protein expressed in 293T cells transfected with an IRTA2 plasmid. MAb F25 also reacted to IRTA2-Fc in the same blot. In contrast, no IRTA2 bands were detected in Karpas 299 and L540 cell lysates.

Fig. 4D shows a summary of the detection of IRTA2 by different methods in various cell lines. There is a clear correlation in IRTA2 expression detected by FACS, mRNA expression detected by RT-PCR, and IRTA2 protein detected by Western blots.

Example 7

Expression of IRTA2 on HCL Cells from Patients

To determine if the MAbs might be useful for examining cells from patients, IRTA2 expression was analyzed in PBMCs of 5 patients with HCL and of 2 normal donors by FACS. In these experiments, the PBMCs were three-color-stained with anti-IRTA2 F56 and CD20 and CD22 or with F56 and CD20 and CD103. It was found that the HCL cells (CD20+, CD22+, and CD103+) from all five patients significantly expressed IRTA2 whereas IRTA2 was not detected on PBMCs of normal donors (CD14+, CD3+ or CD19+). Fig. 5 shows the typical results of a FACS analysis of 2 patients with HCL. Staining with anti-IRTA2 F56 revealed that 27% or 39% of the PBMCs from the 2 patients expressed significant amounts of IRTA2 (Fig. 5A, histograms). IRTA2 was not detected in PBMCs from normal donors. The IRTA2+ cells also expressed CD20, CD22 and CD103 (Fig. 5A, blue dot-blots), indicating almost all of IRTA2 positive cells represent HCL cells. On the other hand, almost all HCL-maker positive cells from the same patients (CD20+/CD22+ or CD20+/CD103+) expressed IRTA2 (Fig. 5B). These data show that all HCL cells in these patients express IRTA2. The IRTA2 signal was

inhibited by the addition of a 100-fold excess amount of IRTA2-Fc (red lines in Fig. 5B) but not by CD30-Fc (blue lines), indicating that IRTA2 staining of the patient samples was antigen-specific.

Thus, the IRTA2 protein is expressed on the surface of many human lymphoma
5 cell lines and on HCL cells from patients. The MABs that bind IRTA2 can be used to identify patients with tumors that express IRTA2. These MABs could be useful for both leukemia diagnosis and IRTA2-targeted immunotherapy. The staining of MAb F56 was always in accordance with that of MAb F119, although these MABs belong to different subclasses (F56, IgG1; F119, IgG2b) and react with different epitopes (formalin-resistant
10 epitope for F56 and formalin-sensitive epitope for F119). In addition, the staining was specifically inhibited by IRTA2-Fc, confirming the specificity of the staining. By using the highly specific staining, IRTA2 expression was detected on leukemia cells from five out of five HCL patients, whereas no IRTA2-positive cells were detected in PBMC from normal donors. Also, almost all IRTA2-positive cells expressed the markers for HCL (CD20+,
15 CD22+, and CD103+) and almost all HCL cells were positive for IRTA2.

IRTA2 expression was detected in eight lymphoma cell lines out of 19 cell lines examined; these are mainly B-NHL cells. The expression profile was confirmed by RT-PCR and Western blotting, indicating that the FACS analysis using the MABs is highly specific. The expression of IRTA2 in OCI-LY7, OCI-LY10, and Karpas 1106 is consistent
20 with mRNA expression detected by DNA microarray. Thus, a FACS analysis is of use to screen IRTA2 expression on cells from patients with B cell malignancies.

The production of 36 anti-IRTA2 MABs is disclosed herein. In this study, a MAB production method was utilized which generates a large number of MABs in one fusion (see Nagata et al., *J Immunol Methods* 280:59-72, 2003). This method involves DNA-
25 immunization followed by a cell boost and generated a large number of anti-IRTA2 MABs. This method yielded a large number of MABs, all of which reacted to the native form of IRTA2 on cells with high affinities (5.4 nM of Kd on average).

Out of the 36 antibodies, eleven cross-reacted with other IRTA family members. This high ratio of cross-reactive MABs was expected as the IRTAs are highly homologous.
30 All IRTA recombinant proteins were used to check the cross-reactivity; 25 IRTA2-specific MABs did not cross-react with other family members. Among the 25 IRTA2-specific MABs, MABs F56 (IgG1) and F25 (IgG2a) are detected formalin-fixed antigen in cell-ELISA and immunofluorescence antibody, and SDS-denatured antigen in Western blots, respectively. Using an immunofluorescence technique, F56 specifically stained the plasma

membranes of the 293T cells transfected with full-length IRTA2 plasmid (Fig. 3A). This result directly demonstrates the plasma membrane localization of IRTA2 protein.

Example 8

5 **Design of Two Independent IRTA2-Specific Sandwich ELISAs**

In order to measure soluble IRTA2 in human serum, four monoclonal antibodies (MAbs) to four different epitopes on the IRTA2 molecule were chosen from the panel of anti-IRTA2 MAbs shown in Table 4, and two independent ELISAs were constructed. ELISA#1 uses antibodies F1 and F25, while ELISA#2 uses antibodies F44 and F54. The
10 performance of the ELISAs using a 1:100 dilution of samples of human serum was characterized.

Initially samples were analyzed from normal donors (n=123) to establish a reference interval. When the 95th percentile values of sIRTA2 level were taken as the cut-off, the reference intervals were <30-375 ng/mL for ELISA#1 and <30-377 ng/mL for ELISA#2.
15 Typical standards curves were obtained from both of the two ELISAs in the range of 0.3-2.1 ng/ml of IRTA2-HFc protein as the standard (Figs. 9A-9B). The ELISAs are able to measure 30 ng/ml to 5.2 µg/ml of soluble IRTA2.

The results of the two ELISAs were well correlated to each other, even though they use different sets of the epitopes on IRTA2 (Fig. 10). No bias and no artifact of the two
20 ELISAs were found.

Example 9

Elevation of Soluble IRTA2 (CD307) level in Hematological Malignancies

The sera of patients with hematological malignancies was screened for the presence
25 of sIRTA2 by using the two ELISAs designed above. The ELISAs were performed as follows: MAb F1 (5 µg/ml for ELISA#1) or MAb F44 (5 µg/ml for ELISA#2) was diluted in PBS. 50 µl/well (50 ng/well) was added to Nunc-Immuno™ Plate MaxSorp™ (Nunc 439454) and the plate was incubated at 4°C overnight. 100 µl/well of blocking buffer (1% bovine serum albumin (BSA)(Roche 100350, Fraction V, protease-free), 0.1% sodium
30 azide in PBS) was added and the plate was incubated at room temperature for at least 30 min. The serum samples and standards (IRTA2-Hfc) were diluted with blocking buffer containing 0.5% mouse serum (normal mouse serum, Zymed) and the plates were washed once with PBS-0.05%Tween (Tween 20, FisherBiotech 337-500 enzyme grade). 50 µl/well of the samples were added and the plate was incubated at 4°C overnight. The plates were
35 washed twice with PBS-Tween, and then 50 µl/well of 0.5 µg/ml of biotin-MAb F25

(ELISA#1) or 1 µg/ml of biotin-MAb F54 (ELISA#2) in blocking buffer containing 0.05% Tween 20 was added. The plates were incubated at room temperature for at least 60 min. The plates were washed three times with PBS-Tween and 50 µl/well of a 1/3000 dilution of HRP-streptosavidin (Biosource) in blocking buffer containing 0.05% Tween 20 was added. 5 The plates were incubated at room temperature for at least 60 min. The plates were washed four times with PBS-Tween and 100 µl/well of TMB-hydrogen peroxide mixture (Pierce TMB substrate kit #34021) was added. The plates were incubated at room temperature for 10-30 min. 50 µl/well of 1M sulfuric acid was added to stop the reaction and the OD was measured at 450 nm with 650 nm as a reference. Standard curves were fitted to the second 10 degree of polynomial curve after log conversion of the concentrations (using SoftMaxPro software, Molecular probes). From the calculated values and the dilutions, the IRTA2 concentrations in the human serum samples were determined. Using ELISA#1, an increase of sIRTA2 levels was found in 34 of 44 HCL (77%), 17 of 21 CLL (81%), 0 of 10 CML (0%), 0 of 9 ALL (0%), 0 of 9 AML (0%) 1 of 4 ATL (25%), 13 of 24 MCL (52%), 0 of 17 15 DLBCL (0%), 3 of HIV-lymphoma (33%), and 0 of 4 HD (0%). The sIRTA2 levels were confirmed by ELISA#2 which independently detects two different epitopes on sIRTA2. Surface IRTA2 expression was also detected in 4 of 7 CLL cells by a FACS analysis using an anti-IRTA2 MAb. Using these ELISAs, an elevated level of IRTA2 was detected in HCL and CLL patients compared to normal (Fig. 11).

20 The finding that sIRTA2 was elevated in various hematological malignancies indicates that sIRTA2 could be a good tumor marker for the prediction and monitoring of these diseases and also suggests that sIRTA2 could be involved in the biology of these malignancies.

25 It will be apparent that the precise details of the methods or compositions described may be varied or modified without departing from the spirit of the described invention. We claim all such modifications and variations that fall within the scope and spirit of the claims below.

CLAIMS

1. A isolated monoclonal antibody that specifically binds an antigenic epitope of the extra-cellular domain of a polypeptide comprising an amino acid sequence set forth as SEQ ID NO: 13.

5

2. The isolated antibody of claim 1, wherein the antibody specifically binds to amino acid residues 1 to 190 of the amino acid sequence set forth as SEQ ID NO:13.

3. The isolated antibody of claim 2, wherein the monoclonal antibody is produced by a hybridoma deposited as American Type Culture Collection (ATCC) Deposit No. ZZZZ, or is a humanized form thereof.

10

4. The isolated antibody of claim 1, wherein the antibody specifically binds to amino acid residues 1 to 109 of the amino acid sequence set forth as SEQ ID NO:13.

15

5. The isolated antibody of claim 4, wherein the antibody comprises a light chain with a CDR1, CDR2 and CDR3 region, and wherein L-CDR1 comprises the amino acid sequence set forth as amino acids 31 to 35 of SEQ ID NO:5, L-CDR2 comprises the amino acid sequence set forth as amino acids 50 to 66 of SEQ ID NO:5, and L-CDR3 comprises the amino acid sequence set forth as amino acids 96 to 101 SEQ ID NO:5.

20

6. The isolated antibody of claim 4, wherein the antibody has a heavy chain with a CDR1, CDR2 and CDR3 region, and wherein H-CDR1 comprises the amino acid sequence set forth as amino acids 24 to 34 of SEQ ID NO:6, H-CDR2 comprises the amino acid sequence set forth as amino acids 50 to 56 of SEQ ID NO:6, and H-CDR3 comprises the amino acid sequence set forth as amino acids 89-97 of SEQ ID NO:6.

25

7. The isolated antibody of claim 1, wherein the antibody specifically binds to amino acid residues 191 to 286 of the amino acid sequence set forth as SEQ ID NO:13.

30

8. The isolated antibody of claim 7, wherein the monoclonal antibody is produced by a hybridoma deposited as American Type Culture Collection (ATCC) Deposit No. YYYY, or is a humanized form thereof.

9. The isolated antibody of claim 7, wherein the antibody comprises a light chain with a CDR1, CDR2 and CDR3 region, and wherein L-CDR1 comprises the amino acid sequence set forth as amino acids 31 to 35 of SEQ ID NO:1, L-CDR2 comprises the amino acid sequence set forth as amino acids 50 to 65 of SEQ ID NO:1, and L-CDR3
5 comprises the amino acid sequence set forth as amino acids 95 to 105 of SEQ ID NO:1.
10. The isolated antibody of claim 7, wherein the antibody has a heavy chain with a CDR1, CDR2 and CDR3 region, and wherein H-CDR1 comprises the amino acid sequence set forth as amino acids 24 to 35 of SEQ ID NO:2, H-CDR2 comprises the amino
10 acid sequence set forth as amino acids 51 to 57 of SEQ ID NO:2, and H-CDR3 comprises the amino acid sequence set forth as amino acids 90 to 98 of SEQ ID NO:2.
11. The isolated antibody of claim 1, wherein the antibody specifically binds to amino acid residues 566 to 746 of the amino acid sequence set forth as SEQ ID NO:13.
15
12. The isolated antibody of claim 11, wherein the monoclonal antibody is produced by a hybridoma deposited as American Type Culture Collection (ATCC) Deposit No. XXXX, or is a humanized form thereof.
13. The isolated antibody of claim 1, wherein the antibody specifically binds to amino acid residues 287 to 565 of the amino acid sequence set forth as SEQ ID NO:13.
20
14. The isolated antibody of claim 13, wherein the antibody comprises a light chain with a CDR1, CDR2 and CDR3 region, and wherein L-CDR1 comprises the amino
25 acid sequence set forth as amino acids 31 to 35 of SEQ ID NO:3, L-CDR2 comprises the amino acid sequence set forth as amino acids 50 to 65 of SEQ ID NO:3, and L-CDR3 comprises the amino acid sequence set forth as amino acids 95 to 102 of SEQ ID NO:3.
15. The isolated antibody of claim 13, wherein the antibody has a heavy chain
30 with a CDR1, CDR2 and CDR3 region, and wherein H-CDR1 comprises the amino acid sequence set forth as amino acids 24 to 34 of SEQ ID NO:4, H-CDR2 comprises the amino acid sequence set forth as amino acids 50 to 56 of SEQ ID NO:4, and H-CDR3 comprises the amino acid sequence set forth as amino acids 89 to 97 of SEQ ID NO:4.

16. The isolated antibody of any one of claims 1-15, wherein the antibody further comprises a human framework region.

17. The isolated antibody of any one of claims 1-15, wherein the antibody
5 further comprises a murine framework region.

18. An isolated functional fragment of the monoclonal antibody of any one of claims 1-13, wherein the functional fragment specifically binds IRTA2.

19. The isolated function fragment of the humanized antibody of claim 16,
10 wherein the fragment comprises an Fv, an Fab, or an F(ab')₂.

20. The isolated antibody of any one of claims 1-15, conjugated to an effector molecule.
15

21. The isolated antibody of claim 20, wherein the effector molecule is a toxin.

22. The isolated antibody of claim 21, wherein the effector molecule comprises ricin A, abrin, diphtheria toxin or a subunit thereof, *Pseudomonas* exotoxin or a portion
20 thereof, saporin, restrictocin or gelonin.

23. The isolated antibody of claim 22, wherein the effector molecule comprises PE38, PE40, PE38KDEL, or PE38REDL.

24. A nucleic acid encoding the antibody of any one of claims 5, 6, 9, 10, 14 or
25 15, or a combination thereof.

25. The nucleic acid of claim 24, operably linked to a promoter.

26. An expression vector comprising the nucleic acid of claim 25.
30

27. A host cell transformed with the expression vector of claim 26.

28. A method for inhibiting the growth of a B cell of a non-Hodgkin's
35 lymphoma or a hairy cell leukemia, comprising contacting the cell with an effective

amount of at least one antibody of any one of claims 1-15 conjugated to an effector molecule, thereby inhibiting the growth of the B cell of the non-Hodgkin's lymphoma or a hairy cell leukemia.

5 29. The method of claim 28, wherein the cell is *in vitro*.

30. The method of claim 28, wherein the cell is *in vivo*.

31. The method of claim 28, wherein the effector molecule is a toxin.

10

32. The method of claim 28, wherein the effector molecule comprises ricin A, abrin, diphtheria toxin or a subunit thereof, *Pseudomonas* exotoxin or a portion thereof, saporin, restrictocin or gelonin.

15 33. The method of claim 28, wherein the effector molecule comprises PE38, PE40, PE38KDEL, and PE38REDL.

34. The method of claim 28, wherein the effector molecule is a chemotherapeutic agent.

20

35. A method for treating a hairy cell leukemia or a B cell lymphoma in a subject, comprising administering to the subject a therapeutically effective amount of at least one antibody of any one of claims 1-15 conjugated to an effector molecule, thereby treating the hairy cell leukemia or non-Hodgkin's lymphoma.

25

36. The method of claim 35, wherein the effector molecule comprises ricin A, abrin, diphtheria toxin or a subunit thereof, *Pseudomonas* exotoxin or a portion thereof, saporin, restrictocin or gelonin.

30 37. The method of claim 35, wherein the antibody is covalently linked to a *Pseudomonas* exotoxin.

38. The method of claim 37, wherein the *Pseudomonas* exotoxin comprises PE35, PE37, PE38 or PE40.

35

39. The method of claim 37, wherein the *Pseudomonas* exotoxin is PE38.

40. Use of an isolated antibody, comprising both the light and the heavy chains of an antibody that binds IRTA2; and an effector molecule comprising a toxin covalently
5 linked to the heavy or the light chain of the antibody for the manufacture of a medicament for the treatment of a hairy cell leukemia or non-Hodgkin's lymphoma.

41. A method of detecting B cell non-Hodgkin's lymphoma or hairy cell leukemia in a subject comprising:
10 a) contacting a biological sample from the subject with at least one antibody of claim 1 that specifically binds IRTA2; and,
b) detecting antibody bound to the sample; thereby detecting B cell non-Hodgkin's lymphoma or hairy cell leukemia in the subject.

15 42. The method of claim 41, wherein the IRTA2 is soluble IRTA2.

43. The method of claim 41, wherein the sample is blood, serum or plasma.

44. The method of claim 41, wherein the biological sample comprises
20 peripheral blood mononuclear cells.

45. A pharmaceutical composition comprising a therapeutically effective amount of the antibody of claim 1 in a pharmaceutically acceptable carrier.

25 46. A kit for the detection or treatment of hairy cell leukemia or B cell non-Hodgkin's lymphoma, comprising a container comprising the antibody of any one of claims 1-15; and instructions for using the kit.

47. A method for detecting soluble IRTA2 in a sample, comprising contacting
30 the sample with one or more antibodies of any of claims 2-15 to form an antibody-IRTA2 complex, and detecting the presence or absence of the complex, wherein presence of the complex detects soluble IRTA2.

48. The method of claim 47, wherein the antibody is labeled.

49. The method of claim 48, wherein detecting comprises the use of immunofluorescence.

50. The method of claim 48, wherein detecting comprises use of an enzyme-linked immunosorbent assay (ELISA).

51. The method of claim 50, wherein the method comprises contacting the sample with an isolated antibody as set forth in claims 2, 4, 7, 11 or 13.

52. The method of claim 50, wherein the ELISA comprises the use of one or more antibodies selected from the group consisting of:

- a. an antibody that is produced by a hybridoma deposited as American Type Culture Collection (ATCC) Deposit No. XXXXX;
- b. an antibody that specifically binds an antigenic epitope of the extracellular domain of a polypeptide comprising the amino acid sequence set forth as SEQ ID NO: 13, wherein the antibody comprises both a light and heavy chain with CDR1, CDR2 and CDR3, wherein L-CDR1 comprises the amino acid sequence set forth as amino acids 31 to 35 of SEQ ID NO:5, L-CDR2 comprises the amino acid sequence set forth as amino acids 50 to 66 of SEQ ID NO:5, and L-CDR3 comprises the amino acid sequence set forth as amino acids 96 to 101 SEQ ID NO:5, and wherein H-CDR1 comprises the amino acid sequence set forth as amino acids 24 to 34 of SEQ ID NO:6, H-CDR2 comprises the amino acid sequence set forth as amino acids 50 to 56 of SEQ ID NO:6, and H-CDR3 comprises the amino acid sequence set forth as amino acids 89-97 of SEQ ID NO:6;
- c. an antibody that is produced by a hybridoma deposited as American Type Culture Collection (ATCC) Deposit No. YYYY;
- d. an antibody that is produced by a hybridoma deposited as American Type Culture Collection (ATCC) Deposit No. ZZZZ;
- e. an antibody that specifically binds an antigenic epitope of the extracellular domain of a polypeptide comprising the amino acid sequence set forth as SEQ ID NO:13, wherein the antibody comprises both a light and heavy chain with CDR1, CDR2 and CDR3, wherein L-CDR1 comprises the amino acid sequence set forth as amino acids 31 to 35 of SEQ ID NO:1, L-CDR2 comprises the amino acid sequence set forth as amino acids 50 to 65 of SEQ ID NO:1, and L-CDR3 comprises the amino acid sequence set forth as amino acids 95 to 105 of SEQ ID NO:1, and wherein H-CDR1 comprises the amino acid sequence set forth as amino acids 24 to 35 of SEQ ID NO:2, H-CDR2 comprises the amino

acid sequence set forth as amino acids 51 to 57 of SEQ ID NO:2, and H-CDR3 comprises the amino acid sequence set forth as amino acids 90 to 98 of SEQ ID NO:2; and,

- f. an antibody that specifically binds an antigenic epitope of the extracellular domain of a polypeptide comprising the amino acid sequence set forth as SEQ ID NO:13, wherein the antibody comprises both a light and heavy chain with CDR1, CDR2 and CDR3, wherein L-CDR1 comprises the amino acid sequence set forth as amino acids 31 to 35 of SEQ ID NO:3, L-CDR2 comprises the amino acid sequence set forth as amino acids 50 to 65 of SEQ ID NO:3, and L-CDR3 comprises the amino acid sequence set forth as amino acids 95 to 102 of SEQ ID NO:3, and wherein H-CDR1 comprises the amino acid sequence set forth as amino acids 24 to 34 of SEQ ID NO:4, H-CDR2 comprises the amino acid sequence set forth as amino acids 50 to 56 of SEQ ID NO:4 and H-CDR3 comprises the amino acid sequence set forth as amino acids 89 to 97 of SEQ ID NO:4.

52. The method of claim 52, wherein the ELISA comprises the use of an antibody produced by a hybridoma deposited as American Type Culture Collection (ATCC) Deposit No. XXXX.

53. The method of claim 52, wherein the ELISA further comprises the use of an antibody that specifically binds an antigenic epitope of the extracellular domain of a polypeptide comprising the amino acid sequence set forth as SEQ ID NO:13, wherein the antibody comprises both a light and heavy chain with CDR1, CDR2 and CDR3, wherein L-CDR1 comprises the amino acid sequence set forth as amino acids 31 to 35 of SEQ ID NO:5, L-CDR2 comprises the amino acid sequence set forth as amino acids 50 to 66 of SEQ ID NO:5, and L-CDR3 comprises the amino acid sequence set forth as amino acids 96 to 101 SEQ ID NO:5, and wherein H-CDR1 comprises the amino acid sequence set forth as amino acids 24 to 34 of SEQ ID NO:6, H-CDR2 comprises the amino acid sequence set forth as amino acids 50 to 56 of SEQ ID NO:6, and H-CDR3 comprises the amino acid sequence set forth as amino acids 89-97 of SEQ ID NO:6.

54. The method of claim 51, wherein the ELISA comprises:
- a) causing a first antibody to adhere to a solid surface; wherein the first antibody is produced by a hybridoma deposited as American Type Culture Collection (ATCC) Deposit No. XXXXX;
- b) contacting the first antibody with the sample to form an antibody-IRTA2 complex;

- c) washing the solid surface to remove any unbound sample;
- d) contacting the complex with a second antibody, wherein the second antibody specifically binds an antigenic epitope of the extracellular domain of a polypeptide comprising the amino acid sequence set forth as SEQ ID NO:13, wherein the antibody
- 5 comprises both a light and heavy chain with CDR1, CDR2 and CDR3, wherein L-CDR1 comprises the amino acid sequence set forth as amino acids 31 to 35 of SEQ ID NO:5, L-CDR2 comprises the amino acid sequence set forth as amino acids 50 to 66 of SEQ ID NO:5, and L-CDR3 comprises the amino acid sequence set forth as amino acids 96 to 101 SEQ ID NO:5, and wherein H-CDR1 comprises the amino acid sequence set forth as amino
- 10 acids 24 to 34 of SEQ ID NO:6, H-CDR2 comprises the amino acid sequence set forth as amino acids 50 to 56 of SEQ ID NO:6, and H-CDR3 comprises the amino acid sequence set forth as amino acids 89-97 of SEQ ID NO:6, and wherein the second antibody is labeled with biotin;
- e) washing the solid surface to remove any unbound second antibody;
- 15 f) incubating HRP-streptavidin with the solid surface;
- g) washing the solid surface to remove any unbound HRP; and,
- h) incubating TMB-hydrogen peroxide with the surface, thereby detecting soluble IRTA2.

- 20 55. The method of claim 51, wherein the ELISA comprises the use of an antibody that specifically binds an antigenic epitope of the extracellular domain of a polypeptide comprising the amino acid sequence set forth as SEQ ID NO:13, wherein the antibody comprises both a light and heavy chain with CDR1, CDR2 and CDR3, wherein L-CDR1 comprises the amino acid sequence set forth as amino acids 31 to 35 of SEQ ID
- 25 NO:5, L-CDR2 comprises the amino acid sequence set forth as amino acids 50 to 66 of SEQ ID NO:5, and L-CDR3 comprises the amino acid sequence set forth as amino acids 96 to 101 SEQ ID NO:5, and wherein H-CDR1 comprises the amino acid sequence set forth as amino acids 24 to 34 of SEQ ID NO:6, H-CDR2 comprises the amino acid sequence set forth as amino acids 50 to 56 of SEQ ID NO:6, and H-CDR3 comprises the amino acid
- 30 sequence set forth as amino acids 89-97 of SEQ ID NO:6.

56. The method of claim 51, wherein the ELISA comprises the use of an antibody produced by a hybridoma deposited as American Type Culture Collection (ATCC) Deposit No. YYYY.

57. The method of claim 56, wherein the ELISA further comprises the use of an antibody produced by a hybridoma deposited as American Type Culture Collection (ATCC) Deposit No. ZZZZZ.

5 58. The method of claim 57, wherein the ELISA comprises:

- a) causing a first antibody to adhere to a solid surface; wherein the first antibody is produced by a hybridoma deposited as American Type Culture Collection (ATCC) Deposit No. YYYYY;
- b) contacting the first antibody with the sample to form an antibody-
10 IRTA2 complex;
- c) washing the solid surface to remove any unbound sample;
- d) contacting the complex with a second antibody, wherein the second antibody is produced by a hybridoma deposited as American Type Culture Collection (ATCC) Deposit No. ZZZZ, and wherein the second antibody is labeled with biotin;
- 15 e) washing the solid surface to remove any unbound second antibody;
- f) incubating HRP-streptavidin with the solid surface;
- g) washing the solid surface to remove any unbound HRP; and,
- h) incubating TMB-hydrogen peroxide with the surface, thereby
detecting soluble IRTA2.

20

59. The method of claim 51, wherein the ELISA comprises the use of an antibody produced by a hybridoma deposited as American Type Culture Collection (ATCC) Deposit No. ZZZZ.

25 60. A hybridoma cell line that produces the monoclonal antibody of any of claims 2-15.

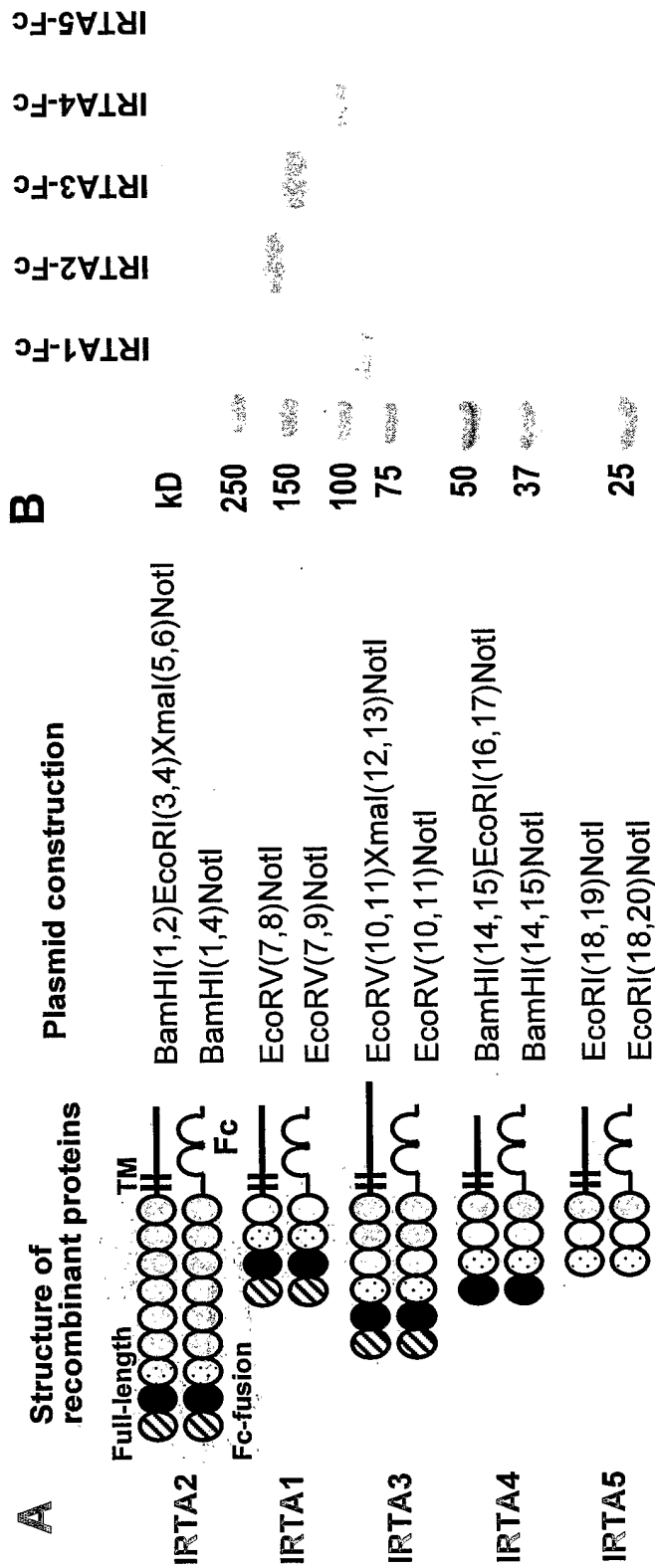
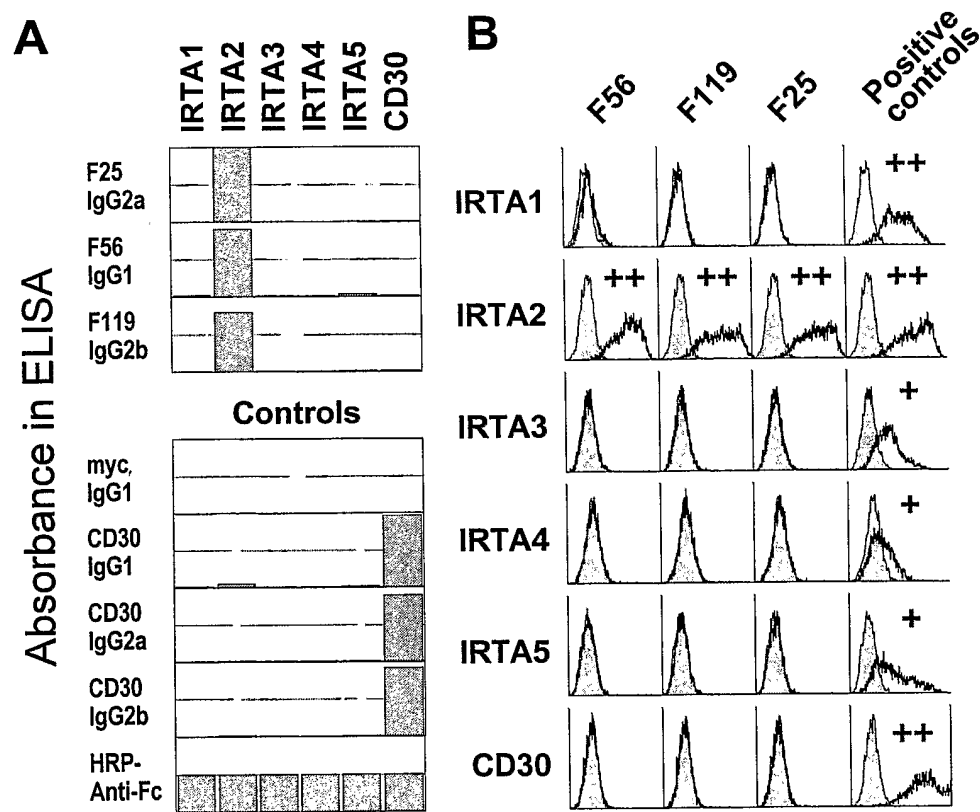


FIG. 1

FIG. 2



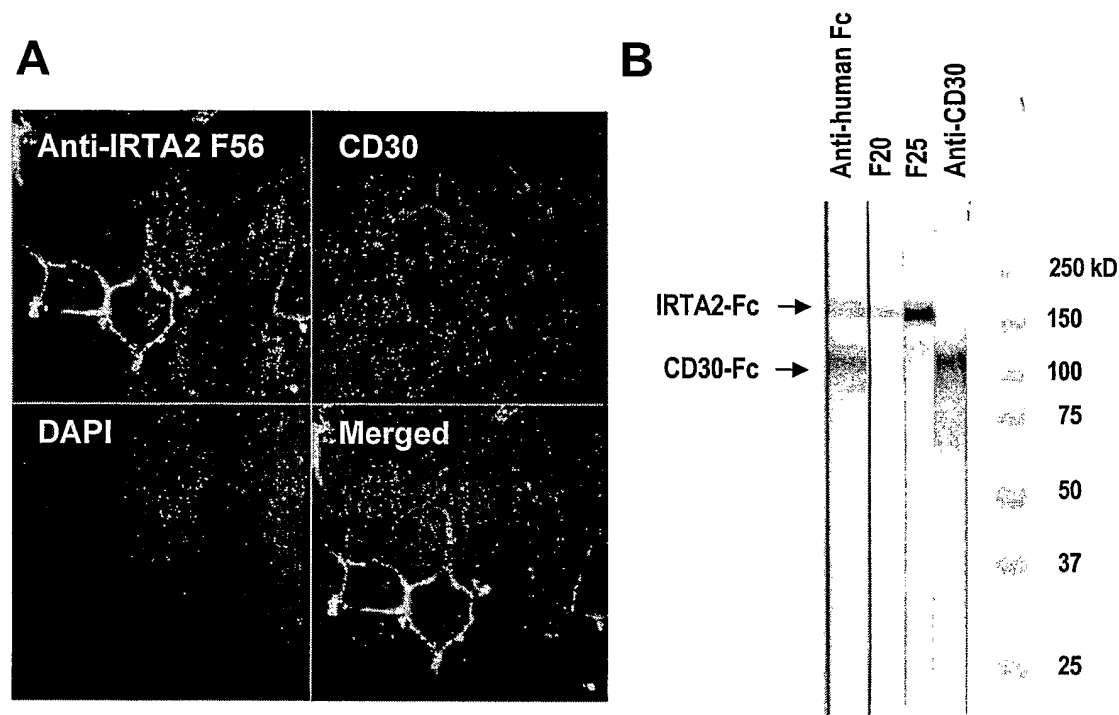


FIG. 3

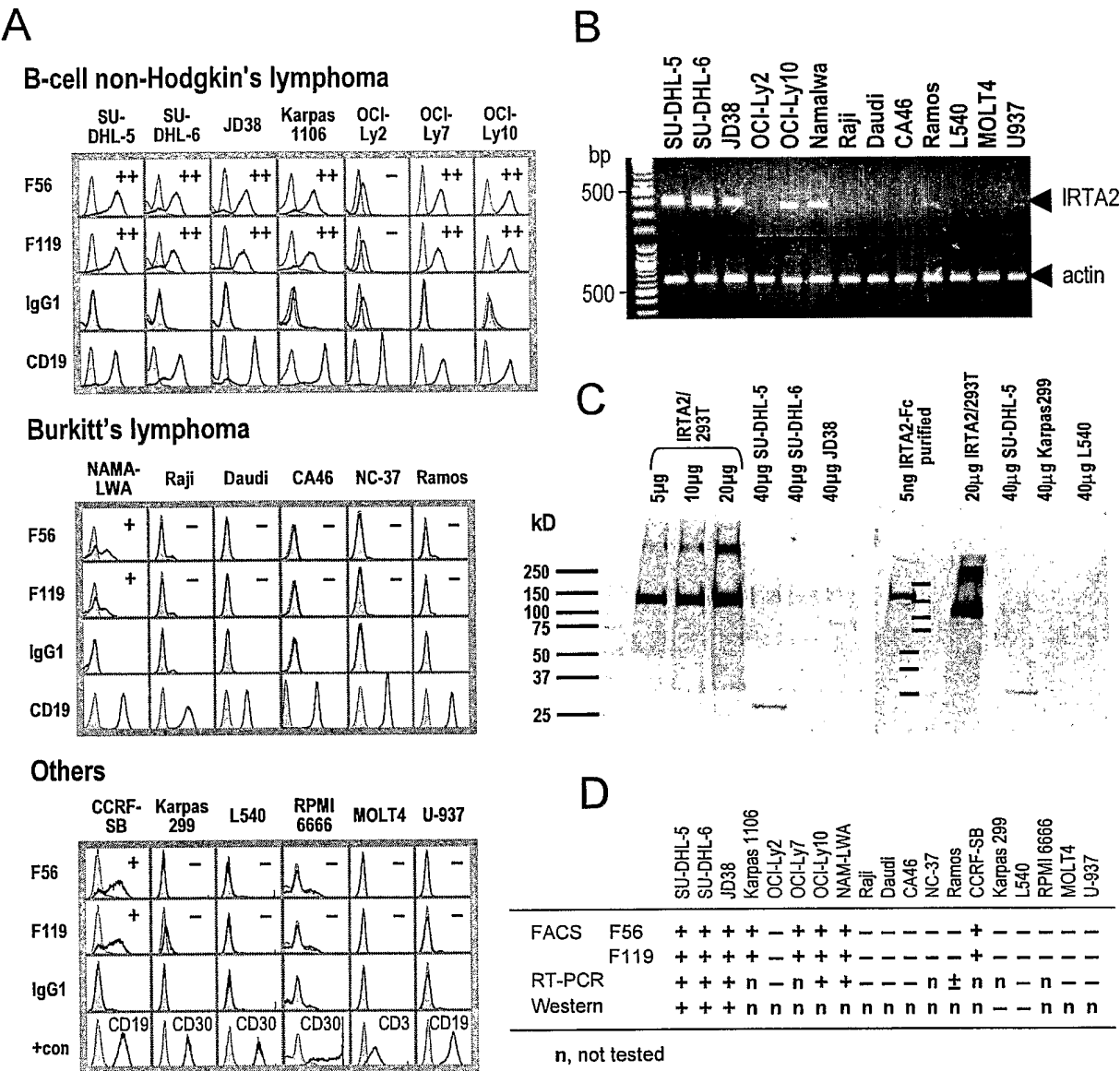


FIG. 4

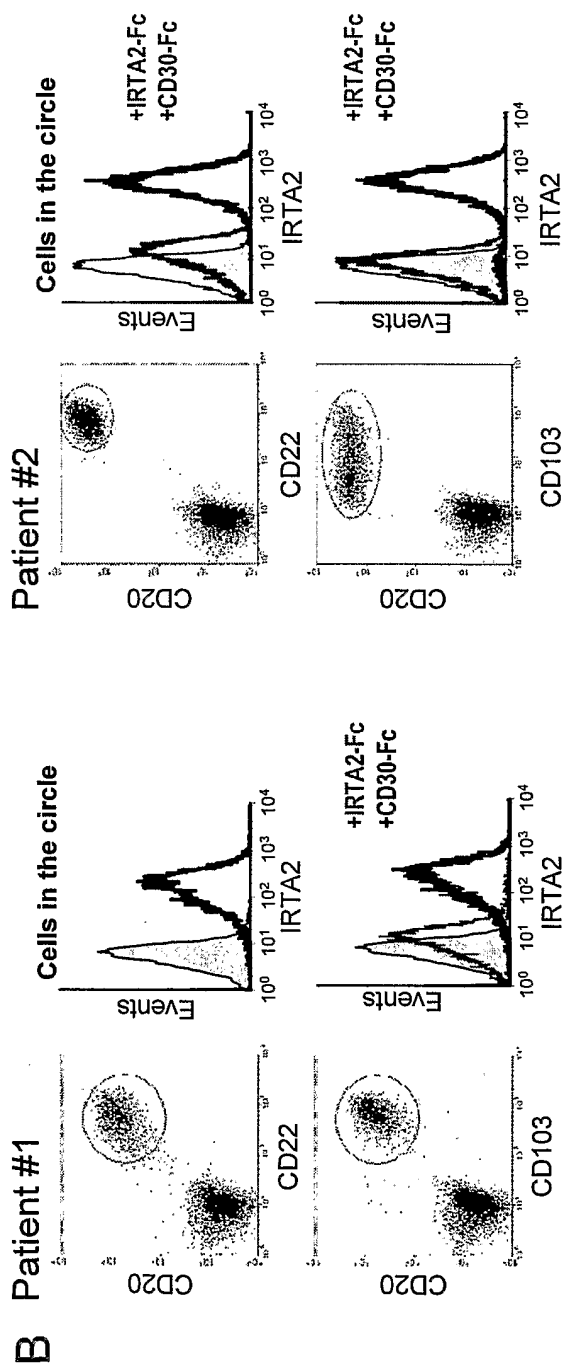
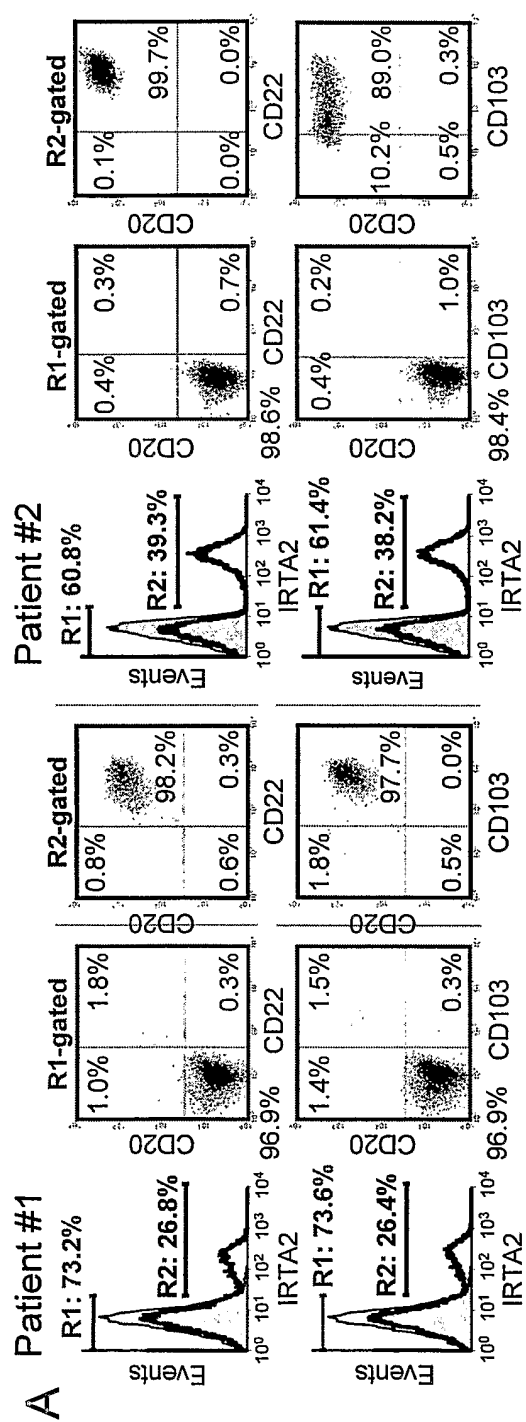


FIG. 5

FIG. 6

VH									
	FR1	CXX	CDR1	W	FR2 (-15-)	CDR2			
	1	2	3		4	5	6		
F119	123456789012345678901234567890	12345AB	67890123456789	012ABC3456789	012ABC3456789012345				
F56	DVQLVESGGGLVQPGGSRKLSCTASGFTFS	SFGMH	WVRQAPEKGLEWVA	YISS--GSNNIYFADIVKG					
F25	DVHLVESGGGLVQPGGSRKLSCAASGFTFS	IFGLH	WVRQAPEKGLEWVA	YISGG--DSNTIYYADIVKG					
	EVQLQQSGPELVKPGTISVKMSCKASGYST	GYFMN	WMMQNHGKSLIEWIG	RINL--YNGDTFYNQKFRD					
VH									
	(-33-)	FR3	CXX	CDR3	WGXS	FR4			
	7	8	9	10	11				
F119	67890123456789012ABC345678901234	567890ABCDEFHJKL2	34567890123						
F56	RFTISRDNPKNTLFLQMTSLRSEDAMYYCAR	SEYSGSSHM----	DY	WG	QGTSTVTSS				
F25	RFTISRDNPKNTLFLQMTSLRSEDAMYYCAR	NSYYAL-----	DY	WG	QGTSTVTSS				
	KATLTVDKSSNTVHMEFRSLASEDSAVYYCAS	SLIYF-----	DY	WG	QGTSTVTSS				

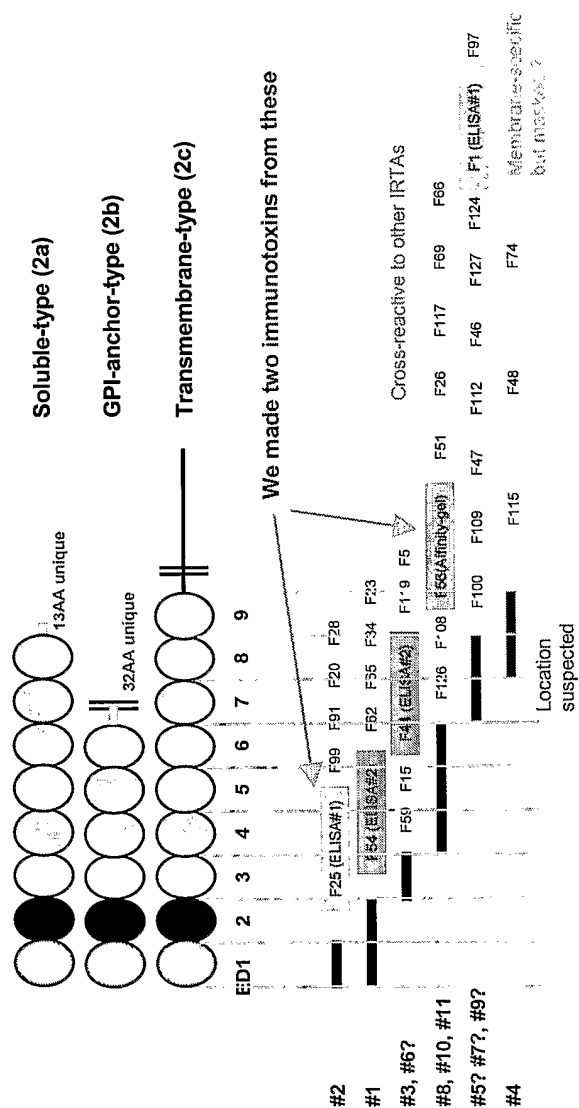
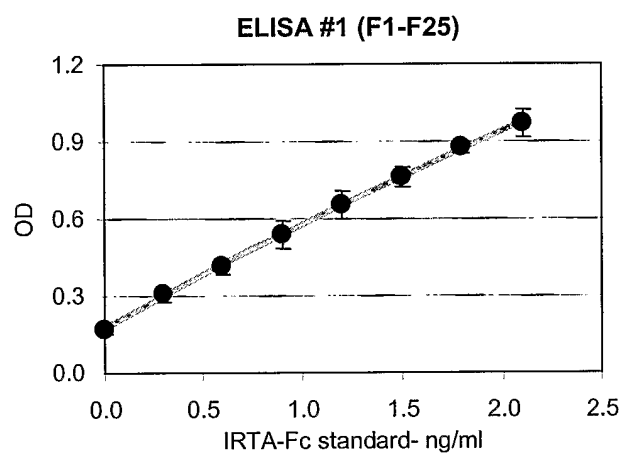


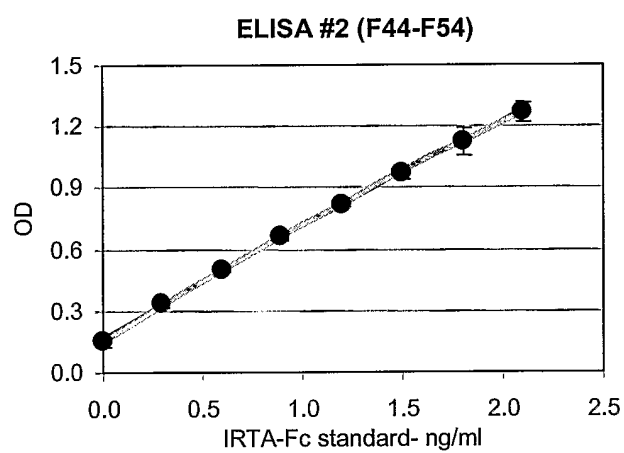
FIG. 8

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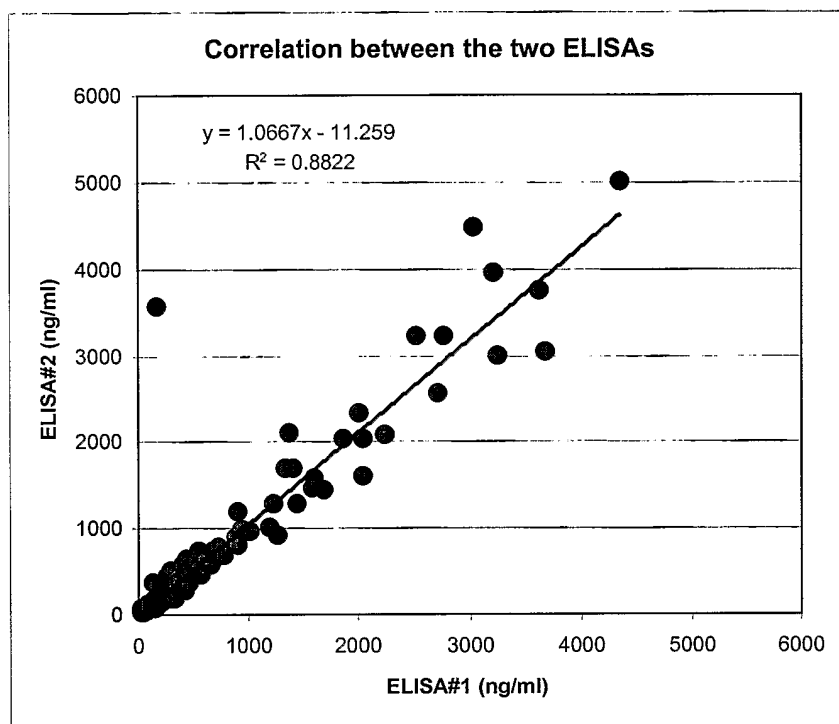
A.



B.

**FIG. 9**

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**FIG. 10**

11/11

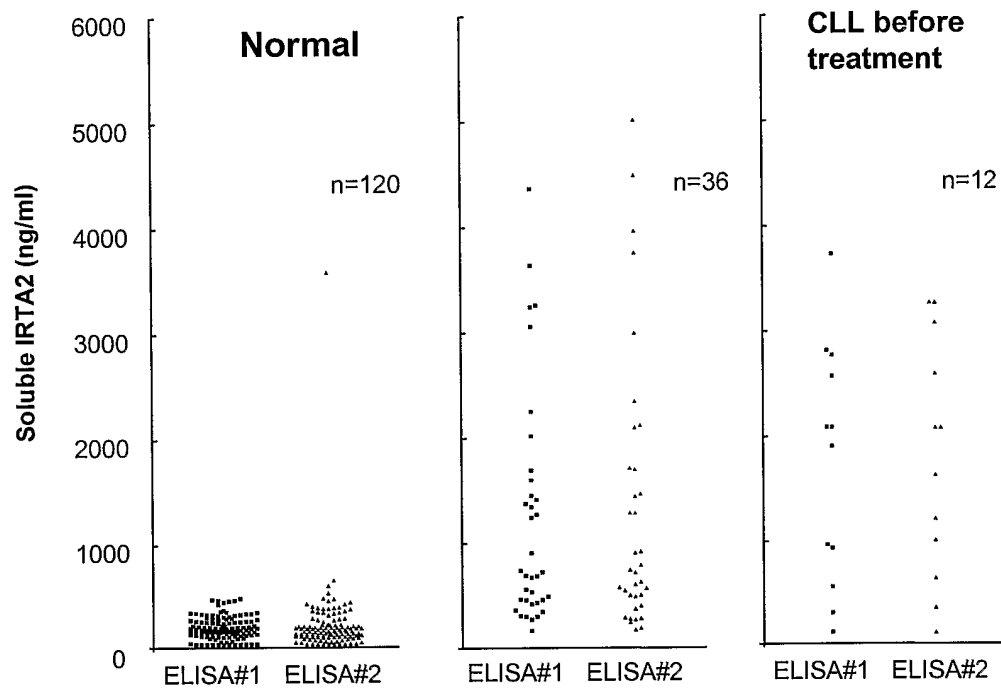


FIG. 11

SEQUENCE LISTING

<110> THE GOVERNMENT OF THE UNITED STATES OF AMERICA AS
REPRESENTED BY THE SECRETARY OF THE DEPARTMENT OF HEALTH AND
HUMAN SERVICES
Pastan, Ira H
Ise, Tomoko
Xiang, Laiman
Nagata, Satoshi

<120> IRTA2 ANTIBODIES AND METHODS OF USE

<130> 4239-69228-02

<150> US 60/615,406

<151> 2004-09-30

<160> 52

<170> PatentIn version 3.3

<210> 1

<211> 120

<212> PRT

<213> Mus musculus

<400> 1

Asp Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Arg Lys Leu Ser Cys Thr Ala Ser Gly Phe Thr Phe Ser Ser Phe
20 25 30

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

Ala Tyr Ile Ser Ser Gly Ser Asn Asn Ile Tyr Phe Ala Asp Thr Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Pro Lys Asn Thr Leu Phe
65 70 75 80

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

Ala Arg Ser Glu Tyr Tyr Gly Ser Ser His Met Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Ser Val Thr Val Ser Ser
115 120

<210> 2

<211> 112

<212> PRT

<213> Mus musculus

<400> 2

Ser Ile Val Met Thr Gln Thr Pro Lys Phe Leu Leu Val Ser Ala Gly
1 5 10 15

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

Val Ala Trp Phe Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile
35 40 45

Tyr Tyr Ala Ser Asn His Tyr Thr Gly Val Pro Asp Arg Phe Thr Gly
50 55 60

Ser Gly Tyr Gly Thr Asp Phe Thr Phe Thr Ile Ser Thr Val Gln Ala
65 70 75 80

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

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala Asp Ala Ala
100 105 110

<210> 3

<211> 118

<212> PRT

<213> Mus musculus

<400> 3

Asp Val His Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Arg Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ile Phe
20 25 30

Gly Leu His Trp Val Arg Gln Ala Pro Glu Lys Gly Leu Glu Trp Val
35 40 45

Ala Tyr Ile Ser Gly Gly Asp Ser Asn Thr Ile Tyr Tyr Ala Asp Thr
50 55 60

Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Pro Lys Asn Thr Leu
65 70 75 80

Phe Leu Gln Met Thr Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr
85 90 95

Cys Ala Arg Asn Ser Tyr Tyr Ala Leu Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Ser Val Thr Val Ser Ser
115

<210> 4
<211> 113
<212> PRT
<213> Mus musculus

<400> 4

Glu Asn Val Phe Thr Gln Ser Pro Ala Ile Met Ser Val Ser Pro Gly
1 5 10 15

Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Ser Ser Ser
20 25 30

Tyr Leu His Trp Tyr Gln Gln Arg Ser Gly Ala Ser Pro Lys Ile Trp
35 40 45

Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser
50 55 60

Gly Ser Gly Thr Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Val Glu
65 70 75 80

Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Gly Tyr Pro
85 90 95

Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala Asp Ala
100 105 110

Ala

<210> 5
<211> 116
<212> PRT
<213> Mus musculus

<400> 5

Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Thr
1 5 10 15

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

Phe Met Asn Trp Met Met Gln Asn His Gly Lys Ser Leu Glu Trp Ile
35 40 45

Gly Arg Ile Asn Leu Tyr Asn Gly Asp Thr Phe Tyr Asn Gln Lys Phe
50 55 60

Lys Asp Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Asn Thr Val His
65 70 75 80

Met Glu Phe Arg Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Ser Ser Leu Tyr Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Thr Phe
100 105 110

Thr Val Ser Ser
115

<210> 6
<211> 112
<212> PRT
<213> Mus musculus

<400> 6

Asp Ile Val Met Thr Gln Ser His Lys Phe Met Ser Thr Ser Val Gly
1 5 10 15

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

Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile
35 40 45

Tyr Trp Ala Ser Thr Arg Gln Thr Gly Val Pro Asp Arg Phe Thr Gly
50 55 60

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

Glu Asp Leu Ser Asp Tyr Phe Cys Gln Gln Tyr Ser Ser Tyr Pro Leu
85 90 95

Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Ala Asp Ala Ala
100 105 110

<210> 7
<211> 360
<212> DNA
<213> Mus musculus

<400> 7
gatgtgcagc tgggtggagtc tgggggaggc ttagtgcagc ctggagggtc ccggaactc 60
tcctgtacag cctctggatt cactttcagt agctttggaa tgcactgggt tcgtcaggct 120
ccagagaagg ggctggagtg ggtcgcatac attagtagtg gcagtaataa catctacttt 180
gcggacacag tgaagggccg attcaccatc tccagagaca atcccaagaa caccctgttc 240

ctgcaaatga ccagtctaag gtctgaggac acggccatgt attactgtgc aagatcggaa 300
tactacggta gtagccatat ggactactgg ggtcaaggaa cctcagtcac cgtctcctca 360

<210> 8
<211> 336
<212> DNA
<213> Mus musculus

<400> 8
agtattgtga tgaccagac tcccaaattc ctgcttgtat cagcaggaga cagggttacc 60
ataacctgca aggccagtca gagtgtgagt aatgatgtag cttgggtcca acagaagcca 120
gggcagtctc ctaaactgct gatatactat gcatccaatc actacactgg agtccctgat 180
cgcttcactg gcagtggata tgggacggat ttcactttca ccatcagcac tgtgcaggct 240
gaagacctgg cagtttattt ctgtcagcag gattatagct ctctccgac gttcgggtgga 300
ggcaccaagc tggaaatcaa acgggctgat gctgca 336

<210> 9
<211> 351
<212> DNA
<213> Mus musculus

<400> 9
gatgtgcacc tgggtggagtc tgggggaggc ttagtgcagc ctggagggtc ccggaaactc 60
tcctgtgcag cctctggatt cactttcagt atctttggat tgcactgggt tcgtcaggct 120
ccagagaagg ggctggagtg ggtcgcatac attagtggtg acagtaatac catctactat 180
gcagacacag tgaagggccg attcaccatc tccagagaca atcccaagaa caccctgttc 240
ctgcaaatga ccagtctaag gtctgaggac acggccatgt attactgtgc aagaaatagc 300
tactatgctc tggactactg gggtaagga acctcagtca cgtctcctc a 351

<210> 10
<211> 339
<212> DNA
<213> Mus musculus

<400> 10
gaaaatgtgt tcaccagtc tccagcaatc atgtctgtat ctccagggtga aaaggtcacc 60
atgacctgca gggccagctc aagtgtcagt tccagttact tgcactggta ccagcagagg 120
tcagggtgct cccccaaaat ctggatttat agcacatcca acttggcttc tggagtcctt 180
gctcgcttca gtggcagtgg gactgggacc tcttactctc tcacaatcag cagtgtggag 240
gctgaagatg ctgccactta ttactgccag cagtacagtg gttaccctg gacgttcggg 300
ggaggcacca agttggaaat caaacgggct gatgctgca 339

<210> 11
<211> 348

<212> DNA

<213> Mus musculus

<400> 11

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gagggttcaac tgcagcagtc tggacctgag ctggtgaagc ctgggacttc agtgaagatg      60
tcctgcaagg cttctgggta ctcatctact ggctacttta tgaactggat gatgcagaac      120
catggaaaga gccttgagtg gattggacgt attaatcttt acaatgggtga tactttctac      180
aaccagaagt tcaaggacaa ggccacattg actgtggaca aatcctctaa cacagtccac      240
atggagttcc ggagcctggc atctgaggac tctgcagtct attattgtgc aagtagtctg      300
tactactttg actactgggg ccaaggcacc actttcacag tctctctca      348

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<210> 12

<211> 336

<212> DNA

<213> Mus musculus

<400> 12

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gacattgtga tgacctcagtc tcacaaattc atgtccacat cagtaggaga caggggtcaat      60
attacctgta aggccagtca ggatgtgggt actaatgtag cctgggtatca acagaaacca      120
gggcaatctc cttaaactact gatttactgg gcatccaccc ggagactgg agtccctgat      180
cgcttcacag gcagtggatc tgggacagat ttcactctca ccattagcaa tgtgcagtct      240
gaagacttgt cagattattt ctgtcagcaa tatagcagct atcctctcac gttcgggtgct      300
gggaccaagc tggagctgaa acgggctgat gctgca      336

```

<210> 13

<211> 759

<212> PRT

<213> Homo sapiens

<400> 13

```

Met Leu Leu Trp Val Ile Leu Leu Val Leu Ala Pro Val Ser Gly Gln
1           5           10           15

```

```

Phe Ala Arg Thr Pro Arg Pro Ile Ile Phe Leu Gln Pro Pro Trp Thr
20           25           30

```

```

Thr Val Phe Gln Gly Glu Arg Val Thr Leu Thr Cys Lys Gly Phe Arg
35           40           45

```

```

Phe Tyr Ser Pro Gln Lys Thr Lys Trp Tyr His Arg Tyr Leu Gly Lys
50           55           60

```

```

Glu Ile Leu Arg Glu Thr Pro Asp Asn Ile Leu Glu Val Gln Glu Ser
65           70           75           80

```

```

Gly Glu Tyr Arg Cys Gln Ala Gln Gly Ser Pro Leu Ser Ser Pro Val
85           90           95

```

His Leu Asp Phe Ser Ser Ala Ser Leu Ile Leu Gln Ala Pro Leu Ser
100 105 110

Val Phe Glu Gly Asp Ser Val Val Leu Arg Cys Arg Ala Lys Ala Glu
115 120 125

Val Thr Leu Asn Asn Thr Ile Tyr Lys Asn Asp Asn Val Leu Ala Phe
130 135 140

Leu Asn Lys Arg Thr Asp Phe His Ile Pro His Ala Cys Leu Lys Asp
145 150 155 160

Asn Gly Ala Tyr Arg Cys Thr Gly Tyr Lys Glu Ser Cys Cys Pro Val
165 170 175

Ser Ser Asn Thr Val Lys Ile Gln Val Gln Glu Pro Phe Thr Arg Pro
180 185 190

Val Leu Arg Ala Ser Ser Phe Gln Pro Ile Ser Gly Asn Pro Val Thr
195 200 205

Leu Thr Cys Glu Thr Gln Leu Ser Leu Glu Arg Ser Asp Val Pro Leu
210 215 220

Arg Phe Arg Phe Phe Arg Asp Asp Gln Thr Leu Gly Leu Gly Trp Ser
225 230 235 240

Leu Ser Pro Asn Phe Gln Ile Thr Ala Met Trp Ser Lys Asp Ser Gly
245 250 255

Phe Tyr Trp Cys Lys Ala Ala Thr Met Pro His Ser Val Ile Ser Asp
260 265 270

Ser Pro Arg Ser Trp Ile Gln Val Gln Ile Pro Ala Ser His Pro Val
275 280 285

Leu Thr Leu Ser Pro Glu Lys Ala Leu Asn Phe Glu Gly Thr Lys Val
290 295 300

Thr Leu His Cys Glu Thr Gln Glu Asp Ser Leu Arg Thr Leu Tyr Arg
305 310 315 320

Phe Tyr His Glu Gly Val Pro Leu Arg His Lys Ser Val Arg Cys Glu
325 330 335

Arg Gly Ala Ser Ile Ser Phe Ser Leu Thr Thr Glu Asn Ser Gly Asn
340 345 350

Tyr Tyr Cys Thr Ala Asp Asn Gly Leu Gly Ala Lys Pro Ser Lys Ala
 355 360 365

Val Ser Leu Ser Val Thr Val Pro Val Ser His Pro Val Leu Asn Leu
 370 375 380

Ser Ser Pro Glu Asp Leu Ile Phe Glu Gly Ala Lys Val Thr Leu His
 385 390 395 400

Cys Glu Ala Gln Arg Gly Ser Leu Pro Ile Leu Tyr Gln Phe His His
 405 410 415

Glu Asp Ala Ala Leu Glu Arg Arg Ser Ala Asn Ser Ala Gly Gly Val
 420 425 430

Ala Ile Ser Phe Ser Leu Thr Ala Glu His Ser Gly Asn Tyr Tyr Cys
 435 440 445

Thr Ala Asp Asn Gly Phe Gly Pro Gln Arg Ser Lys Ala Val Ser Leu
 450 455 460

Ser Ile Thr Val Pro Val Ser His Pro Val Leu Thr Leu Ser Ser Ala
 465 470 475 480

Glu Ala Leu Thr Phe Glu Gly Ala Thr Val Thr Leu His Cys Glu Val
 485 490 495

Gln Arg Gly Ser Pro Gln Ile Leu Tyr Gln Phe Tyr His Glu Asp Met
 500 505 510

Pro Leu Trp Ser Ser Ser Thr Pro Ser Val Gly Arg Val Ser Phe Ser
 515 520 525

Phe Ser Leu Thr Glu Gly His Ser Gly Asn Tyr Tyr Cys Thr Ala Asp
 530 535 540

Asn Gly Phe Gly Pro Gln Arg Ser Glu Val Val Ser Leu Phe Val Thr
 545 550 555 560

Val Pro Val Ser Arg Pro Ile Leu Thr Leu Arg Val Pro Arg Ala Gln
 565 570 575

Ala Val Val Gly Asp Leu Leu Glu Leu His Cys Glu Ala Pro Arg Gly
 580 585 590

Ser Pro Pro Ile Leu Tyr Trp Phe Tyr His Glu Asp Val Thr Leu Gly
 595 600 605

Ser Ser Ser Ala Pro Ser Gly Gly Glu Ala Ser Phe Asn Leu Ser Leu
610 615 620

Thr Ala Glu His Ser Gly Asn Tyr Ser Cys Glu Ala Asn Asn Gly Leu
625 630 635 640

Val Ala Gln His Ser Asp Thr Ile Ser Leu Ser Val Ile Val Pro Val
645 650 655

Ser Arg Pro Ile Leu Thr Phe Arg Ala Pro Arg Ala Gln Ala Val Val
660 665 670

Gly Asp Leu Leu Glu Leu His Cys Glu Ala Leu Arg Gly Ser Ser Pro
675 680 685

Ile Leu Tyr Trp Phe Tyr His Glu Asp Val Thr Leu Gly Lys Ile Ser
690 695 700

Ala Pro Ser Gly Gly Gly Ala Ser Phe Asn Leu Ser Leu Thr Thr Glu
705 710 715 720

His Ser Gly Ile Tyr Ser Cys Glu Ala Asp Asn Gly Leu Glu Ala Gln
725 730 735

Arg Ser Glu Met Val Thr Leu Lys Val Ala Gly Glu Trp Ala Leu Pro
740 745 750

Thr Ser Ser Thr Ser Glu Asn
755

<210> 14
<211> 515
<212> PRT
<213> Homo sapiens

<400> 14

Met Leu Leu Trp Ala Ser Leu Leu Ala Phe Ala Pro Val Cys Gly Gln
1 5 10 15

Ser Ala Ala Ala His Lys Pro Val Ile Ser Val His Pro Pro Trp Thr
20 25 30

Thr Phe Phe Lys Gly Glu Arg Val Thr Leu Thr Cys Asn Gly Phe Gln
35 40 45

Phe Tyr Ala Thr Glu Lys Thr Thr Trp Tyr His Arg His Tyr Trp Gly
50 55 60

Glu Lys Leu Thr Leu Thr Pro Gly Asn Thr Leu Glu Val Arg Glu Ser
65 70 75 80

Gly Leu Tyr Arg Cys Gln Ala Arg Gly Ser Pro Arg Ser Asn Pro Val
85 90 95

Arg Leu Leu Phe Ser Ser Asp Ser Leu Ile Leu Gln Ala Pro Tyr Ser
100 105 110

Val Phe Glu Gly Asp Thr Leu Val Leu Arg Cys His Arg Arg Arg Lys
115 120 125

Glu Lys Leu Thr Ala Val Lys Tyr Thr Trp Asn Gly Asn Ile Leu Ser
130 135 140

Ile Ser Asn Lys Ser Trp Asp Leu Leu Ile Pro Gln Ala Ser Ser Asn
145 150 155 160

Asn Asn Gly Asn Tyr Arg Cys Ile Gly Tyr Gly Asp Glu Asn Asp Val
165 170 175

Phe Arg Ser Asn Phe Lys Ile Ile Lys Ile Gln Glu Leu Phe Pro His
180 185 190

Pro Glu Leu Lys Ala Thr Asp Ser Gln Pro Thr Glu Gly Asn Ser Val
195 200 205

Asn Leu Ser Cys Glu Thr Gln Leu Pro Pro Glu Arg Ser Asp Thr Pro
210 215 220

Leu His Phe Asn Phe Phe Arg Asp Gly Glu Val Ile Leu Ser Asp Trp
225 230 235 240

Ser Thr Tyr Pro Glu Leu Gln Leu Pro Thr Val Trp Arg Glu Asn Ser
245 250 255

Gly Ser Tyr Trp Cys Gly Ala Glu Thr Val Arg Gly Asn Ile His Lys
260 265 270

His Ser Pro Ser Leu Gln Ile His Val Gln Arg Ile Pro Val Ser Gly
275 280 285

Val Leu Leu Glu Thr Gln Pro Ser Gly Gly Gln Ala Val Glu Gly Glu
290 295 300

Met Leu Val Leu Val Cys Ser Val Ala Glu Gly Thr Gly Asp Thr Thr
305 310 315 320

Phe Ser Trp His Arg Glu Asp Met Gln Glu Ser Leu Gly Arg Lys Thr
325 330 335

Gln Arg Ser Leu Arg Ala Glu Leu Glu Leu Pro Ala Ile Arg Gln Ser
 340 345 350

His Ala Gly Gly Tyr Tyr Cys Thr Ala Asp Asn Ser Tyr Gly Pro Val
 355 360 365

Gln Ser Met Val Leu Asn Val Thr Val Arg Glu Thr Pro Gly Asn Arg
 370 375 380

Asp Gly Leu Val Ala Ala Gly Ala Thr Gly Gly Leu Leu Ser Ala Leu
 385 390 395 400

Leu Leu Ala Val Ala Leu Leu Phe His Cys Trp Arg Arg Arg Lys Ser
 405 410 415

Gly Val Gly Phe Leu Gly Asp Glu Thr Arg Leu Pro Pro Ala Pro Gly
 420 425 430

Pro Gly Glu Ser Ser His Ser Ile Cys Pro Ala Gln Val Glu Leu Gln
 435 440 445

Ser Leu Tyr Val Asp Val His Pro Lys Lys Gly Asp Leu Val Tyr Ser
 450 455 460

Glu Ile Gln Thr Thr Gln Leu Gly Glu Glu Glu Glu Ala Asn Thr Ser
 465 470 475 480

Arg Thr Leu Leu Glu Asp Lys Asp Val Ser Val Val Tyr Ser Glu Val
 485 490 495

Lys Thr Gln His Pro Asp Asn Ser Ala Gly Lys Ile Ser Ser Lys Asp
 500 505 510

Glu Glu Ser
 515

<210> 15
 <211> 734
 <212> PRT
 <213> Homo sapiens

<400> 15

Met Leu Leu Trp Leu Leu Leu Leu Ile Leu Thr Pro Gly Arg Glu Gln
 1 5 10 15

Ser Gly Val Ala Pro Lys Ala Val Leu Leu Leu Asn Pro Pro Trp Ser
 20 25 30

Thr Ala Phe Lys Gly Glu Lys Val Ala Leu Ile Cys Ser Ser Ile Ser

35	40	45
His Ser Leu Ala Gln Gly Asp Thr Tyr Trp Tyr His Asp Glu Lys Leu		
50	55	60
Leu Lys Ile Lys His Asp Lys Ile Gln Ile Thr Glu Pro Gly Asn Tyr		
65	70	75
Gln Cys Lys Thr Arg Gly Ser Ser Leu Ser Asp Ala Val His Val Glu		
	85	90
Phe Ser Pro Asp Trp Leu Ile Leu Gln Ala Leu His Pro Val Phe Glu		
	100	105
Gly Asp Asn Val Ile Leu Arg Cys Gln Gly Lys Asp Asn Lys Asn Thr		
	115	120
His Gln Lys Val Tyr Tyr Lys Asp Gly Lys Gln Leu Pro Asn Ser Tyr		
	130	135
Asn Leu Glu Lys Ile Thr Val Asn Ser Val Ser Arg Asp Asn Ser Lys		
	145	150
Tyr His Cys Thr Ala Tyr Arg Lys Phe Tyr Ile Leu Asp Ile Glu Val		
	165	170
Thr Ser Lys Pro Leu Asn Ile Gln Val Gln Glu Leu Phe Leu His Pro		
	180	185
Val Leu Arg Ala Ser Ser Ser Thr Pro Ile Glu Gly Ser Pro Met Thr		
	195	200
Leu Thr Cys Glu Thr Gln Leu Ser Pro Gln Arg Pro Asp Val Gln Leu		
	210	215
Gln Phe Ser Leu Phe Arg Asp Ser Gln Thr Leu Gly Leu Gly Trp Ser		
	225	230
Arg Ser Pro Arg Leu Gln Ile Pro Ala Met Trp Thr Glu Asp Ser Gly		
	245	250
Ser Tyr Trp Cys Glu Val Glu Thr Val Thr His Ser Ile Lys Lys Arg		
	260	265
Ser Leu Arg Ser Gln Ile Arg Val Gln Arg Val Pro Val Ser Asn Val		
	275	280
Asn Leu Glu Ile Arg Pro Thr Gly Gly Gln Leu Ile Glu Gly Glu Asn		
	290	300

Met Val Leu Ile Cys Ser Val Ala Gln Gly Ser Gly Thr Val Thr Phe
305 310 315 320

Ser Trp His Lys Glu Gly Arg Val Arg Ser Leu Gly Arg Lys Thr Gln
325 330 335

Arg Ser Leu Leu Ala Glu Leu His Val Leu Thr Val Lys Glu Ser Asp
340 345 350

Ala Gly Arg Tyr Tyr Cys Ala Ala Asp Asn Val His Ser Pro Ile Leu
355 360 365

Ser Thr Trp Ile Arg Val Thr Val Arg Ile Pro Val Ser His Pro Val
370 375 380

Leu Thr Phe Arg Ala Pro Arg Ala His Thr Val Val Gly Asp Leu Leu
385 390 395 400

Glu Leu His Cys Glu Ser Leu Arg Gly Ser Pro Pro Ile Leu Tyr Arg
405 410 415

Phe Tyr His Glu Asp Val Thr Leu Gly Asn Ser Ser Ala Pro Ser Gly
420 425 430

Gly Gly Ala Ser Phe Asn Leu Ser Leu Thr Ala Glu His Ser Gly Asn
435 440 445

Tyr Ser Cys Asp Ala Asp Asn Gly Leu Gly Ala Gln His Ser His Gly
450 455 460

Val Ser Leu Arg Val Thr Val Pro Val Ser Arg Pro Val Leu Thr Leu
465 470 475 480

Arg Ala Pro Gly Ala Gln Ala Val Val Gly Asp Leu Leu Glu Leu His
485 490 495

Cys Glu Ser Leu Arg Gly Ser Phe Pro Ile Leu Tyr Trp Phe Tyr His
500 505 510

Glu Asp Asp Thr Leu Gly Asn Ile Ser Ala His Ser Gly Gly Gly Ala
515 520 525

Ser Phe Asn Leu Ser Leu Thr Thr Glu His Ser Gly Asn Tyr Ser Cys
530 535 540

Glu Ala Asp Asn Gly Leu Gly Ala Gln His Ser Lys Val Val Thr Leu
545 550 555 560

Asn Val Thr Gly Thr Ser Arg Asn Arg Thr Gly Leu Thr Ala Ala Gly
565 570 575

Ile Thr Gly Leu Val Leu Ser Ile Leu Val Leu Ala Ala Ala Ala Ala
580 585 590

Leu Leu His Tyr Ala Arg Ala Arg Arg Lys Pro Gly Gly Leu Ser Ala
595 600 605

Thr Gly Thr Ser Ser His Ser Pro Ser Glu Cys Gln Glu Pro Ser Ser
610 615 620

Ser Arg Pro Ser Arg Ile Asp Pro Gln Glu Pro Thr His Ser Lys Pro
625 630 635 640

Leu Ala Pro Met Glu Leu Glu Pro Met Tyr Ser Asn Val Asn Pro Gly
645 650 655

Asp Ser Asn Pro Ile Tyr Ser Gln Ile Trp Ser Ile Gln His Thr Lys
660 665 670

Glu Asn Ser Ala Asn Cys Pro Met Met His Gln Glu His Glu Glu Leu
675 680 685

Thr Val Leu Tyr Ser Glu Leu Lys Lys Thr His Pro Asp Asp Ser Ala
690 695 700

Gly Glu Ala Ser Ser Arg Gly Arg Ala His Glu Glu Asp Asp Glu Glu
705 710 715 720

Asn Tyr Glu Asn Val Pro Arg Val Leu Leu Ala Ser Asp His
725 730

<210> 16
<211> 508
<212> PRT
<213> Homo sapiens

<400> 16

Met Leu Leu Trp Ser Leu Leu Val Ile Phe Asp Ala Val Thr Glu Gln
1 5 10 15

Ala Asp Ser Leu Thr Leu Val Ala Pro Ser Ser Val Phe Glu Gly Asp
20 25 30

Ser Ile Val Leu Lys Cys Gln Gly Glu Gln Asn Trp Lys Ile Gln Lys
35 40 45

Met Ala Tyr His Lys Asp Asn Lys Glu Leu Ser Val Phe Lys Lys Phe

50		55		60
Ser Asp Phe Leu Ile Gln Ser Ala Val Leu Ser Asp Ser Gly Asn Tyr				
65		70		75
				80
Phe Cys Ser Thr Lys Gly Gln Leu Phe Leu Trp Asp Lys Thr Ser Asn				
	85		90	95
Ile Val Lys Ile Lys Val Gln Glu Leu Phe Gln Arg Pro Val Leu Thr				
	100		105	110
Ala Ser Ser Phe Gln Pro Ile Glu Gly Gly Pro Val Ser Leu Lys Cys				
	115		120	125
Glu Thr Arg Leu Ser Pro Gln Arg Leu Asp Val Gln Leu Gln Phe Cys				
	130		135	140
Phe Phe Arg Glu Asn Gln Val Leu Gly Ser Gly Trp Ser Ser Ser Pro				
145		150	155	160
Glu Leu Gln Ile Ser Ala Val Trp Ser Glu Asp Thr Gly Ser Tyr Trp				
	165		170	175
Cys Lys Ala Glu Thr Val Thr His Arg Ile Arg Lys Gln Ser Leu Gln				
	180		185	190
Ser Gln Ile His Val Gln Arg Ile Pro Ile Ser Asn Val Ser Leu Glu				
	195		200	205
Ile Arg Ala Pro Gly Gly Gln Val Thr Glu Gly Gln Lys Leu Ile Leu				
	210		215	220
Leu Cys Ser Val Ala Gly Gly Thr Gly Asn Val Thr Phe Ser Trp Tyr				
225		230	235	240
Arg Glu Ala Thr Gly Thr Ser Met Gly Lys Lys Thr Gln Arg Ser Leu				
	245		250	255
Ser Ala Glu Leu Glu Ile Pro Ala Val Lys Glu Ser Asp Ala Gly Lys				
	260		265	270
Tyr Tyr Cys Arg Ala Asp Asn Gly His Val Pro Ile Gln Ser Lys Val				
	275		280	285
Val Asn Ile Pro Val Arg Ile Pro Val Ser Arg Pro Val Leu Thr Leu				
	290		295	300
Arg Ser Pro Gly Ala Gln Ala Ala Val Gly Asp Leu Leu Glu Leu His				
305		310	315	320

Glu Asp Val Thr Leu Gly Asn Ser Ser Ala Pro Ser Gly Gly Gly Ala
340 345 350

Ser Phe Asn Leu Ser Leu Thr Ala Glu His Ser Gly Asn Tyr Ser Cys
355 360 365

Glu Ala Asn Asn Gly Leu Gly Ala Gln Cys Ser Glu Ala Val Pro Val
370 375 380

Ser Ile Ser Gly Pro Asp Gly Tyr Arg Arg Asp Leu Met Thr Ala Gly
385 390 395 400

Val Leu Trp Gly Leu Phe Gly Val Leu Gly Phe Thr Gly Val Ala Leu
405 410 415

Leu Leu Tyr Ala Leu Phe His Lys Ile Ser Gly Glu Ser Ser Ala Thr
420 425 430

Asn Glu Pro Arg Gly Ala Ser Arg Pro Asn Pro Gln Glu Phe Thr Tyr
435 440 445

Ser Ser Pro Thr Pro Asp Met Glu Glu Leu Gln Pro Val Tyr Val Asn
450 455 460

Val Gly Ser Val Asp Val Asp Val Val Tyr Ser Gln Val Trp Ser Met
465 470 475 480

Gln Gln Pro Glu Ser Ser Ala Asn Ile Arg Thr Leu Leu Glu Asn Lys
485 490 495

Asp Ser Gln Val Ile Tyr Ser Ser Val Lys Lys Ser
500 505

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<210> 17
<211> 429
<212> PRT
<213> Homo sapiens
```

<400> 17

Met Leu Pro Arg Leu Leu Leu Leu Ile Cys Ala Pro Leu Cys Glu Pro
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 35 40 45

Gln Phe Gln Phe Cys Phe Phe Arg Asp Thr Arg Ala Leu Gly Pro Gly
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Trp Ser Ser Ser Pro Lys Leu Gln Ile Ala Ala Met Trp Lys Glu Asp
 65 70 75 80

Thr Gly Ser Tyr Trp Cys Glu Ala Gln Thr Met Ala Ser Lys Val Leu
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Arg Ser Arg Arg Ser Gln Ile Asn Val His Arg Val Pro Val Ala Asp
 100 105 110

Val Ser Leu Glu Thr Gln Pro Pro Gly Gly Gln Val Met Glu Gly Asp
 115 120 125

Arg Leu Val Leu Ile Cys Ser Val Ala Met Gly Thr Gly Asp Ile Thr
 130 135 140

Phe Leu Trp Tyr Lys Gly Ala Val Gly Leu Asn Leu Gln Ser Lys Thr
 145 150 155 160

Gln Arg Ser Leu Thr Ala Glu Tyr Glu Ile Pro Ser Val Arg Glu Ser
 165 170 175

Asp Ala Glu Gln Tyr Tyr Cys Val Ala Glu Asn Gly Tyr Gly Pro Ser
 180 185 190

Pro Ser Gly Leu Val Ser Ile Thr Val Arg Ile Pro Val Ser Arg Pro
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Ile Leu Met Leu Arg Ala Pro Arg Ala Gln Ala Ala Val Glu Asp Val
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Leu Glu Leu His Cys Glu Ala Leu Arg Gly Ser Pro Pro Ile Leu Tyr
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Trp Phe Tyr His Glu Asp Ile Thr Leu Gly Ser Arg Ser Ala Pro Ser
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Ala Val Thr Leu Asn Phe Thr Val Pro Thr Gly Ala Arg Ser Asn His

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 Glu Phe Thr Tyr Leu Asn Ser Pro Thr Pro Gly Gln Leu Gln Pro Ile
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 35 40 45
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 Ser Ile Thr Ser Asp Gly Leu Thr Ile Arg Leu Glu Gly Gly Val Glu
 65 70 75 80
 Pro Asn Lys Pro Val Arg Tyr Ser Tyr Thr Arg Gln Ala Arg Gly Ser
 85 90 95

Trp Ser Leu Asn Trp Leu Val Pro Ile Gly His Glu Lys Pro Ser Asn
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Ile Lys Val Phe Ile His Glu Leu Asn Ala Gly Asn Gln Leu Ser His
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 130 135 140

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Met Gln Pro Thr Leu Ala Ile Ser His Ala Gly Val Ser Val Val Met
 165 170 175

Ala Gln Thr Gln Pro Arg Arg Glu Lys Arg Trp Ser Glu Trp Ala Ser
 180 185 190

Gly Lys Val Leu Cys Leu Leu Asp Pro Leu Asp Gly Val Tyr Asn Tyr
 195 200 205

Leu Ala Gln Gln Arg Cys Asn Leu Asp Asp Thr Trp Glu Gly Lys Ile
 210 215 220

Tyr Arg Val Leu Ala Gly Asn Pro Ala Lys His Asp Leu Asp Ile Lys
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Pro Thr Val Ile Ser His Arg Leu His Phe Pro Glu Gly Gly Ser Leu
 245 250 255

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Thr Arg His Arg Gln Pro Arg Gly Trp Glu Gln Leu Glu Gln Cys Gly
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Tyr Pro Val Gln Arg Leu Val Ala Leu Tyr Leu Ala Ala Arg Leu Ser
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Gly Tyr Val Phe Val Gly Tyr His Gly Thr Phe Leu Glu Ala Ala Gln
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Ser Ile Val Phe Gly Gly Val Arg Ala Arg Ser Gln Asp Leu Asp Ala
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Tyr Ala Gln Asp Gln Glu Pro Asp Ala Arg Gly Arg Ile Arg Asn Gly
 485 490 495

Ala Leu Leu Arg Val Tyr Val Pro Arg Ser Ser Leu Pro Gly Phe Tyr
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Arg Thr Ser Leu Thr Leu Ala Ala Pro Glu Ala Ala Gly Glu Val Glu
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Arg Leu Ile Gly His Pro Leu Pro Leu Arg Leu Asp Ala Ile Thr Gly
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Pro Glu Glu Glu Gly Gly Arg Leu Glu Thr Ile Leu Gly Trp Pro Leu
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Applicant's or agent's file reference	4239-6922802	International Application No.	PCT/US2005/034444
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**INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL**

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 33, lines 10-19.	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐	
Name of depositary institution AMERICAN TYPE CULTURE COLLECTION (ATCC)	
Address of depositary institution (including postal code and country) 10801 UNIVERSITY BLVD. MANASSAS, VIRGINIA 20110-2209 UNITED STATES OF AMERICA	
Date of deposit September 22, 2005	Accession Numbers F44 - ATCC No. PTA-7046 F1 - ATCC No. PTA-7047 F54 - ATCC No. PTA-7048
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet ☐	
Expert opinion is requested under Rule 28(4) of the Implementing Regulations of the European Patent Convention (IREPC) and equivalent national provisions in other jurisdictions.	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

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