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(54) Title: HUMAN ANTIBODIES THAT BIND MULTIPLE IRTA FAMILY PROTEINS, AND USES THEREOF

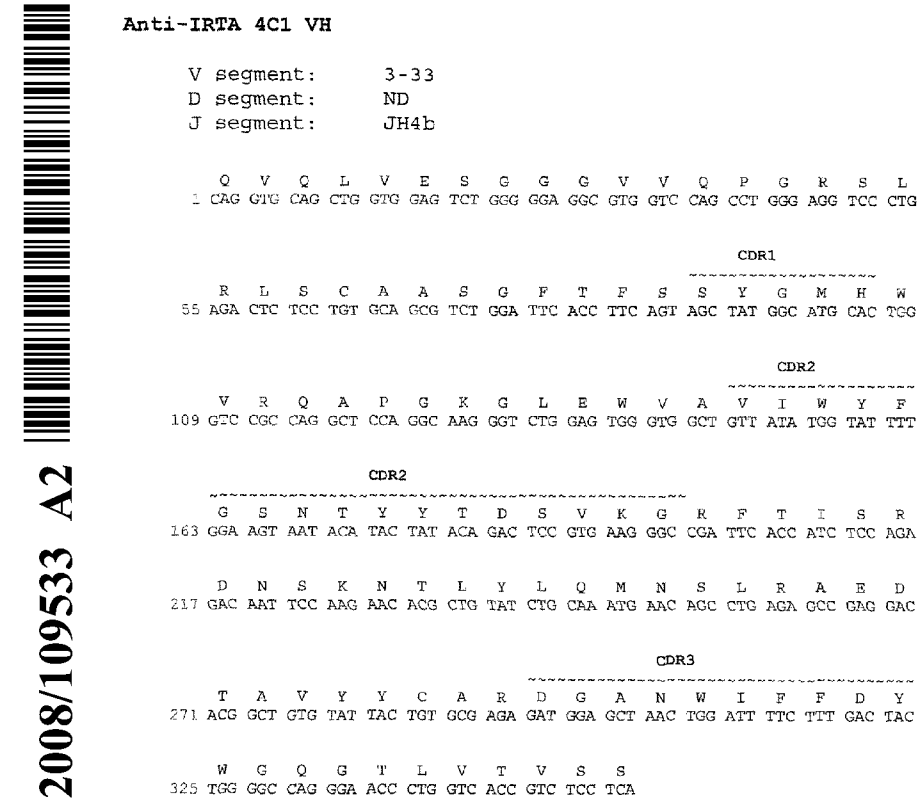

FIGURE 1A

(57) Abstract: The present disclosure provides isolated monoclonal antibodies that bind to multiple IRTA family proteins with high affinity, particularly human monoclonal antibodies. Preferred antibodies of the invention specifically bind an IRTA4 protein and at least one other IRTA family protein. Nucleic acid molecules encoding the antibodies of this disclosure, expression vectors, host cells and methods for expressing the antibodies of this disclosure are also provided. Immunoconjugates, bispecific molecules and pharmaceutical compositions comprising the antibodies of this disclosure are also provided. This disclosure also provides methods for detecting IRTA proteins, as well as methods for treating various cancers, such as B cell lymphomas, using anti-IRTA antibodies of this disclosure.

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HUMAN ANTIBODIES THAT BIND MULTIPLE IRTA FAMILY PROTEINS, AND USES THEREOF

Cross-Reference to Related Applications

This application claims priority to U.S. Provisional application Serial No. 60/892,789, filed on March 2, 2007, the contents of which are expressly incorporated herein by reference.

Background

The Immune Receptor Translocation Associated (IRTA) genes/proteins, also known as Fc Receptor Homolog (FcRH) genes, consist of a five-member family of immunoglobulin-like cell surface receptors (Miller *et al.*, (2002) *Blood.* 99:2662; Davis *et al.*, (2002) *Immunological Reviews.* 190:123). The IRTAs were initially discovered by analysis of the breakpoints of a multiple myeloma cell line which contained a 1q21 chromosomal rearrangement (Hatzivassiliou *et al.*, (2001) *Immunity.* 14:277). Each of the IRTA glycoproteins contains from 3 to 9 extracellular Ig-like domains (Miller, 2002, *supra*). Sequence alignment of these extracellular Ig-like domains from the five IRTA family members defined five subtypes of Ig-like domains, referred to as subtypes A through E (Miller, 2002, *supra*). The extent of sequence identity of corresponding Ig-like domains varies within the range of 45% to 83% among IRTA family members (Miller, 2002, *supra*).

IRTAs are also characterized by having a cytoplasmic domain containing 3 to 5 tyrosine residues contained within particular motifs, suggesting the presence of immunotyrosine inhibitory motifs (ITIM) and immunotyrosine activation-like (ITAM-like) motifs (Miller, 2002, *supra*; Hatzivassiliou, 2001, *supra*).

IRTAs are expressed in peripheral lymphoid tissues, including lymph nodes, tonsils, resting peripheral B cells and normal germinal center B cells (Davis *et al.*, (2001) *Proc. Natl. Acad. Sci. USA* 98:9772). IRTA 2, 3, 4, and 5 are all expressed at high levels in spleen, whereas, by comparison, IRTA 1 has been detected in lower levels in the spleen. IRTA expression has been analyzed within the B cell compartment of human tonsil tissue. IRTA 1 is expressed outside of lymphoid follicles in the marginal zone pattern and in intraepithelial lymphocytes. IRTA2 and IRTA3 are expressed within the germinal center, with highest expression in the

centocyte-rich light zone. IRTA4 and IRTA5 are expressed highest within mantle zones, indicating expression in naive B cells. (Miller, 2002, *supra*)

IRTA5 is unique among the IRTAs in that it has a charged glutamic acid residue in the transmembrane region, suggesting it may heterodimerize with a protein containing a positively charged amino acid in a nearby position, as is the case for many ITAM-bearing proteins (Miller, 2002, *supra*). The IRTA genes have been shown to be highly expressed in B cell non-Hodgkin's lymphoma, chronic lymphocytic leukemias, follicular lymphomas, diffuse large cell lymphomas of B lineage, and multiple myelomas (Davis, 2001, *supra*).

Summary

The present disclosure provides isolated monoclonal antibodies, in particular human monoclonal antibodies that specifically bind to at least two IRTA family proteins. Thus, the invention provides antibodies that exhibit specific cross-reactivity to different IRTA family proteins, as well as exhibiting other desirable properties. These properties include high affinity binding to IRTA family proteins, antibody dependent cellular cytotoxicity (ADCC) activity and the ability to bind to various B cell tumor cells and cell lines. The antibodies of the invention can be used, for example, to detect IRTA family proteins or to treat IRTA+ tumors, such as B cell lymphomas.

In one aspect, the instant disclosure pertains to an isolated monoclonal antibody, or an antigen-binding portion thereof, wherein the antibody specifically binds at least two IRTA family proteins. For example, in one embodiment, an antibody of invention specifically binds an IRTA4 protein and at least one other IRTA family protein. In a preferred embodiment, the antibody specifically binds at least an IRTA4 protein and an IRTA5 protein, preferably human IRTA4 and IRTA5. In a preferred embodiment, the antibody specifically binds at least an IRTA3 protein, an IRTA4 protein and an IRTA5 protein, preferably human IRTA3, IRTA4 and IRTA5 proteins. In another preferred embodiment, the antibody specifically binds at least an IRTA2 protein, an IRTA4 protein and an IRTA5 protein, preferably human IRTA2, IRTA4 and IRTA5 proteins.

The antibodies described herein that specifically cross-react with two or more IRTA family proteins can be, for example, human monoclonal antibodies.

Alternatively, the antibodies can be, for example, chimeric or humanized monoclonal antibodies.

Preferably, the antibody is capable of binding at least one B cell tumor cell or cell line, such as one or more B cell tumor cell lines selected from the group consisting of Daudi (ATCC CCL-213), SU-DHL-6 (DSMZ ACC 572) or a B cell tumor cell such as a chronic lymphocytic leukemia (CLL) cell. Preferred antibodies are capable of binding a Daudi B cell tumor cell line.

Preferably, the antibody exhibits antibody dependent cellular cytotoxicity (ADCC), such as ADCC activity against the SU-DHL-6 cell line.

In another aspect, the invention pertains to an isolated human monoclonal antibody, or antigen binding portion thereof, wherein the antibody cross-competes for binding to an IRTA family protein with a reference antibody, wherein the reference antibody comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 13 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 15; or

(b) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 14 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

In a preferred embodiment, the reference antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 13 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 15. In another preferred embodiment, the reference antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 14 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

In another aspect, the invention pertains to an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a heavy chain variable region that is the product of or derived from a human V_H 3-33 gene or a human V_H 3-7 gene, wherein the antibody specifically binds at least two IRTA family proteins. In another aspect, the invention pertains to an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a light chain variable region that is the product of or derived from a human V_K L18 gene or a human V_K A27 gene, wherein the antibody specifically binds at least two IRTA family proteins. In a preferred

embodiment, the invention provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising:

(a) a heavy chain variable region that is the product of or derived from a human V_H 3-33 gene and a light chain variable region that is the product of or derived from a human V_K L18 gene; or

(b) a heavy chain variable region that is the product of or derived from a human V_H 3-7 gene and a light chain variable region that is the product of or derived from a human V_K A27 gene;

wherein the antibody specifically binds at least two IRTA family proteins.

A particularly preferred antibody, or antigen-binding portion thereof, comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO: 1;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO: 3;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO: 5;

(d) a light chain variable region CDR1 comprising SEQ ID NO: 7;

(e) a light chain variable region CDR2 comprising SEQ ID NO: 9; and

(f) a light chain variable region CDR3 comprising SEQ ID NO: 11.

Another particularly preferred antibody, or antigen-binding portion thereof, comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO: 2;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO: 4;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO: 6;

(d) a light chain variable region CDR1 comprising SEQ ID NO: 8;

(e) a light chain variable region CDR2 comprising SEQ ID NO: 10; and

(f) a light chain variable region CDR3 comprising SEQ ID NO: 12.

In another aspect, the invention pertains to an isolated monoclonal antibody, or antigen binding portion thereof, comprising:

(a) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 13-14; and

(b) a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 15-16;

wherein the antibody specifically binds at least two IRTA family proteins.

A preferred combination comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 13; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 15.

Another preferred combination comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 14; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

The antibodies of this disclosure can be, for example, full-length antibodies, for example of an IgG1 or IgG4 isotype. Alternatively, the antibodies can be antibody fragments, such as Fab, Fab' or Fab'2 fragments, or single chain antibodies.

This disclosure also provides an immunoconjugate comprising an antibody of this disclosure, or antigen-binding portion thereof, linked to a therapeutic agent, such as a cytotoxin or a radioactive isotope. This disclosure also provides a bispecific molecule comprising an antibody, or antigen-binding portion thereof, of this disclosure, linked to a second functional moiety having a different binding specificity than said antibody, or antigen binding portion thereof.

Compositions comprising an antibody, or antigen-binding portion thereof, or immunoconjugate or bispecific molecule of this disclosure and a pharmaceutically acceptable carrier are also provided.

Nucleic acid molecules encoding the antibodies, or antigen-binding portions thereof, of this disclosure are also encompassed by this disclosure, as well as expression vectors comprising such nucleic acids and host cells comprising such expression vectors. Methods for preparing anti-IRTA antibodies using the host cells comprising such expression vectors are also provided and may include the steps of (i) expressing the antibody in the host cell and (ii) isolating the antibody from the host cell.

Another aspect of this disclosure pertains to a method of inhibiting growth of tumor cells expressing an IRTA protein. The method comprises contacting the cells with the antibody, or antigen-binding portion thereof, of the invention in an amount effective to inhibit growth of the tumor cells. The tumor cell can be, for example, a B

cell lymphoma, such as a non-Hodgkin's lymphoma. In certain embodiments, the antibody, or antigen-binding portion thereof, is conjugated to a therapeutic agent, such as a cytotoxin.

In yet another aspect, the invention pertains to a method for preparing an anti-IRTA antibody. The method comprises:

(a) providing: (i) a heavy chain variable region antibody sequence comprising a CDR1 sequence selected from the group consisting of SEQ ID NOs: 1-2, a CDR2 sequence selected from the group consisting of SEQ ID NOs: 3-4, and/or a CDR3 sequence selected from the group consisting of SEQ ID NOs: 5-6; and/or (ii) a light chain variable region antibody sequence comprising a CDR1 sequence selected from the group consisting of SEQ ID NOs: 7-8, a CDR2 sequence selected from the group consisting of SEQ ID NOs: 9-10, and/or a CDR3 sequence selected from the group consisting of SEQ ID NOs: 11-12;

(b) altering at least one amino acid residue within the heavy chain variable region antibody sequence and/or the light chain variable region antibody sequence to create at least one altered antibody sequence; and

(c) expressing the altered antibody sequence as a protein.

Other features and advantages of the instant disclosure will be apparent from the following detailed description and examples, which should not be construed as limiting. The contents of all references, Genbank entries, patents and published patent applications cited throughout this application are expressly incorporated herein by reference.

Brief Description of the Drawings

Figure 1A shows the nucleotide sequence (SEQ ID NO: 17) and amino acid sequence (SEQ ID NO: 13) of the heavy chain variable region of the 4C1 human monoclonal antibody. The CDR1 (SEQ ID NO: 1), CDR2 (SEQ ID NO: 3) and CDR3 (SEQ ID NO: 5) regions are delineated and the V, D and J germline derivations are indicated.

Figure 1B shows the nucleotide sequence (SEQ ID NO: 19) and amino acid sequence (SEQ ID NO: 15) of the kappa light chain variable region of the 4C1 human monoclonal antibody. The CDR1 (SEQ ID NO: 7), CDR2 (SEQ ID NO: 9) and

CDR3 (SEQ ID NO: 11) regions are delineated and the V and J germline derivations are indicated.

Figure 2A shows the nucleotide sequence (SEQ ID NO: 18) and amino acid sequence (SEQ ID NO: 14) of the heavy chain variable region of the 1B10 human monoclonal antibody. The CDR1 (SEQ ID NO: 2), CDR2 (SEQ ID NO: 4) and CDR3 (SEQ ID NO: 6) regions are delineated and the V, D and J germline derivations are indicated.

Figure 2B shows the nucleotide sequence (SEQ ID NO: 20) and amino acid sequence (SEQ ID NO: 16) of the kappa light chain variable region of the 1B10 human monoclonal antibody. The CDR1 (SEQ ID NO: 8), CDR2 (SEQ ID NO: 10) and CDR3 (SEQ ID NO: 12) regions are delineated and the V and J germline derivations are indicated.

Figure 3 shows the alignment of the amino acid sequence of the heavy chain variable regions of 4C1 (SEQ ID NO: 13) with the human germline V_H 3-33 amino acid sequence (SEQ ID NO: 21).

Figure 4 shows the alignment of the amino acid sequence of the light chain variable region of 4C1 (SEQ ID NO: 15) with the human germline V_k L18 amino acid sequence (SEQ ID NO:23).

Figure 5 shows the alignment of the amino acid sequence of the heavy chain variable regions of 1B10 (SEQ ID NO: 14) with the human germline V_H 3-7 amino acid sequence (SEQ ID NO: 22).

Figure 6 shows the alignment of the amino acid sequence of the light chain variable region of 1B10 (SEQ ID NO: 16) with the human germline V_k A27 amino acid sequence (SEQ ID NO:24).

Figure 7 is a graph showing the activity of human anti-IRTA antibodies 4C1 and 1B10 in an antibody dependent cellular cytotoxicity (ADCC) assay. Antibody 2G5 is used as a control.

Detailed Description of this disclosure

The present disclosure relates to isolated monoclonal antibodies, particularly human monoclonal antibodies, which bind multiple IRTA family proteins, in particular multiple human IRTA family protein. That is, the antibodies of the invention have specificity for at least two IRTA family proteins and may have

specificity for three, four or five IRTA family proteins. In certain embodiments, the antibodies of this disclosure are derived from particular heavy and light chain germline sequences and/or comprise particular structural features such as CDR regions comprising particular amino acid sequences. This disclosure provides isolated antibodies, methods of making such antibodies, immunoconjugates and bispecific molecules comprising such antibodies and pharmaceutical compositions containing the antibodies, immunoconjugates or bispecific molecules of this disclosure. This disclosure also relates to methods of using the antibodies, such as to detect IRTA proteins, as well as to modulate B cell activity in diseases or disorders associated with expression of IRTA proteins, such as IRTA+ tumors. Accordingly, this disclosure also provides methods of using the anti-IRTA antibodies of this disclosure to inhibit the growth of IRTA+ tumor cells, for example, to treat B cell lymphomas.

In order that the present disclosure may be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the detailed description.

As used herein, the term "IRTA" refers to Immune Receptor Translocation Associated (IRTA) genes/proteins, and encompasses all members of the IRTA family, including IRTA1, IRTA2, IRTA3, IRTA4 and IRTA5. Thus, the general terms "IRTA" or "IRTA family protein" or "IRTA family member" is used to refer to any member of the IRTA family, while the terms "IRTA1", "IRTA2", "IRTA3", "IRTA4" and "IRTA5" refer to specific members of the family.

The term "IRTA" includes variants, isoforms, homologs, orthologs and paralogs. For example, antibodies specific for an IRTA protein may, in certain cases, cross-react with an IRTA protein from species other than human. In other embodiments, the antibodies specific for a human IRTA protein may be completely specific for the human IRTA protein and may not exhibit species or other types of cross-reactivity. An antibody that "specifically binds at least two IRTA family proteins" refers to an antibody that has specificity for IRTA family proteins (*i.e.*, lacks specific binding to non-IRTA proteins) and, moreover, exhibits specific binding to, or cross-reactivity with, at least two members of the IRTA family of proteins, *i.e.*, at

least two proteins selected from the group consisting of IRTA1, IRTA2, IRTA3, IRTA4 and IRTA5.

The term "human IRTA1" refers to human sequence IRTA1, such as the protein having the complete amino acid sequence of human IRTA1 having Genbank accession number NP_112572, encoded by the nucleotide sequence having Genbank accession number NM_031282. The term "human IRTA2" refers to human sequence IRTA2, such as the protein having the complete amino acid sequence of human IRTA2 having Genbank accession number NP_112571, encoded by the nucleotide sequence having Genbank accession number NM_031281. The term "human IRTA3" refers to human sequence IRTA3, such as the protein having the complete amino acid sequence of human IRTA3 having Genbank accession number AAL59390, encoded by the nucleotide sequence having Genbank accession number AF459027. The term "human IRTA4" refers to human sequence IRTA4, such as the protein having the complete amino acid sequence of human IRTA4 having Genbank accession number AAL60249, encoded by the nucleotide sequence having Genbank accession number AF459633. The term "human IRTA5" refers to human sequence IRTA5, such as the protein having the complete amino acid sequence of human IRTA5 having Genbank accession number AAL60250, encoded by the nucleotide sequence having Genbank accession number AF459634.

The human IRTA1, IRTA2, IRTA3, IRTA4 and/or IRTA5 sequences may differ from the human IRTA1, IRTA2, IRTA3, IRTA4 and/or IRTA5 sequences set forth in the Genbank accession numbers described above by having, for example, conserved mutations or mutations in non-conserved regions, wherein the IRTA protein has substantially the same biological function as the human IRTA1, IRTA2, IRTA3, IRTA4 and/or IRTA5 sequence set forth in the Genbank accession numbers described above. For example, a biological function of a human IRTA protein is having an epitope in the extracellular domain of the human IRTA protein that is specifically bound by an antibody of the instant disclosure.

A particular human IRTA protein sequence will generally be at least 90% identical in amino acids sequence to the human IRTA protein sequence set forth in the Genbank accession number described above and contains amino acid residues that identify the amino acid sequence as being human when compared to IRTA protein amino acid sequences of other species (*e.g.*, murine). In certain cases, a human IRTA

protein may be at least 95%, or even at least 96%, 97%, 98%, or 99% identical in amino acid sequence to the IRTA sequence set forth in the Genbank accession number described above. In certain embodiments, a human IRTA protein sequence (e.g., human IRTA1) will display no more than 10 amino acid differences from the corresponding IRTA protein sequence (e.g., human IRTA1) set forth in the Genbank accession number described above. In certain embodiments, the human IRTA protein may display no more than 5, or even no more than 4, 3, 2, or 1 amino acid difference from the human IRTA sequence set forth in the Genbank accession number described above. Percent identity can be determined as described herein.

The term "immune response" refers to the action of, for example, lymphocytes, antigen presenting cells, phagocytic cells, granulocytes, and soluble macromolecules produced by the above cells or the liver (including antibodies, cytokines, and complement) that results in selective damage to, destruction of, or elimination from the human body of invading pathogens, cells or tissues infected with pathogens, cancerous cells, or, in cases of autoimmunity or pathological inflammation, normal human cells or tissues.

A “signal transduction pathway” refers to the biochemical relationship between a variety of signal transduction molecules that play a role in the transmission of a signal from one portion of a cell to another portion of a cell. As used herein, the phrase "cell surface receptor" includes, for example, molecules and complexes of molecules capable of receiving a signal and the transmission of such a signal across the plasma membrane of a cell. Examples of a "cell surface receptor" of the present disclosure are the IRTA1, IRTA2, IRTA3, IRTA4 and IRTA5 proteins.

The term “antibody” as referred to herein includes whole antibodies and any antigen binding fragment (*i.e.*, “antigen-binding portion”) or single chains thereof. An "antibody" refers to a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, or an antigen binding portion thereof. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, C_H1, C_H2 and C_H3. Each light chain is comprised of a light chain variable region (abbreviated herein as V_L) and a light chain constant region. The light chain constant region is comprised of one domain, C_L. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed

complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (Clq) of the classical complement system.

The term "antigen-binding portion" of an antibody (or simply "antibody portion"), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (*e.g.*, an IRTA protein). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L and C_H1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment linked by a disulfide bridge at the hinge region (see, FUNDAMENTAL IMMUNOLOGY (Paul ed., 3.sup.rd ed. 1993); (iii) a Fd fragment consisting of the V_H and C_H1 domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a V_H domain; (vi) an isolated complementarity determining region (CDR); and (vii) a nanobody, a heavy chain variable region containing a single variable domain and two constant domains. Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv); *see e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

The abbreviation "V_k", as used herein, refers to the variable domain of a kappa light chain, whereas the abbreviation "V_λ", as used herein, refers to the variable domain of a lambda light chain. The abbreviation "V_L", as used herein, refers to the variable domain of an immunoglobulin light chain and thus encompasses both V_k and V_λ light chains.

An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other antibodies having different antigenic specificities (*e.g.*, an isolated antibody that specifically binds an IRTA family member is substantially free of antibodies that specifically bind antigens other than IRTA family members). An isolated antibody that specifically binds an IRTA family member may, however, have cross-reactivity to other antigens, such as IRTA proteins from other species. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

The terms "monoclonal antibody" or "monoclonal antibody composition" as used herein refer to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope.

The term "human antibody", as used herein, is intended to include antibodies having variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. Furthermore, if the antibody contains a constant region, the constant region also is derived from human germline immunoglobulin sequences. The human antibodies of this disclosure may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

The term "human monoclonal antibody" refers to antibodies displaying a single binding specificity, which have variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. In one embodiment, the human monoclonal antibodies are produced by a hybridoma which includes a B cell obtained from a transgenic nonhuman animal, *e.g.*, a

transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene fused to an immortalized cell.

The term "recombinant human antibody", as used herein, includes all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as (a) antibodies isolated from an animal (*e.g.,* a mouse) that is transgenic or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom (described further below), (b) antibodies isolated from a host cell transformed to express the human antibody, *e.g.,* from a transfectoma, (c) antibodies isolated from a recombinant, combinatorial human antibody library, and (d) antibodies prepared, expressed, created or isolated by any other means that involve splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable regions in which the framework and CDR regions are derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies can be subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the V_H and V_L regions of the recombinant antibodies are sequences that, while derived from and related to human germline V_H and V_L sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

As used herein, "isotype" refers to the antibody class (*e.g.,* IgM or IgGl) that is encoded by the heavy chain constant region genes.

The phrases "an antibody recognizing an antigen" and "an antibody specific for an antigen" are used interchangeably herein with the term "an antibody which binds specifically to an antigen."

The term "human antibody derivatives" refers to any modified form of the human antibody, *e.g.,* a conjugate of the antibody and another agent or antibody.

The term "humanized antibody" is intended to refer to antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. Additional framework region modifications may be made within the human framework sequences.

The term "chimeric antibody" is intended to refer to antibodies in which the variable region sequences are derived from one species and the constant region sequences are derived from another species, such as an antibody in which the variable

region sequences are derived from a mouse antibody and the constant region sequences are derived from a human antibody.

As used herein, an antibody that "specifically binds to a human IRTA protein" is intended to refer to an antibody that binds to a human IRTA protein family member (and possibly an IRTA protein from one or more non-human species) but does not substantially bind to non-IRTA proteins. Preferably, the antibody binds to a human IRTA protein with a K_D of 1×10^{-7} M or less, more preferably 5×10^{-8} M or less, more preferably 3×10^{-8} M or less, more preferably 1×10^{-8} M or less, more preferably 5×10^{-9} M or less or even more preferably 1×10^{-9} M or less.

The term "does not substantially bind" to a protein or cells, as used herein, means does not bind or does not bind with a high affinity to the protein or cells, *i.e.* binds to the protein or cells with a K_D of 1×10^{-6} M or more, more preferably 1×10^{-5} M or more, more preferably 1×10^{-4} M or more, more preferably 1×10^{-3} M or more, even more preferably 1×10^{-2} M or more.

The term "K_{assoc}" or "K_a", as used herein, is intended to refer to the association rate of a particular antibody-antigen interaction, whereas the term "K_{dis}" or "K_d," as used herein, is intended to refer to the dissociation rate of a particular antibody-antigen interaction. The term "K_D", as used herein, is intended to refer to the dissociation constant, which is obtained from the ratio of K_d to K_a (i.e., K_d/K_a) and is expressed as a molar concentration (M). K_D values for antibodies can be determined using methods well established in the art. A preferred method for determining the K_D of an antibody is by using surface plasmon resonance, preferably using a biosensor system such as a Biacore® system.

As used herein, the term "high affinity" for an IgG antibody refers to an antibody having a K_D of 1×10^{-7} M or less, more preferably 5×10^{-8} M or less, even more preferably 1×10^{-8} M or less, even more preferably 5×10^{-9} M or less and even more preferably 1×10^{-9} M or less for a target antigen. However, "high affinity" binding can vary for other antibody isotypes. For example, "high affinity" binding for an IgM isotype refers to an antibody having a K_D of 10^{-6} M or less, more preferably 10^{-7} M or less, even more preferably 10^{-8} M or less.

As used herein, the term "B cell tumor cell or cell line" refers to tumor cells from a patient having a B cell tumor and to cell line derived from a B cell tumor, such as a Burkitt's lymphoma cell line or a non-Hodgkin's lymphoma cell line and the like.

Non-limiting examples of B cell tumor cell lines include Daudi (ATCC CCL-213), Raji (ATCC CCL-86), SU-DHL-6 (DSMZ ACC 572), Granta 519 (DSMZ ACC 342), JeKo (DSMZ ACC 553) and P3HR1 (ATCC HTB-62). A non-limiting example of B cell tumor cells are chronic lymphocytic leukemia (CLL) cells.

As used herein, the term "subject" includes any human or nonhuman animal. The term "nonhuman animal" includes all vertebrates, *e.g.,* mammals and non-mammals, such as nonhuman primates, sheep, dogs, cats, horses, cows, chickens, amphibians, reptiles, etc.

Various aspects of this disclosure are described in further detail in the following subsections.

Anti-IRTA Antibodies That Bind Multiple IRTA Family Proteins

The antibodies of this disclosure are characterized by particular functional features or properties of the antibodies. For example, the antibodies specifically bind to more than one IRTA family protein. Preferably, an antibody of this disclosure binds to an IRTA protein with high affinity, for example with a K_D of 1 x 10^{-7} M or less, more preferably with a K_D of 1 x 10^{-8} M or less and even more preferably with a K_D of 1 x 10^{-9} M or less. The anti-IRTA antibodies of this disclosure preferably exhibit one or more of the following characteristics:

(i) binding to at least two IRTA family proteins, preferably at least two human IRTA family proteins;

(ii) binding to an IRTA4 protein and at least one other IRTA family protein;

(iii) high affinity binding to at least two IRTA family proteins;

(iv) an ability to bind at least one B cell tumor cell or cell line;

(v) an ability to bind a Daudi B cell tumor cell line; and/or

(vi) antibody dependent cellular cytotoxicity (ADCC) activity.

In preferred embodiments, the antibody exhibits at least two of the properties listed as (i) – (vi). In even more preferred embodiments, the antibody exhibits three or more of the properties listed as (i) – (vi).

In a preferred embodiment, the invention provides an isolated monoclonal antibody, or an antigen-binding portion thereof, wherein the antibody specifically binds at least two IRTA family proteins. In a particularly preferred embodiment, the antibody specifically binds an IRTA4 protein and at least one other

IRTA family protein. For example, a preferred antibody specifically binds at least an IRTA4 protein and an IRTA5 protein (*e.g.*, human IRTA4 and IRTA5 proteins). Another preferred antibody specifically binds at least an IRTA3 protein, an IRTA4 protein and an IRTA5 protein (*e.g.*, human IRTA3, IRTA4 and IRTA5 proteins). The 4C1 antibody described herein is an example of a monoclonal antibody that specifically binds at least an IRTA3 protein, an IRTA4 protein and an IRTA5 protein. Another preferred antibody specifically binds at least an IRTA2 protein, an IRTA4 protein and an IRTA5 protein (*e.g.*, human IRTA2, IRTA4 and IRTA5 proteins). The 1B10 antibody described herein is an example of a monoclonal antibody that specifically binds at least an IRTA2 protein, an IRTA4 protein and an IRTA5 protein.

The binding of an antibody of the invention to various IRTA proteins preferably is tested by a flow cytometry assay in which the antibody is reacted with cell lines that exclusively express either IRTA1, IRTA2, IRTA3, IRTA4 or IRTA5, such as CHO cells that have been transfected to express either IRTA1, IRTA2, IRTA3, IRTA4 or IRTA5 (see, *e.g.*, Example 3 for a suitable assay). Additionally or alternatively, the binding of the antibody can be tested in BIAcore binding assays (see , *e.g.*, Example 4 for suitable assays). However, it has been observed that for certain antibodies the BIAcore results may differ somewhat from the flow cytometry results. For example, by flow cytometry the 1B10 antibody exhibits specificity for IRTA2, IRTA4 and IRTA5, whereas by BIAcore analysis the antibody exhibit binding to each of these three IRTA proteins but also exhibits specific binding for IRTA3. Thus, if a single binding assay is to be selected to test binding specificity, flow cytometry (against cells expressing each of the IRTA proteins individually) is to be used to evaluate binding specificity.

The antibodies of the invention preferably bind at least one B cell tumor cell or cell line. Non-limiting examples of B cell tumor cell lines to which an antibody of the invention may bind include Daudi (ATCC CCL-213), Raji (ATCC CCL-86), SU-DHL-6 (DSMZ ACC 572), Granta 519 (DSMZ ACC 342), JeKo (DSMZ ACC 553) and P3HR1 (ATCC HTB-62) cell lines. A non-limiting example of a type of B cell tumor cell that can be used is CLL cells obtained from a CLL patient. Preferably, the antibody binds at least one B cell tumor cell or cell line selected from the group consisting of Daudi, SU-DHL-6 and CLL cells. Even more preferably, the antibody binds at least a Daudi B cell tumor cell line.

Preferred antibodies of the invention are human monoclonal antibodies. Additionally or alternatively, the antibodies can be, for example, chimeric or humanized monoclonal antibodies.

Preferably, an antibody of this disclosure binds to an IRTA protein with a K_D of 5×10^{-8} M or less, binds to an IRTA protein with a K_D of 2×10^{-8} M or less, binds to an IRTA protein with a K_D of 5×10^{-9} M or less, binds to an IRTA protein with a K_D of 4×10^{-9} M or less, binds to an IRTA protein with a K_D of 3×10^{-9} M or less, binds to an IRTA protein with a K_D of 2×10^{-9} M or less, binds to an IRTA protein with a K_D of 1×10^{-9} M or less, binds to an IRTA protein with a K_D of 5×10^{-10} M or less, binds to an IRTA protein with a K_D of 1×10^{-10} M or less, binds to an IRTA protein with a K_D of 5×10^{-11} M or less or binds to an IRTA protein with a K_D of 1×10^{-11} M or less.

Preferably, an antibody of this disclosure exhibits antibody dependent cellular cytotoxicity (ADCC) against a cell or cell line that expresses one or more IRTA proteins. A preferred target cell for testing ADCC is an SU-DHL-6 cell line.

Standard assays to evaluate the binding affinity of the antibodies toward IRTA proteins are known in the art, including for example, ELISAs, flow cytometric analysis and/or BIAcore analysis with recombinant IRTA proteins (see Examples 3 and 4). Standard assays for evaluating ADCC activity also are known in the art and include, for example, the assay described in Example 5.

Monoclonal Antibodies 4C1 and 1B10

Preferred antibodies of this disclosure are the human monoclonal antibodies 4C1 and 1B10, isolated and structurally characterized as described in Examples 1 and 2. The V_H amino acid sequences of 4C1 and 1B10 are shown in SEQ ID NOs: 13 and 14, respectively. The V_K amino acid sequences of 4C1 and 1B10 are shown in SEQ ID NOs: 15 and 16, respectively.

Given that each of these antibodies can bind to IRTA family members, the V_H and V_L sequences can be "mixed and matched" to create other anti-IRTA binding molecules of this disclosure. IRTA binding of such "mixed and matched" antibodies can be tested using the binding assays described above and in the Examples (*e.g.*, ELISA or flow cytometry). Preferably, when V_H and V_L chains are mixed and

matched, a V_H sequence from a particular V_H/V_L pairing is replaced with a structurally similar V_H sequence. Likewise, preferably a V_L sequence from a particular V_H/V_L pairing is replaced with a structurally similar V_L sequence.

Accordingly, in one aspect, this disclosure provides an isolated monoclonal antibody, or antigen binding portion thereof comprising:

(a) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 13-14; and

(b) a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 15-16;

wherein the antibody specifically binds an IRTA protein, preferably at least two IRTA family proteins, more preferably at least two human IRTA family proteins. Preferred heavy and light chain combinations include:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 13 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 15; or

(b) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 14 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

In another aspect, this disclosure provides antibodies that comprise the heavy chain and light chain CDR1s, CDR2s and CDR3s of 4C1 or 1B10, or combinations thereof. The amino acid sequences of the V_H CDR1s of 4C1 and 1B10 are shown in SEQ ID NOs: 1-2, respectively. The amino acid sequences of the V_H CDR2s of 4C1 and 1B10 are shown in SEQ ID NOs: 3-4, respectively. The amino acid sequences of the V_H CDR3s of 4C1 and 1B10 are shown in SEQ ID NOs: 5-6, respectively. The amino acid sequences of the V_K CDR1s of 4C1 and 1B10 are shown in SEQ ID NOs: 7-8, respectively. The amino acid sequences of the V_K CDR2s of 4C1 and 1B10 are shown in SEQ ID NOs: 9-10. The amino acid sequences of the V_K CDR3s of 4C1 and 1B10 are shown in SEQ ID NOs: 11-12, respectively. The CDR regions are delineated using the Kabat system (Kabat, E. A., *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242).

Given that each of these antibodies can bind to IRTA family members and that antigen-binding specificity is provided primarily by the CDR1, CDR2, and CDR3

regions, the V_H CDR1, CDR2, and CDR3 sequences and V_L CDR1, CDR2, and CDR3 sequences can be "mixed and matched" (*i.e.*, CDRs from different antibodies can be mixed and match, although each antibody must contain a V_H CDR1, CDR2, and CDR3 and a V_L CDR1, CDR2, and CDR3) to create other anti-IRTA binding molecules of this disclosure. IRTA binding of such "mixed and matched" antibodies can be tested using the binding assays described above and in the Examples (*e.g.*, ELISAs, Biacore® analysis). Preferably, when V_H CDR sequences are mixed and matched, the CDR1, CDR2 and/or CDR3 sequence from a particular V_H sequence is replaced with a structurally similar CDR sequence(s). Likewise, when V_L CDR sequences are mixed and matched, the CDR1, CDR2 and/or CDR3 sequence from a particular V_L sequence preferably is replaced with a structurally similar CDR sequence(s). It will be readily apparent to the ordinarily skilled artisan that novel V_H and V_L sequences can be created by substituting one or more V_H and/or V_L CDR region sequences with structurally similar sequences from the CDR sequences disclosed herein for monoclonal antibodies 4C1 and 1B10.

Accordingly, in another aspect, this disclosure provides an isolated monoclonal antibody, or antigen binding portion thereof comprising:

(a) a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-2;

(b) a heavy chain variable region CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 3-4;

(c) a heavy chain variable region CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 5-6;

(d) a light chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 7-8;

(e) a light chain variable region CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 9-10; and

(f) a light chain variable region CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 11-12;

wherein the antibody specifically binds an IRTA protein, preferably at least two IRTA family proteins, more preferably at least two human IRTA family proteins.

In a preferred embodiment, the antibody comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO: 1;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO: 3;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO: 5;

(d) a light chain variable region CDR1 comprising SEQ ID NO: 7;

(e) a light chain variable region CDR2 comprising SEQ ID NO: 9; and

(f) a light chain variable region CDR3 comprising SEQ ID NO: 11.

In another preferred embodiment, the antibody comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO: 2;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO: 4;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO: 6;

(d) a light chain variable region CDR1 comprising SEQ ID NO: 8;

(e) a light chain variable region CDR2 comprising SEQ ID NO: 10; and

(f) a light chain variable region CDR3 comprising SEQ ID NO: 12.

It is well known in the art that the CDR3 domain, independently from the CDR1 and/or CDR2 domain(s), alone can determine the binding specificity of an antibody for a cognate antigen and that multiple antibodies can predictably be generated having the same binding specificity based on a common CDR3 sequence. *See*, for example, Klimka *et al.*, *British J. of Cancer* 83(2):252-260 (2000) (describing the production of a humanized anti-CD30 antibody using only the heavy chain variable domain CDR3 of murine anti-CD30 antibody Ki-4); Beiboer *et al.*, *J. Mol. Biol.* 296:833-849 (2000) (describing recombinant epithelial glycoprotein-2 (EGP-2) antibodies using only the heavy chain CDR3 sequence of the parental murine MOC-31 anti-EGP-2 antibody); Rader *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 95:8910-8915 (1998) (describing a panel of humanized anti-integrin $\alpha_v\beta_3$ antibodies using a heavy and light chain variable CDR3 domain of a murine anti-integrin $\alpha_v\beta_3$ antibody LM609

wherein each member antibody comprises a distinct sequence outside the CDR3 domain and capable of binding the same epitope as the parent murine antibody with affinities as high or higher than the parent murine antibody); Barbas *et al.*, *J. Am. Chem. Soc.* 116:2161-2162 (1994) (disclosing that the CDR3 domain provides the most significant contribution to antigen binding); Barbas *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 92:2529-2533 (1995) (describing the grafting of heavy chain CDR3 sequences of three Fabs (SI-1, SI-40, and SI-32) against human placental DNA onto the heavy chain of an anti-tetanus toxoid Fab thereby replacing the existing heavy chain CDR3 and demonstrating that the CDR3 domain alone conferred binding specificity); Ditzel *et al.*, *J. Immunol.* 157:739-749 (1996) (describing grafting studies wherein transfer of only the heavy chain CDR3 of a parent polyspecific Fab LNA3 to a heavy chain of a monospecific IgG tetanus toxoid-binding Fab p313 antibody was sufficient to retain binding specificity of the parent Fab); Berezov *et al.*, *BIAjournal* 8:Scientific Review 8 (2001) (describing peptide mimetics based on the CDR3 of an anti-HER2 monoclonal antibody; Igarashi *et al.*, *J. Biochem (Tokyo)* 117:452-7 (1995) (describing a 12 amino acid synthetic polypeptide corresponding to the CDR3 domain of an anti-phosphatidylserine antibody); Bourgeois *et al.*, *J. Virol* 72:807-10 (1998) (showing that a single peptide derived form the heavy chain CDR3 domain of an anti-respiratory syncytial virus (RSV) antibody was capable of neutralizing the virus *in vitro*); Levi *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 90:4374-8 (1993) (describing a peptide based on the heavy chain CDR3 domain of a murine anti-HIV antibody); Polymenis and Stoller, *J. Immunol.* 152:5218-5329 (1994) (describing enabling binding of an scFv by grafting the heavy chain CDR3 region of a Z-DNA-binding antibody) and Xu and Davis, *Immunity* 13:37-45 (2000) (describing that diversity at the heavy chain CDR3 is sufficient to permit otherwise identical IgM molecules to distinguish between a variety of hapten and protein antigens). See also, US Patents Nos. 6,951,646; 6,914,128; 6,090,382; 6,818,216; 6,156,313; 6,827,925; 5,833,943; 5,762,905 and 5,760,185, describing patented antibodies defined by a single CDR domain. Each of these references is hereby incorporated by reference in its entirety.

Accordingly, the present disclosure provides monoclonal antibodies comprising one or more heavy and/or light chain CDR3 domains from an antibody derived from a human or non-human animal, wherein the monoclonal antibody is

capable of specifically binding to multiple IRTA family members. Within certain aspects, the present disclosure provides monoclonal antibodies comprising one or more heavy and/or light chain CDR3 domain from a non-human antibody, such as a mouse or rat antibody, wherein the monoclonal antibody is capable of specifically binding to multiple IRTA family members. Within some embodiments, such inventive antibodies comprising one or more heavy and/or light chain CDR3 domain from a non-human antibody (a) are capable of competing for binding with; (b) retain the functional characteristics; (c) bind to the same epitope; and/or (d) have a similar binding affinity as the corresponding parental non-human antibody.

Within other aspects, the present disclosure provides monoclonal antibodies comprising one or more heavy and/or light chain CDR3 domain from a human antibody, such as, for example, a human antibody obtained from a non-human animal, wherein the human antibody is capable of specifically binding to multiple IRTA family members. Within other aspects, the present disclosure provides monoclonal antibodies comprising one or more heavy and/or light chain CDR3 domain from a first human antibody, such as, for example, a human antibody obtained from a non-human animal, wherein the first human antibody is capable of specifically binding to multiple IRTA family members and wherein the CDR3 domain from the first human antibody replaces a CDR3 domain in a human antibody that is lacking binding specificity for IRTA proteins to generate a second human antibody that is capable of specifically binding to multiple IRTA family members. Within some embodiments, such inventive antibodies comprising one or more heavy and/or light chain CDR3 domain from the first human antibody (a) are capable of competing for binding with; (b) retain the functional characteristics; (c) bind to the same epitope; and/or (d) have a similar binding affinity as the corresponding parental first human antibody.

Antibodies Having Particular Germline Sequences

In certain embodiments, an antibody of this disclosure comprises a heavy chain variable region from a particular germline heavy chain immunoglobulin gene and/or a light chain variable region from a particular germline light chain immunoglobulin gene.

For example, in a preferred embodiment, this disclosure provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a heavy chain variable region that is the product of or derived from a human V_H 3-33 gene or a human V_H 3-7gene, wherein the antibody specifically binds an IRTA protein, preferably at least two IRTA family proteins, more preferably at least two human IRTA family proteins. In another preferred embodiment, this disclosure provides an isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a light chain variable region that is the product of or derived from a human V_K L18 gene or a human V_K A27 gene, wherein the antibody specifically binds an IRTA protein, preferably at least two IRTA family proteins, more preferably at least two human IRTA family proteins. In yet another preferred embodiment, this disclosure provides an isolated monoclonal antibody, or antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable region that is the product of or derived from a human V_H 3-33 gene and comprises a light chain variable region that is the product of or derived from a human V_K L18 gene, wherein the antibody specifically binds an IRTA protein, preferably at least two IRTA family proteins, more preferably at least two human IRTA family proteins. In yet another preferred embodiment, this disclosure provides an isolated monoclonal antibody, or antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable region that is the product of or derived from a human V_H 3-7 gene and comprises a light chain variable region that is the product of or derived from a human V_K A27 gene, wherein the antibody specifically binds an IRTA protein, preferably at least two IRTA family proteins, more preferably at least two human IRTA family proteins. Such antibodies also may possess one or more of the functional characteristics described in detail above, such as high affinity binding to IRTA family proteins and/or binding to at least one, or more, B cell tumor cell lines.

An example of an antibody having V_H and V_L of V_H 3-33 and V_K L18, respectively, is the 4C1 antibody. An example of an antibody having V_H and V_L of V_H 3-7 and V_K A27, respectively, is the 1B10 antibody.

As used herein, a human antibody comprises heavy or light chain variable regions that is "the product of" or "derived from" a particular germline sequence if the variable regions of the antibody are obtained from a system that uses human germline immunoglobulin genes. Such systems include immunizing a transgenic mouse

carrying human immunoglobulin genes with the antigen of interest or screening a human immunoglobulin gene library displayed on phage with the antigen of interest. A human antibody that is "the product of" or "derived from" a human germline immunoglobulin sequence can be identified as such by comparing the amino acid sequence of the human antibody to the amino acid sequences of human germline immunoglobulins and selecting the human germline immunoglobulin sequence that is closest in sequence (*i.e.*, greatest % identity) to the sequence of the human antibody. A human antibody that is "the product of" or "derived from" a particular human germline immunoglobulin sequence may contain amino acid differences as compared to the germline sequence, due to, for example, naturally-occurring somatic mutations or intentional introduction of site-directed mutation. However, a selected human antibody typically is at least 90% identical in amino acids sequence to an amino acid sequence encoded by a human germline immunoglobulin gene and contains amino acid residues that identify the human antibody as being human when compared to the germline immunoglobulin amino acid sequences of other species (*e.g.*, murine germline sequences). In certain cases, a human antibody may be at least 95%, or even at least 96%, 97%, 98%, or 99% identical in amino acid sequence to the amino acid sequence encoded by the germline immunoglobulin gene. Typically, a human antibody derived from a particular human germline sequence will display no more than 10 amino acid differences from the amino acid sequence encoded by the human germline immunoglobulin gene. In certain cases, the human antibody may display no more than 5, or even no more than 4, 3, 2, or 1 amino acid difference from the amino acid sequence encoded by the germline immunoglobulin gene.

Homologous Antibodies

In yet another embodiment, an antibody of this disclosure comprises heavy and light chain variable regions comprising amino acid sequences that are homologous to the amino acid sequences of the preferred antibodies described herein, and wherein the antibodies retain the desired functional properties of the anti-IRTA antibodies of this disclosure.

For example, this disclosure provides an isolated monoclonal antibody, or antigen binding portion thereof, comprising a heavy chain variable region and a light chain variable region, wherein:

(a) the heavy chain variable region comprises an amino acid sequence that is at least 80% homologous to an amino acid sequence selected from the group consisting of SEQ ID NOs: 13-14;

(b) the light chain variable region comprises an amino acid sequence that is at least 80% homologous to an amino acid sequence selected from the group consisting of SEQ ID NOs: 15-16;

(c) the antibody specifically binds to an IRTA protein, preferably at least two IRTA family proteins, more preferably at least two human IRTA family proteins.

Additionally or alternatively, the antibody may possess one or more of the following functional properties discussed above, such as high affinity binding to IRTA family proteins and/or the ability to bind at least one, or more, B cell tumor cell lines.

In various embodiments, the antibody can be, for example, a human antibody, a humanized antibody or a chimeric antibody.

In other embodiments, the V_H and/or V_L amino acid sequences may be 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous to the sequences set forth above. An antibody having V_H and V_L regions having high (*i.e.*, 80% or greater) homology to the V_H and V_L regions of the sequences set forth above, can be obtained by mutagenesis (*e.g.,* site-directed or PCR-mediated mutagenesis) of nucleic acid molecules encoding SEQ ID NOs: 17-18 or 19-20, followed by testing of the encoded altered antibody for retained function (*i.e.*, the functions set forth above) using the functional assays described herein.

As used herein, the percent homology between two amino acid sequences is equivalent to the percent identity between the two sequences. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % homology = # of identical positions/total # of positions x 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be

accomplished using a mathematical algorithm, as described in the non-limiting examples below.

The percent identity between two amino acid sequences can be determined using the algorithm of E. Meyers and W. Miller (*Comput. Appl. Biosci.*, 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman and Wunsch (*J. Mol. Biol.* 48:444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at http://www.gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

Additionally or alternatively, the protein sequences of the present disclosure can further be used as a "query sequence" to perform a search against public databases to, for example, to identify related sequences. Such searches can be performed using the XBLAST program (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to the antibody molecules of this disclosure. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.,* XBLAST and NBLAST) are useful. See www.ncbi.nlm.nih.gov.

Antibodies with Conservative Modifications

In certain embodiments, an antibody of this disclosure comprises a heavy chain variable region comprising CDR1, CDR2 and CDR3 sequences and a light chain variable region comprising CDR1, CDR2 and CDR3 sequences, wherein one or more of these CDR sequences comprise specified amino acid sequences based on the preferred antibodies described herein (*e.g.,* 4C1 or 1B10), or conservative modifications thereof, and wherein the antibodies retain the desired functional properties of the anti-IRTA antibodies of this disclosure. It is understood in the art that certain conservative sequence modification can be made which do not remove

antigen binding. See, for example, Brummell *et al.* (1993) *Biochem* 32:1180-8 (describing mutational analysis in the CDR3 heavy chain domain of antibodies specific for *Salmonella*); de Wildt *et al.* (1997) *Prot. Eng.* 10:835-41 (describing mutation studies in anti-UA1 antibodies); Komissarov *et al.* (1997) *J. Biol. Chem.* 272:26864-26870 (showing that mutations in the middle of HCDR3 led to either abolished or diminished affinity); Hall *et al.* (1992) *J. Immunol.* 149:1605-12 (describing that a single amino acid change in the CDR3 region abolished binding activity); Kelley and O'Connell (1993) *Biochem.* 32:6862-35 (describing the contribution of Tyr residues in antigen binding); Adib-Conquy *et al.* (1998) *Int. Immunol.* 10:341-6 (describing the effect of hydrophobicity in binding) and Beers *et al.* (2000) *Clin. Can. Res.* 6:2835-43 (describing HCDR3 amino acid mutants). Accordingly, this disclosure provides an isolated monoclonal antibody, or antigen binding portion thereof, comprising a heavy chain variable region comprising CDR1, CDR2, and CDR3 sequences and a light chain variable region comprising CDR1, CDR2, and CDR3 sequences, wherein:

(a) the heavy chain variable region CDR3 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs: 5-6, and conservative modifications thereof;

(b) the light chain variable region CDR3 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequence of SEQ ID NOs: 11-12, and conservative modifications thereof; and

(c) the antibody specifically binds an IRTA protein, preferably at least two IRTA family proteins, more preferably at least two human IRTA family proteins.

Additionally or alternatively, the antibody may possess one or more of the following functional properties described above, such as high affinity binding to IRTA family proteins and/or the ability to bind at least one, or more, B cell tumor cell lines.

In a preferred embodiment, the heavy chain variable region CDR2 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs: 3-4, and conservative modifications thereof; and the light chain variable region CDR2 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs: 9-10, and

conservative modifications thereof. In another preferred embodiment, the heavy chain variable region CDR1 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs: 1-2, and conservative modifications thereof; and the light chain variable region CDR1 sequence comprises an amino acid sequence selected from the group consisting of amino acid sequences of SEQ ID NOs: 7-8, and conservative modifications thereof.

In various embodiments, the antibody can be, for example, human antibodies, humanized antibodies or chimeric antibodies.

As used herein, the term "conservative sequence modifications" is intended to refer to amino acid modifications that do not significantly affect or alter the binding characteristics of the antibody containing the amino acid sequence. Such conservative modifications include amino acid substitutions, additions and deletions. Modifications can be introduced into an antibody of this disclosure by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions are ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, one or more amino acid residues within the CDR regions of an antibody of this disclosure can be replaced with other amino acid residues from the same side chain family and the altered antibody can be tested for retained function (*i.e.*, the functions set forth above) using the functional assays described herein.

Antibodies that Bind to the Same Epitope as Anti-IRTA Antibodies

In another embodiment, this disclosure provides antibodies that bind to the same epitope on IRTA family members as any of the anti-IRTA monoclonal antibodies of this disclosure (*i.e.*, antibodies that have the ability to cross-compete for

binding to IRTA family members with any of the monoclonal antibodies of this disclosure). In preferred embodiments, the reference antibody for cross-competition studies can be the monoclonal antibody 4C1 (having V_H and V_L sequences as shown in SEQ ID NOs: 13 and 15, respectively), or the monoclonal antibody 1B10 (having V_H and V_L sequences as shown in SEQ ID NOs: 14 and 16, respectively).

Such cross-competing antibodies can be identified based on their ability to cross-compete with 4C1 and 1B10 in standard IRTA binding assays. For example, standard ELISA assays can be used in which a recombinant IRTA family protein is immoblized on the plate, one of the antibodies is fluorescently labeled and the ability of non-labeled antibodies to compete off the binding of the labeled antibody is evaluated. Additionally or alternatively, BIAcore analysis can be used to assess the ability of the antibodies to cross-compete. The ability of a test antibody to inhibit the binding of, for example, 4C1 and 1B10, to human IRTA family proteins demonstrates that the test antibody can compete with 4C1 and 1B10 for binding to human IRTA family proteins and thus binds to the same epitope on human IRTA family proteins as 4C1 and 1B10. In a preferred embodiment, the antibody that binds to the same epitope on IRTA family proteins as 4C1 or 1B10 is a human monoclonal antibody. Such human monoclonal antibodies can be prepared and isolated as described in the Examples.

Engineered and Modified Antibodies

An antibody of this disclosure further can be prepared using an antibody having one or more of the V_H and/or V_L sequences disclosed herein as starting material to engineer a modified antibody, which modified antibody may have altered properties from the starting antibody. An antibody can be engineered by modifying one or more residues within one or both variable regions (*i.e.*, V_H and/or V_L), for example within one or more CDR regions and/or within one or more framework regions. Additionally or alternatively, an antibody can be engineered by modifying residues within the constant region(s), for example to alter the effector function(s) of the antibody.

In certain embodiments, CDR grafting can be used to engineer variable regions of antibodies. Antibodies interact with target antigens predominantly through

amino acid residues that are located in the six heavy and light chain complementarity determining regions (CDRs). For this reason, the amino acid sequences within CDRs are more diverse between individual antibodies than sequences outside of CDRs. Because CDR sequences are responsible for most antibody-antigen interactions, it is possible to express recombinant antibodies that mimic the properties of specific naturally occurring antibodies by constructing expression vectors that include CDR sequences from the specific naturally occurring antibody grafted onto framework sequences from a different antibody with different properties (see, *e.g.,* Riechmann, L. *et al.* (1998) *Nature* 332:323-327; Jones, P. *et al.* (1986) *Nature* 321:522-525; Queen, C. *et al.* (1989) *Proc. Natl. Acad. See. U.S.A.* 86:10029-10033; U.S. Patent No. 5,225,539 to Winter, and U.S. Patent Nos. 5,530,101; 5,585,089; 5,693,762 and 6,180,370 to Queen *et al.*)

Accordingly, another embodiment of this disclosure pertains to an isolated monoclonal antibody, or antigen binding portion thereof, comprising a heavy chain variable region comprising CDR1, CDR2, and CDR3 sequences comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-2, SEQ ID NOs: 3-4, and SEQ ID NOs: 5-6, respectively, and a light chain variable region comprising CDR1, CDR2, and CDR3 sequences comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 7-8, SEQ ID NOs: 9-10, and SEQ ID NOs: 11-12, respectively. Thus, such antibodies contain the V_H and V_L CDR sequences of monoclonal antibodies 4C1 or 1B10 yet may contain different framework sequences from these antibodies.

Such framework sequences can be obtained from public DNA databases or published references that include germline antibody gene sequences. For example, germline DNA sequences for human heavy and light chain variable region genes can be found in the "VBase" human germline sequence database (available on the Internet at www.mrc-cpe.cam.ac.uk/vbase), as well as in Kabat, E. A., *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242; Tomlinson, I. M., *et al.* (1992) "The Repertoire of Human Germline V_H Sequences Reveals about Fifty Groups of V_H Segments with Different Hypervariable Loops" *J. Mol. Biol.* 227:776-798; and Cox, J. P. L. *et al.* (1994) "A Directory of Human Germ-line V_H Segments Reveals a Strong Bias in their Usage" *Eur. J. Immunol.* 24:827-836; the contents of each of which are

expressly incorporated herein by reference. As another example, the germline DNA sequences for human heavy and light chain variable region genes can be found in the Genbank database. For example, the following heavy chain germline sequences found in the HCo7 HuMAb mouse are available in the accompanying Genbank accession numbers: 1-69 (NG_0010109, NT_024637 and BC070333), 3-33 (NG_0010109 and NT_024637) and 3-7 (NG_0010109 and NT_024637). As another example, the following heavy chain germline sequences found in the HCo12 HuMAb mouse are available in the accompanying Genbank accession numbers: 1-69 (NG_0010109, NT_024637 and BC070333), 5-51 (NG_0010109 and NT_024637), 4-34 (NG_0010109 and NT_024637), 3-30.3 (CAJ556644) and 3-23 (AJ406678).

Antibody protein sequences are compared against a compiled protein sequence database using one of the sequence similarity searching methods called the Gapped BLAST (Altschul *et al.* (1997) *Nucleic Acids Research* 25:3389-3402), which is well known to those skilled in the art. BLAST is a heuristic algorithm in that a statistically significant alignment between the antibody sequence and the database sequence is likely to contain high-scoring segment pairs (HSP) of aligned words. Segment pairs whose scores cannot be improved by extension or trimming is called a *hit*. Briefly, the nucleotide sequences of VBASE origin (*http://vbase.mrc-cpe.cam.ac.uk/vbase1/list2.php*) are translated and the region between and including FR1 through FR3 framework region is retained. The database sequences have an average length of 98 residues. Duplicate sequences which are exact matches over the entire length of the protein are removed. A BLAST search for proteins using the program blastp with default, standard parameters except the low complexity filter, which is turned off, and the substitution matrix of BLOSUM62, filters for top 5 hits yielding sequence matches. The nucleotide sequences are translated in all six frames and the frame with no stop codons in the matching segment of the database sequence is considered the potential hit. This is in turn confirmed using the BLAST program tblastx, which translates the antibody sequence in all six frames and compares those translations to the VBASE nucleotide sequences dynamically translated in all six frames.

The identities are exact amino acid matches between the antibody sequence and the protein database over the entire length of the sequence. The positives (identities + substitution match) are not identical but amino acid substitutions guided

by the BLOSUM62 substitution matrix. If the antibody sequence matches two of the database sequences with same identity, the hit with most positives would be decided to be the matching sequence hit.

Preferred framework sequences for use in the antibodies of this disclosure are those that are structurally similar to the framework sequences used by selected antibodies of this disclosure, *e.g.,* similar to the V_H 3-33 (SEQ ID NO: 21) or V_H 3-7 (SEQ ID NO: 22) framework sequences and/or the V_K L18 (SEQ ID NO: 23) or V_K A27 (SEQ ID NO: 24) framework sequences used by preferred monoclonal antibodies of this disclosure. The V_H CDR1, CDR2, and CDR3 sequences, and the V_K CDR1, CDR2, and CDR3 sequences, can be grafted onto framework regions that have the identical sequence as that found in the germline immunoglobulin gene from which the framework sequence derive, or the CDR sequences can be grafted onto framework regions that contain one or more mutations as compared to the germline sequences. For example, it has been found that in certain instances it is beneficial to mutate residues within the framework regions to maintain or enhance the antigen binding ability of the antibody (see *e.g.,* U.S. Patent Nos. 5,530,101; 5,585,089; 5,693,762 and 6,180,370 to Queen *et al.*).

Another type of variable region modification is to mutate amino acid residues within the V_H and/or V_L CDR1, CDR2 and/or CDR3 regions to thereby improve one or more binding properties (*e.g.,* affinity) of the antibody of interest. Site-directed mutagenesis or PCR-mediated mutagenesis can be performed to introduce the mutation(s) and the effect on antibody binding, or other functional property of interest, can be evaluated in *in vitro* or *in vivo* assays as described herein and provided in the Examples. Preferably conservative modifications (as discussed above) are introduced. The mutations may be amino acid substitutions, additions or deletions, but are preferably substitutions. Moreover, typically no more than one, two, three, four or five residues within a CDR region are altered.

Accordingly, in another embodiment, the instant disclosure provides isolated anti-IRTA monoclonal antibodies, or antigen binding portions thereof, comprising a heavy chain variable region comprising: (a) a V_H CDR1 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-2, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs: 1-2; (b) a V_H CDR2 region comprising an

amino acid sequence selected from the group consisting of SEQ ID NOs: 3-4, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs: 3-4; (c) a V_H CDR3 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 5-6, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs: 5-6; (d) a V_L CDR1 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 7-8, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs: 7-8; (e) a V_L CDR2 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 9-10, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs: 9-10; and (f) a V_L CDR3 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 11-12, or an amino acid sequence having one, two, three, four or five amino acid substitutions, deletions or additions as compared to SEQ ID NOs: 11-12.

Engineered antibodies of this disclosure include those in which modifications have been made to framework residues within V_H and/or V_L, *e.g.* to improve the properties of the antibody. Typically such framework modifications are made to decrease the immunogenicity of the antibody. For example, one approach is to "backmutate" one or more framework residues to the corresponding germline sequence. More specifically, an antibody that has undergone somatic mutation may contain framework residues that differ from the germline sequence from which the antibody is derived. Such residues can be identified by comparing the antibody framework sequences to the germline sequences from which the antibody is derived.

Another type of framework modification involves mutating one or more residues within the framework region, or even within one or more CDR regions, to remove T cell epitopes to thereby reduce the potential immunogenicity of the antibody. This approach is also referred to as "deimmunization" and is described in further detail in U.S. Patent Publication No. 20030153043 by Carr *et al.*

In addition or alternative to modifications made within the framework or CDR regions, antibodies of this disclosure may be engineered to include modifications within the Fc region, typically to alter one or more functional properties of the

antibody, such as serum half-life, complement fixation, Fc receptor binding, and/or antigen-dependent cellular cytotoxicity. Furthermore, an antibody of this disclosure may be chemically modified (*e.g.*, one or more chemical moieties can be attached to the antibody) or be modified to alter its glycosylation, again to alter one or more functional properties of the antibody. Each of these embodiments is described in further detail below. The numbering of residues in the Fc region is that of the EU index of Kabat.

In one embodiment, the hinge region of CH1 is modified such that the number of cysteine residues in the hinge region is altered, *e.g.*, increased or decreased. This approach is described further in U.S. Patent No. 5,677,425 by Bodmer *et al.* The number of cysteine residues in the hinge region of CH1 is altered to, for example, facilitate assembly of the light and heavy chains or to increase or decrease the stability of the antibody.

In another embodiment, the Fc hinge region of an antibody is mutated to decrease the biological half life of the antibody. More specifically, one or more amino acid mutations are introduced into the CH2-CH3 domain interface region of the Fc-hinge fragment such that the antibody has impaired Staphylococcyl protein A (SpA) binding relative to native Fc-hinge domain SpA binding. This approach is described in further detail in U.S. Patent No. 6,165,745 by Ward *et al.*

In another embodiment, the antibody is modified to increase its biological half life. Various approaches are possible. For example, one or more of the following mutations can be introduced: T252L, T254S, T256F, as described in U.S. Patent No. 6,277,375 to Ward. Alternatively, to increase the biological half life, the antibody can be altered within the CH1 or CL region to contain a salvage receptor binding epitope taken from two loops of a CH2 domain of an Fc region of an IgG, as described in U.S. Patent Nos. 5,869,046 and 6,121,022 by Presta *et al.*

In yet other embodiments, the Fc region is altered by replacing at least one amino acid residue with a different amino acid residue to alter the effector function(s) of the antibody. For example, one or more amino acids selected from amino acid residues 234, 235, 236, 237, 297, 318, 320 and 322 can be replaced with a different amino acid residue such that the antibody has an altered affinity for an effector ligand but retains the antigen-binding ability of the parent antibody. The effector ligand to which affinity is altered can be, for example, an Fc receptor or the C1 component of

complement. This approach is described in further detail in U.S. Patent Nos. 5,624,821 and 5,648,260, both by Winter *et al.*

In another example, one or more amino acids selected from amino acid residues 329, 331 and 322 can be replaced with a different amino acid residue such that the antibody has altered C1q binding and/or reduced or abolished complement dependent cytotoxicity (CDC). This approach is described in further detail in U.S. Patent Nos. 6,194,551 by Idusogie *et al.*

In another example, one or more amino acid residues within amino acid positions 231 and 239 are altered to thereby alter the ability of the antibody to fix complement. This approach is described further in PCT Publication WO 94/29351 by Bodmer *et al.*

In yet another example, the Fc region is modified to increase the ability of the antibody to mediate antibody dependent cellular cytotoxicity (ADCC) and/or to increase the affinity of the antibody for an Fcγ receptor by modifying one or more amino acids at the following positions: 238, 239, 248, 249, 252, 254, 255, 256, 258, 265, 267, 268, 269, 270, 272, 276, 278, 280, 283, 285, 286, 289, 290, 292, 293, 294, 295, 296, 298, 301, 303, 305, 307, 309, 312, 315, 320, 322, 324, 326, 327, 329, 330, 331, 333, 334, 335, 337, 338, 340, 360, 373, 376, 378, 382, 388, 389, 398, 414, 416, 419, 430, 434, 435, 437, 438 or 439. This approach is described further in PCT Publication WO 00/42072 by Presta. Moreover, the binding sites on human IgG1 for FcγR1, FcγRII, FcγRIII and FcRn have been mapped and variants with improved binding have been described (see Shields, R.L. *et al.* (2001) *J. Biol. Chem.* 276:6591-6604). Specific mutations at positions 256, 290, 298, 333, 334 and 339 were shown to improve binding to FcγRIII. Additionally, the following combination mutants were shown to improve FcγRIII binding: T256A/S298A, S298A/E333A, S298A/K224A and S298A/E333A/K334A.

In still another embodiment, the glycosylation of an antibody is modified. For example, an aglycoslated antibody can be made (*i.e.*, the antibody lacks glycosylation). Glycosylation can be altered to, for example, increase the affinity of the antibody for antigen. Such carbohydrate modifications can be accomplished by, for example, altering one or more sites of glycosylation within the antibody sequence. For example, one or more amino acid substitutions can be made that result in elimination of one or more variable region framework glycosylation sites to thereby

eliminate glycosylation at that site. Such aglycosylation may increase the affinity of the antibody for antigen. Such an approach is described in further detail in U.S. Patent Nos. 5,714,350 and 6,350,861 by Co *et al.*

Additionally or alternatively, an antibody can be made that has an altered type of glycosylation, such as a hypofucosylated antibody having reduced amounts of fucosyl residues or an antibody having increased bisecting GlcNac structures. Such altered glycosylation patterns have been demonstrated to increase the ADCC ability of antibodies. Such carbohydrate modifications can be accomplished by, for example, expressing the antibody in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery have been described in the art and can be used as host cells in which to express recombinant antibodies of this disclosure to thereby produce an antibody with altered glycosylation. For example, the cell lines Ms704, Ms705, and Ms709 lack the fucosyltransferase gene, FUT8 (alpha (1,6) fucosyltransferase), such that antibodies expressed in the Ms704, Ms705, and Ms709 cell lines lack fucose on their carbohydrates. The Ms704, Ms705, and Ms709 $FUT8^{-/-}$ cell lines were created by the targeted disruption of the FUT8 gene in CHO/DG44 cells using two replacement vectors (see U.S. Patent Publication No. 20040110704 by Yamane *et al.* and Yamane-Ohnuki *et al.* (2004) *Biotechnol Bioeng* 87:614-22). As another example, EP 1,176,195 by Hanai *et al.* describes a cell line with a functionally disrupted FUT8 gene, which encodes a fucosyl transferase, such that antibodies expressed in such a cell line exhibit hypofucosylation by reducing or eliminating the alpha 1,6 bond-related enzyme. Hanai *et al.* also describe cell lines which have a low enzyme activity for adding fucose to the N-acetylglucosamine that binds to the Fc region of the antibody or does not have the enzyme activity, for example the rat myeloma cell line YB2/0 (ATCC CRL 1662). PCT Publication WO 03/035835 by Presta describes a variant CHO cell line, Lec13 cells, with reduced ability to attach fucose to Asn(297)-linked carbohydrates, also resulting in hypofucosylation of antibodies expressed in that host cell (see also Shields, R.L. *et al.* (2002) *J. Biol. Chem.* 277:26733-26740). Antibodies with a modified glycosylation profile can also be produced in chicken eggs, as described in US Patent Application No. PCT/US06/05853. Alternatively, antibodies with a modified glycosylation profile can be produced in plant cells, such as *Lemna*. Methods for production of antibodies in a plant system are disclosed in the U.S. Patent application corresponding to Alston &

Bird LLP attorney docket No. 040989/314911, filed on August 11, 2006. PCT Publication WO 99/54342 by Umana *et al.* describes cell lines engineered to express glycoprotein-modifying glycosyl transferases (*e.g.*, beta(1,4)-N-acetylglucosaminyltransferase III (GnTIII)) such that antibodies expressed in the engineered cell lines exhibit increased bisecting GlcNac structures which results in increased ADCC activity of the antibodies (see also Umana *et al.* (1999) *Nat. Biotech.* 17:176-180). Alternatively, the fucose residues of the antibody may be cleaved off using a fucosidase enzyme. For example, the fucosidase alpha-L-fucosidase removes fucosyl residues from antibodies (Tarentino, A.L. *et al.* (1975) *Biochem.* 14:5516-23).

Another modification of the antibodies herein that is contemplated by this disclosure is pegylation. An antibody can be pegylated to, for example, increase the biological (*e.g.*, serum) half life of the antibody. To pegylate an antibody, the antibody, or fragment thereof, typically is reacted with polyethylene glycol (PEG), such as a reactive ester or aldehyde derivative of PEG, under conditions in which one or more PEG groups become attached to the antibody or antibody fragment. Preferably, the pegylation is carried out via an acylation reaction or an alkylation reaction with a reactive PEG molecule (or an analogous reactive water-soluble polymer). As used herein, the term "polyethylene glycol" is intended to encompass any of the forms of PEG that have been used to derivatize other proteins, such as mono (C1-C10) alkoxy- or aryloxy-polyethylene glycol or polyethylene glycol-maleimide. In certain embodiments, the antibody to be pegylated is an aglycosylated antibody. Methods for pegylating proteins are known in the art and can be applied to the antibodies of this disclosure. See for example, EP 0 154 316 by Nishimura *et al.* and EP 0 401 384 by Ishikawa *et al.*

Antibody Physical Properties

The antibodies of the present disclosure may be further characterized by the various physical properties of the anti-IRTA antibodies. Various assays may be used to detect and/or differentiate different classes of antibodies based on these physical properties.

In some embodiments, antibodies of the present disclosure may contain one or more glycosylation sites in either the light or heavy chain variable region. The

presence of one or more glycosylation sites in the variable region may result in increased immunogenicity of the antibody or an alteration of the pK of the antibody due to altered antigen binding (Marshall *et al* (1972) *Annu Rev Biochem* 41:673-702; Gala FA and Morrison SL (2004) *J Immunol* 172:5489-94; Wallick *et al* (1988) *J Exp Med* 168:1099-109; Spiro RG (2002) *Glycobiology* 12:43R-56R; Parekh *et al* (1985) *Nature* 316:452-7; Mimura *et al.* (2000) *Mol Immunol* 37:697-706). Glycosylation has been known to occur at motifs containing an N-X-S/T sequence. Variable region glycosylation may be tested using a Glycoblot assay, which cleaves the antibody to produce a Fab, and then tests for glycosylation using an assay that measures periodate oxidation and Schiff base formation. Alternatively, variable region glycosylation may be tested using Dionex light chromatography (Dionex-LC), which cleaves saccharides from a Fab into monosaccharides and analyzes the individual saccharide content. In some instances, it is preferred to have an anti-IRTA antibody that does not contain variable region glycosylation. This can be achieved either by selecting antibodies that do not contain the glycosylation motif in the variable region or by mutating residues within the glycosylation motif using standard techniques well known in the art.

In a preferred embodiment, the antibodies of the present disclosure do not contain asparagine isomerism sites. A deamidation or isoaspartic acid effect may occur on N-G or D-G sequences, respectively. The deamidation or isoaspartic acid effect results in the creation of isoaspartic acid which decreases the stability of an antibody by creating a kinked structure off a side chain carboxy terminus rather than the main chain. The creation of isoaspartic acid can be measured using an iso-quant assay, which uses a reverse-phase HPLC to test for isoaspartic acid.

Each antibody will have a unique isoelectric point (pI), but generally antibodies will fall in the pH range of between 6 and 9.5. The pI for an IgG1 antibody typically falls within the pH range of 7-9.5 and the pI for an IgG4 antibody typically falls within the pH range of 6-8. Antibodies may have a pI that is outside this range. Although the effects are generally unknown, there is speculation that antibodies with a pI outside the normal range may have some unfolding and instability under *in vivo* conditions. The isoelectric point may be tested using a capillary isoelectric focusing assay, which creates a pH gradient and may utilize laser focusing for increased accuracy (Janini *et al* (2002) *Electrophoresis* 23:1605-11; Ma *et al.* (2001) *Chromatographia* 53:S75-89; Hunt *et al* (1998) *J Chromatogr A*

800:355-67). In some instances, it is preferred to have an anti-IRTA antibody that contains a pI value that falls in the normal range. This can be achieved either by selecting antibodies with a pI in the normal range, or by mutating charged surface residues using standard techniques well known in the art.

Each antibody will have a melting temperature that is indicative of thermal stability (Krishnamurthy R and Manning MC (2002) *Curr Pharm Biotechnol* 3:361-71). A higher thermal stability indicates greater overall antibody stability *in vivo*. The melting point of an antibody may be measure using techniques such as differential scanning calorimetry (Chen *et al* (2003) *Pharm Res* 20:1952-60; Ghirlando *et al* (1999) *Immunol Lett* 68:47-52). T_{M1} indicates the temperature of the initial unfolding of the antibody. T_{M2} indicates the temperature of complete unfolding of the antibody. Generally, it is preferred that the T_{M1} of an antibody of the present disclosure is greater than 60°C, preferably greater than 65°C, even more preferably greater than 70°C. Alternatively, the thermal stability of an antibody may be measure using circular dichroism (Murray *et al.* (2002) *J. Chromatogr Sci* 40:343-9).

In a preferred embodiment, antibodies are selected that do not rapidly degrade. Fragmentation of an anti-IRTA antibody may be measured using capillary electrophoresis (CE) and MALDI-MS, as is well understood in the art (Alexander AJ and Hughes DE (1995) *Anal Chem* 67:3626-32).

In another preferred embodiment, antibodies are selected that have minimal aggregation effects. Aggregation may lead to triggering of an unwanted immune response and/or altered or unfavorable pharmacokinetic properties. Generally, antibodies are acceptable with aggregation of 25% or less, preferably 20% or less, even more preferably 15% or less, even more preferably 10% or less and even more preferably 5% or less. Aggregation may be measured by several techniques well known in the art, including size-exclusion column (SEC) high performance liquid chromatography (HPLC), and light scattering to identify monomers, dimers, trimers or multimers.

Methods of Engineering Antibodies

As discussed above, the anti-IRTA antibodies having V_H and V_L sequences disclosed herein can be used to create new anti-IRTA antibodies by modifying the V_H

and/or V_L sequences, or the constant region(s) attached thereto. Thus, in another aspect of this disclosure, the structural features of an anti-IRTA antibody of this disclosure, *e.g.* 4C1 or 1B10, are used to create structurally related anti-IRTA antibodies that retain at least one functional property of the antibodies of this disclosure, such as binding to multiple IRTA family members. For example, one or more CDR regions of 4C1 or 1B10, or mutations thereof, can be combined recombinantly with known framework regions and/or other CDRs to create additional, recombinantly-engineered, anti-IRTA antibodies of this disclosure, as discussed above. Other types of modifications include those described in the previous section. The starting material for the engineering method is one or more of the V_H and/or V_L sequences provided herein, or one or more CDR regions thereof. To create the engineered antibody, it is not necessary to actually prepare (*i.e.*, express as a protein) an antibody having one or more of the V_H and/or V_L sequences provided herein, or one or more CDR regions thereof. Rather, the information contained in the sequence(s) is used as the starting material to create a "second generation" sequence(s) derived from the original sequence(s) and then the "second generation" sequence(s) is prepared and expressed as a protein.

Accordingly, in another embodiment, this disclosure provides a method for preparing an anti-IRTA antibody comprising:

(a) providing: (i) a heavy chain variable region antibody sequence comprising a CDR1 sequence selected from the group consisting of SEQ ID NOs: 1-2, a CDR2 sequence selected from the group consisting of SEQ ID NOs: 3-4, and/or a CDR3 sequence selected from the group consisting of SEQ ID NOs: 5-6; and/or (ii) a light chain variable region antibody sequence comprising a CDR1 sequence selected from the group consisting of SEQ ID NOs: 7-8, a CDR2 sequence selected from the group consisting of SEQ ID NOs: 9-10, and/or a CDR3 sequence selected from the group consisting of SEQ ID NOs: 11-12;

(b) altering at least one amino acid residue within the heavy chain variable region antibody sequence and/or the light chain variable region antibody sequence to create at least one altered antibody sequence; and

(c) expressing the altered antibody sequence as a protein.

Standard molecular biology techniques can be used to prepare and express the altered antibody sequence.

Preferably, the antibody encoded by the altered antibody sequence(s) is one that retains one, some or all of the functional properties of the anti-IRTA antibodies described herein, which functional properties include, but are not limited to:

(i) binding to at least two IRTA family proteins, preferably at least two human IRTA family proteins;

(ii) binding to an IRTA4 protein and at least one other IRTA family protein;

(iii) high affinity binding to at least two IRTA family proteins;

(iv) an ability to bind at least one B cell tumor cell or cell line;

(v) an ability to bind a Daudi B cell tumor cell line; and/or

(vi) exhibiting antibody dependent cellular cytotoxicity (ADCC) activity.

The functional properties of the altered antibodies can be assessed using standard assays available in the art and/or described herein, such as those set forth in the Examples.

In certain embodiments of the methods of engineering antibodies of this disclosure, mutations can be introduced randomly or selectively along all or part of an anti-IRTA antibody coding sequence and the resulting modified anti-IRTA antibodies can be screened for binding activity and/or other functional properties as described herein. Mutational methods have been described in the art. For example, PCT Publication WO 02/092780 by Short describes methods for creating and screening antibody mutations using saturation mutagenesis, synthetic ligation assembly, or a combination thereof. Alternatively, PCT Publication WO 03/074679 by Lazar *et al.* describes methods of using computational screening methods to optimize physiochemical properties of antibodies.

Nucleic Acid Molecules Encoding Antibodies of this Disclosure

Another aspect of this disclosure pertains to nucleic acid molecules that encode the antibodies of this disclosure. The nucleic acids may be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form. A nucleic acid is "isolated" or "rendered substantially pure" when purified away from other cellular components or other contaminants, *e.g.,* other cellular nucleic acids or proteins, by standard techniques, including alkaline/SDS treatment, CsCl banding, column chromatography, agarose gel electrophoresis and others well known in the art.

See, F. Ausubel, *et al.*, ed. (1987) Current Protocols in Molecular Biology, Greene Publishing and Wiley Interscience, New York. A nucleic acid of this disclosure can be, for example, DNA or RNA and may or may not contain intronic sequences. In a preferred embodiment, the nucleic acid is a cDNA molecule.

Nucleic acids of this disclosure can be obtained using standard molecular biology techniques. For antibodies expressed by hybridomas (*e.g.*, hybridomas prepared from transgenic mice carrying human immunoglobulin genes as described further below), cDNAs encoding the light and heavy chains of the antibody made by the hybridoma can be obtained by standard PCR amplification or cDNA cloning techniques. For antibodies obtained from an immunoglobulin gene library (*e.g.*, using phage display techniques), a nucleic acid encoding such antibodies can be recovered from the gene library.

Preferred nucleic acids molecules of this disclosure are those encoding the V_H and V_L sequences of the 4C1 and 1B10 monoclonal antibodies. DNA sequences encoding the V_H sequences of 4C1 and 1B10 are shown in SEQ ID NOs: 17-18, respectively. DNA sequences encoding the V_L sequences of 4C1 and 1B10 are shown in SEQ ID NOs: 19-20, respectively.

Once DNA fragments encoding V_H and V_L segments are obtained, these DNA fragments can be further manipulated by standard recombinant DNA techniques, for example to convert the variable region genes to full-length antibody chain genes, to Fab fragment genes or to a scFv gene. In these manipulations, a V_L- or V_H-encoding DNA fragment is operatively linked to another DNA fragment encoding another protein, such as an antibody constant region or a flexible linker. The term "operatively linked", as used in this context, is intended to mean that the two DNA fragments are joined such that the amino acid sequences encoded by the two DNA fragments remain in-frame.

The isolated DNA encoding the V_H region can be converted to a full-length heavy chain gene by operatively linking the VH-encoding DNA to another DNA molecule encoding heavy chain constant regions (CH1, CH2 and CH3). The sequences of human heavy chain constant region genes are known in the art (see *e.g.*, Kabat, E. A., el al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard

PCR amplification. The heavy chain constant region can be an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM or IgD constant region, but most preferably is an IgG1 or IgG4 constant region. For a Fab fragment heavy chain gene, the V_H-encoding DNA can be operatively linked to another DNA molecule encoding only the heavy chain CH1 constant region.

The isolated DNA encoding the V_L region can be converted to a full-length light chain gene (as well as a Fab light chain gene) by operatively linking the V_L-encoding DNA to another DNA molecule encoding the light chain constant region, CL. The sequences of human light chain constant region genes are known in the art (see *e.g.,* Kabat, E. A., *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. In preferred embodiments, the light chain constant region can be a kappa or lambda constant region.

To create a scFv gene, the V_H- and V_L-encoding DNA fragments are operatively linked to another fragment encoding a flexible linker, *e.g.,* encoding the amino acid sequence $(Gly_4\text{-}Ser)_3$, such that the V_H and V_L sequences can be expressed as a contiguous single-chain protein, with the V_L and V_H regions joined by the flexible linker (see *e.g.,* Bird *et al.* (1988) *Science* 242:423-426; Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883; McCafferty *et al.*, (1990) *Nature* 348:552-554).

Production of Monoclonal Antibodies of this Disclosure

Monoclonal antibodies (mAbs) of the present disclosure can be produced by a variety of techniques, including conventional monoclonal antibody methodology *e.g.,* the standard somatic cell hybridization technique of Kohler and Milstein (1975) *Nature* 256: 495. Although somatic cell hybridization procedures are preferred, in principle, other techniques for producing monoclonal antibody can be employed *e.g.,* viral or oncogenic transformation of B lymphocytes.

The preferred animal system for preparing hybridomas is the murine system. Hybridoma production in the mouse is a very well-established procedure. Immunization protocols and techniques for isolation of immunized splenocytes for

fusion are known in the art. Fusion partners (*e.g.*, murine myeloma cells) and fusion procedures are also known.

Chimeric or humanized antibodies of the present disclosure can be prepared based on the sequence of a non-human monoclonal antibody prepared as described above. DNA encoding the heavy and light chain immunoglobulins can be obtained from the non-human hybridoma of interest and engineered to contain non-murine (*e.g.*, human) immunoglobulin sequences using standard molecular biology techniques. For example, to create a chimeric antibody, murine variable regions can be linked to human constant regions using methods known in the art (see *e.g.*, U.S. Patent No. 4,816,567 to Cabilly *et al.*). To create a humanized antibody, murine CDR regions can be inserted into a human framework using methods known in the art (see *e.g.*, U.S. Patent No. 5,225,539 to Winter, and U.S. Patent Nos. 5,530,101; 5,585,089; 5,693,762 and 6,180,370 to Queen *et al.*).

In a preferred embodiment, the antibodies of this disclosure are human monoclonal antibodies. Such human monoclonal antibodies directed against IRTA family members can be generated using transgenic or transchromosomic mice carrying parts of the human immune system rather than the mouse system. These transgenic and transchromosomic mice include mice referred to herein as the HuMAb Mouse® and KM Mouse®, respectively, and are collectively referred to herein as "human Ig mice."

The HuMAb Mouse® (Medarex®, Inc.) contains human immunoglobulin gene miniloci that encode unrearranged human heavy (μ and γ) and κ light chain immunoglobulin sequences, together with targeted mutations that inactivate the endogenous μ and κ chain loci (see *e.g.*, Lonberg, *et al.* (1994) *Nature* 368(6474): 856-859). Accordingly, the mice exhibit reduced expression of mouse IgM or κ, and in response to immunization, the introduced human heavy and light chain transgenes undergo class switching and somatic mutation to generate high affinity human IgGκ monoclonal antibodies (Lonberg, N. *et al.* (1994), *supra*; reviewed in Lonberg, N. (1994) *Handbook of Experimental Pharmacology* 113:49-101; Lonberg, N. and Huszar, D. (1995) *Intern. Rev. Immunol.* 13: 65-93, and Harding, F. and Lonberg, N. (1995) *Ann. N.Y. Acad. Sci.* 764:536-546). Preparation and use of the HuMAb Mouse®, and the genomic modifications carried by such mice, is further described in Taylor, L. *et al.* (1992) *Nucleic Acids Research* 20:6287-6295; Chen, J. *et al.* (1993)

International Immunology 5: 647-656; Tuaillon *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:3720-3724; Choi *et al.* (1993) *Nature Genetics* 4:117-123; Chen, J. *et al.* (1993) *EMBO J.* 12: 821-830; Tuaillon *et al.* (1994) *J. Immunol.* 152:2912-2920; Taylor, L. *et al.* (1994) *International Immunology* 6: 579-591; and Fishwild, D. *et al.* (1996) *Nature Biotechnology* 14: 845-851, the contents of all of which are hereby specifically incorporated by reference in their entirety. See further, U.S. Patent Nos. 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,789,650; 5,877,397; 5,661,016; 5,814,318; 5,874,299; and 5,770,429; all to Lonberg and Kay; U.S. Patent No. 5,545,807 to Surani *et al.*; PCT Publication Nos. WO 92/03918, WO 93/12227, WO 94/25585, WO 97/13852, WO 98/24884 and WO 99/45962, all to Lonberg and Kay; and PCT Publication No. WO 01/14424 to Korman *et al.*

In another embodiment, human antibodies of this disclosure can be raised using a mouse that carries human immunoglobulin sequences on transgenes and transchomosomes, such as a mouse that carries a human heavy chain transgene and a human light chain transchromosome. This mouse is referred to herein as a "KM mouse®," and is described in detail in PCT Publication WO 02/43478 to Ishida *et al.*

Still further, alternative transgenic animal systems expressing human immunoglobulin genes are available in the art and can be used to raise anti-IRTA antibodies of this disclosure. For example, an alternative transgenic system referred to as the Xenomouse (Abgenix, Inc.) can be used; such mice are described in, for example, U.S. Patent Nos. 5,939,598; 6,075,181; 6,114,598; 6,150,584 and 6,162,963 to Kucherlapati *et al.*

Moreover, alternative transchromosomic animal systems expressing human immunoglobulin genes are available in the art and can be used to raise anti-IRTA antibodies of this disclosure. For example, mice carrying both a human heavy chain transchromosome and a human light chain tranchromosome, referred to as "TC mice" can be used; such mice are described in Tomizuka *et al.* (2000) *Proc. Natl. Acad. Sci. USA* 97:722-727. Furthermore, cows carrying human heavy and light chain transchromosomes have been described in the art (*e.g.*, Kuroiwa *et al.* (2002) *Nature Biotechnology* 20:889-894 and PCT application No. WO 2002/092812) and can be used to raise anti-IRTA antibodies of this disclosure.

Human monoclonal antibodies of this disclosure can also be prepared using phage display methods for screening libraries of human immunoglobulin genes. Such

phage display methods for isolating human antibodies are established in the art. See for example: U.S. Patent Nos. 5,223,409; 5,403,484; and 5,571,698 to Ladner *et al.*; U.S. Patent Nos. 5,427,908 and 5,580,717 to Dower *et al.*; U.S. Patent Nos. 5,969,108 and 6,172,197 to McCafferty *et al.*; and U.S. Patent Nos. 5,885,793; 6,521,404; 6,544,731; 6,555,313; 6,582,915 and 6,593,081 to Griffiths *et al.*

Human monoclonal antibodies of this disclosure can also be prepared using SCID mice into which human immune cells have been reconstituted such that a human antibody response can be generated upon immunization. Such mice are described in, for example, U.S. Patent Nos. 5,476,996 and 5,698,767 to Wilson *et al.*

In another embodiment, human anti-IRTA antibodies are prepared using a combination of human Ig mouse and phage display techniques, as described in U.S. Patent No. 6,794,132 by Buechler *et al.* More specifically, the method first involves raising an anti-IRTA antibody response in a human Ig mouse (such as a HuMab mouse or KM mouse as described above) by immunizing the mouse with one or more IRTA antigens, followed by isolating nucleic acids encoding human antibody chains from lymphatic cells of the mouse and introducing these nucleic acids into a display vector (*e.g.*, phage) to provide a library of display packages. Thus, each library member comprises a nucleic acid encoding a human antibody chain and each antibody chain is displayed from the display package. The library then is screened with IRTA family proteins to isolate library members that specifically bind to multiple IRTA family proteins. Nucleic acid inserts of the selected library members then are isolated and sequenced by standard methods to determine the light and heavy chain variable sequences of the selected IRTA binders. The variable regions can be converted to full-length antibody chains by standard recombinant DNA techniques, such as cloning of the variable regions into an expression vector that carries the human heavy and light chain constant regions such that the V_H region is operatively linked to the C_H region and the V_L region is operatively linked to the C_L region.

Immunization of Human Ig Mice

When human Ig mice are used to raise human antibodies of this disclosure, such mice can be immunized with a purified or enriched preparation of an IRTA antigen and/or recombinant IRTA protein, or cells expressing an IRTA protein, or an

IRTA fusion protein, as described by Lonberg, N. *et al.* (1994) *Nature* 368(6474): 856-859; Fishwild, D. *et al.* (1996) *Nature Biotechnology* 14: 845-851; and PCT Publication WO 98/24884 and WO 01/14424. Preferably, the mice will be 6-16 weeks of age upon the first infusion. For example, a purified or recombinant preparation (5-50 μg) of IRTA antigen can be used to immunize the human Ig mice intraperitoneally. Most preferably, the immunogen used to raise the antibodies of this disclosure is an IRTA fusion protein comprising the extracellular domain of an IRTA protein (e.g., an IRTA2 protein, an IRTA4 protein), fused to a non-IRTA polypeptide (described further in Example 1).

Detailed procedures to generate fully human monoclonal antibodies that bind multiple IRTA family proteins are described in Example 1 below. Cumulative experience with various antigens has shown that the transgenic mice respond when initially immunized intraperitoneally (IP) with antigen in complete Freund's adjuvant, followed by every other week IP immunizations (up to a total of 6) with antigen in incomplete Freund's adjuvant. However, adjuvants other than Freund's are also found to be effective. In addition, whole cells in the absence of adjuvant are found to be highly immunogenic. The immune response can be monitored over the course of the immunization protocol with plasma samples being obtained by retroorbital bleeds. The plasma can be screened by ELISA (as described below), and mice with sufficient titers of anti-IRTA human immunoglobulin can be used for fusions. Mice can be boosted intravenously with antigen 3 days before sacrifice and removal of the spleen. It is expected that 2-3 fusions for each immunization may need to be performed. Between 6 and 24 mice are typically immunized for each antigen. Usually both HCo7 and HCo12 strains are used. In addition, both HCo7 and HCo12 transgene can be bred together into a single mouse having two different human heavy chain transgenes (HCo7/HCo12). Alternatively or additionally, the KM Mouse® strain can be used, as described in Example 1.

Generation of Hybridomas Producing Human Monoclonal Antibodies of this disclosure

To generate hybridomas producing human monoclonal antibodies of this disclosure, splenocytes and/or lymph node cells from immunized mice can be isolated

and fused to an appropriate immortalized cell line, such as a mouse myeloma cell line. The resulting hybridomas can be screened for the production of antigen-specific antibodies. For example, single cell suspensions of splenic lymphocytes from immunized mice can be fused to one-sixth the number of P3X63-Ag8.653 nonsecreting mouse myeloma cells (ATCC, CRL 1580) with 50% PEG. Alternatively, the single cell suspension of splenic lymphocytes from immunized mice can be fused using an electric field based electrofusion method, using a CytoPulse large chamber cell fusion electroporator (CytoPulse Sciences, Inc., Glen Burnie Maryland). Cells are plated at approximately 2 x 10^5 in flat bottom microtiter plate, followed by a two week incubation in selective medium containing 20% fetal Clone Serum, 18% "653" conditioned media, 5% origen (IGEN), 4 mM L-glutamine, 1 mM sodium pyruvate, 5mM HEPES, 0.055 mM 2-mercaptoethanol, 50 units/ml penicillin, 50 mg/ml streptomycin, 50 mg/ml gentamycin and 1X HAT (Sigma; the HAT is added 24 hours after the fusion). After approximately two weeks, cells can be cultured in medium in which the HAT is replaced with HT. Individual wells can then be screened by ELISA for human monoclonal IgM and IgG antibodies. Once extensive hybridoma growth occurs, medium can be observed usually after 10-14 days. The antibody secreting hybridomas can be replated, screened again, and if still positive for human IgG, the monoclonal antibodies can be subcloned at least twice by limiting dilution. The stable subclones can then be cultured *in vitro* to generate small amounts of antibody in tissue culture medium for characterization.

To purify human monoclonal antibodies, selected hybridomas can be grown in two-liter spinner-flasks for monoclonal antibody purification. Supernatants can be filtered and concentrated before affinity chromatography with protein A-sepharose (Pharmacia, Piscataway, N.J.). Eluted IgG can be checked by gel electrophoresis and high performance liquid chromatography to ensure purity. The buffer solution can be exchanged into PBS, and the concentration can be determined by OD280 using 1.43 extinction coefficient. The monoclonal antibodies can be aliquoted and stored at -80° C.

Generation of Transfectomas Producing Monoclonal Antibodies of this Disclosure

Antibodies of this disclosure also can be produced in a host cell transfectoma using, for example, a combination of recombinant DNA techniques and gene transfection methods as is well known in the art (*e.g.*, Morrison, S. (1985) *Science* 229:1202).

For example, to express the antibodies, or antibody fragments thereof, DNAs encoding partial or full-length light and heavy chains, can be obtained by standard molecular biology techniques (*e.g.*, PCR amplification or cDNA cloning using a hybridoma that expresses the antibody of interest) and the DNAs can be inserted into expression vectors such that the genes are operatively linked to transcriptional and translational control sequences. In this context, the term "operatively linked" is intended to mean that an antibody gene is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain gene and the antibody heavy chain gene can be inserted into separate vector or, more typically, both genes are inserted into the same expression vector. The antibody genes are inserted into the expression vector by standard methods (*e.g.*, ligation of complementary restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present). The light and heavy chain variable regions of the antibodies described herein can be used to create full-length antibody genes of any antibody isotype by inserting them into expression vectors already encoding heavy chain constant and light chain constant regions of the desired isotype such that the V_H segment is operatively linked to the C_H segment(s) within the vector and the V_L segment is operatively linked to the C_L segment within the vector. Additionally or alternatively, the recombinant expression vector can encode a signal peptide that facilitates secretion of the antibody chain from a host cell. The antibody chain gene can be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the antibody chain gene. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (*i.e.*, a signal peptide from a non-immunoglobulin protein).

In addition to the antibody chain genes, the recombinant expression vectors of this disclosure carry regulatory sequences that control the expression of the antibody chain genes in a host cell. The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.,* polyadenylation signals) that control the transcription or translation of the antibody chain genes. Such regulatory sequences are described, for example, in Goeddel (Gene Expression Technology. Methods in Enzymology 185, Academic Press, San Diego, CA (1990)). It will be appreciated by those skilled in the art that the design of the expression vector, including the selection of regulatory sequences, may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. Preferred regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from cytomegalovirus (CMV), Simian Virus 40 (SV40), adenovirus, (*e.g.,* the adenovirus major late promoter (AdMLP) and polyoma. Alternatively, nonviral regulatory sequences may be used, such as the ubiquitin promoter or β-globin promoter. Still further, regulatory elements composed of sequences from different sources, such as the SRα promoter system, which contains sequences from the SV40 early promoter and the long terminal repeat of human T cell leukemia virus type 1 (Takebe, Y. *et al.* (1988) *Mol. Cell. Biol.* 8:466-472).

In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors of this disclosure may carry additional sequences, such as sequences that regulate replication of the vector in host cells (*e.g.,* origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see, *e.g.,* U.S. Pat. Nos. 4,399,216, 4,634,665 and 5,179,017, all by Axel *et al.*). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr- host cells with methotrexate selection/amplification) and the neo gene (for G418 selection).

For expression of the light and heavy chains, the expression vector(s) encoding the heavy and light chains is transfected into a host cell by standard

techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, *e.g.*, electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is theoretically possible to express the antibodies of this disclosure in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells, and most preferably mammalian host cells, is the most preferred because such eukaryotic cells, and in particular mammalian cells, are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody. Prokaryotic expression of antibody genes has been reported to be ineffective for production of high yields of active antibody (Boss, M. A. and Wood, C. R. (1985) *Immunology Today* 6:12-13).

Preferred mammalian host cells for expressing the recombinant antibodies of this disclosure include Chinese Hamster Ovary (CHO cells) (including $dhfr^-$ CHO cells, described in Urlaub and Chasin, (1980) *Proc. Natl. Acad. Sci. USA* 77:4216-4220, used with a DHFR selectable marker, *e.g.*, as described in R. J. Kaufman and P. A. Sharp (1982) *J. Mol. Biol.* 159:601-621), NSO myeloma cells, COS cells and SP2 cells. In particular, for use with NSO myeloma cells, another preferred expression system is the GS gene expression system disclosed in WO 87/04462 (to Wilson), WO 89/01036 (to Bebbington) and EP 338,841 (to Bebbington). When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

Characterization of Antibody Binding to Antigen

Antibodies of the invention can be tested for binding to one or more IRTA family proteins by, for example, standard ELISA. Briefly, microtiter plates are coated with purified and/or recombinant IRTA protein (*e.g.*, an IRTA fusion protein as described in Example 1) at 0.25μg/ml in PBS, and then blocked with 5% bovine

serum albumin in PBS. Dilutions of antibody (*e.g.,* dilutions of plasma from IRTA-immunized mice) are added to each well and incubated for 1-2 hours at 37°C. The plates are washed with PBS/Tween and then incubated with secondary reagent (*e.g.,* for human antibodies, a goat-anti-human IgG Fc-specific polyclonal reagent) conjugated to alkaline phosphatase for 1 hour at 37°C. After washing, the plates are developed with pNPP substrate (1 mg/ml), and analyzed at OD of 405-650. Preferably, mice that develop the highest titers will be used for fusions.

An ELISA assay as described above can also be used to screen for hybridomas that show positive reactivity with an IRTA protein. Hybridomas that bind with high avidity to an IRTA protein are subcloned and further characterized. One clone from each hybridoma, which retains the reactivity of the parent cells (by ELISA), can be chosen for making a 5-10 vial cell bank stored at -140°C, and for antibody purification.

To purify anti-IRTA antibodies, selected hybridomas can be grown in two-liter spinner-flasks for monoclonal antibody purification. Supernatants can be filtered and concentrated before affinity chromatography with protein A-sepharose (Pharmacia, Piscataway, NJ). Eluted IgG can be checked by gel electrophoresis and high performance liquid chromatography to ensure purity. The buffer solution can be exchanged into PBS, and the concentration can be determined by OD280 using 1.43 extinction coefficient. The monoclonal antibodies can be aliquoted and stored at -80°C.

To determine if the selected anti-IRTA monoclonal antibodies bind to unique epitopes, each antibody can be biotinylated using commercially available reagents (Pierce, Rockford, IL). Competition studies using unlabeled monoclonal antibodies and biotinylated monoclonal antibodies can be performed using IRTA protein coated-ELISA plates as described above. Biotinylated mAb binding can be detected with a strep-avidin-alkaline phosphatase probe.

To determine the isotype of purified antibodies, isotype ELISAs can be performed using reagents specific for antibodies of a particular isotype. For example, to determine the isotype of a human monoclonal antibody, wells of microtiter plates can be coated with 1 μg/ml of anti-human immunoglobulin overnight at 4°C. After blocking with 1% BSA, the plates are reacted with 1 μg /ml or less of test monoclonal antibodies or purified isotype controls, at ambient temperature for one to two hours.

The wells can then be reacted with either human IgG1 or human IgM-specific alkaline phosphatase-conjugated probes. Plates are developed and analyzed as described above.

Anti-IRTA human IgGs can be further tested for reactivity with an IRTA antigen by Western blotting. Briefly, an IRTA protein can be prepared and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis. After electrophoresis, the separated antigens are transferred to nitrocellulose membranes, blocked with 10% fetal calf serum, and probed with the monoclonal antibodies to be tested. Human IgG binding can be detected using anti-human IgG alkaline phosphatase and developed with BCIP/NBT substrate tablets (Sigma Chem. Co., St. Louis, Mo.).

The binding specificity of an antibody of this disclosure may also be determined by monitoring binding of the antibody to cells expressing an IRTA protein, for example by flow cytometry. A cell line that naturally expresses an IRTA protein, such as Daudi cells, may be used or a cell line, such as a CHO cell line, may be transfected with an expression vector encoding a transmembrane form of an IRTA protein. The transfected protein may comprise a tag, such as a myc-tag, preferably at the N-terminus, for detection using an antibody to the tag. Binding of an antibody of this disclosure to an IRTA protein may be determined by incubating the transfected cells with the antibody, and detecting bound antibody. Binding of an antibody to the tag on the transfected protein may be used as a positive control.

Immunoconjugates

In another aspect, the present disclosure features an anti-IRTA antibody, or a fragment thereof, conjugated to a therapeutic moiety, such as a cytotoxin, a drug (*e.g.*, an immunosuppressant) or a radiotoxin. Such conjugates are referred to herein as "immunoconjugates". Immunoconjugates that include one or more cytotoxins are referred to as "immunotoxins." A cytotoxin or cytotoxic agent includes any agent that is detrimental to (*e.g.*, kills) cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs

thereof. Therapeutic agents also include, for example, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine).

Other preferred examples of therapeutic cytotoxins that can be conjugated to an antibody of this disclosure include duocarmycins, calicheamicins, maytansines and auristatins, and derivatives thereof. An example of a calicheamicin antibody conjugate is commercially available (Mylotarg®; American Home Products).

Cytotoxins can be conjugated to antibodies of this disclosure using linker technology available in the art. Examples of linker types that have been used to conjugate a cytotoxin to an antibody include, but are not limited to, hydrazones, thioethers, esters, disulfides and peptide-containing linkers. A linker can be chosen that is, for example, susceptible to cleavage by low pH within the lysosomal compartment or susceptible to cleavage by proteases, such as proteases preferentially expressed in tumor tissue such as cathepsins (*e.g.*, cathepsins B, C, D).

For further discussion of types of cytotoxins, linkers and methods for conjugating therapeutic agents to antibodies, see also Saito, G. *et al.* (2003) *Adv. Drug Deliv. Rev.* 55:199-215; Trail, P.A. *et al.* (2003) *Cancer Immunol. Immunother.* 52:328-337; Payne, G. (2003) *Cancer Cell* 3:207-212; Allen, T.M. (2002) *Nat. Rev. Cancer* 2:750-763; Pastan, I. and Kreitman, R. J. (2002) *Curr. Opin. Investig. Drugs* 3:1089-1091; Senter, P.D. and Springer, C.J. (2001) *Adv. Drug Deliv. Rev.* 53:247-264.

Antibodies of the present disclosure also can be conjugated to a radioactive isotope to generate cytotoxic radiopharmaceuticals, also referred to as radioimmunoconjugates. Examples of radioactive isotopes that can be conjugated to antibodies for use diagnostically or therapeutically include, but are not limited to, iodine^{131}, indium^{111}, yttrium^{90} and lutetium^{177}. Method for preparing radioimmunconjugates are established in the art. Examples of

radioimmunoconjugates are commercially available, including Zevalin® (IDEC Pharmaceuticals) and Bexxar® (Corixa Pharmaceuticals), and similar methods can be used to prepare radioimmunoconjugates using the antibodies of this disclosure.

The antibody conjugates of this disclosure can be used to modify a given biological response, and the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, an enzymatically active toxin, or active fragment thereof, such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor or interferon-γ; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, *e.g.,* Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*, *Immunol. Rev.*, 62:119-58 (1982).

Bispecific Molecules

In another aspect, the present disclosure features bispecific molecules comprising an anti-IRTA antibody, or a fragment thereof, of this disclosure. An antibody of this disclosure, or antigen-binding portions thereof, can be derivatized or linked to another functional molecule, *e.g.,* another peptide or protein (*e.g.,* another antibody or ligand for a receptor) to generate a bispecific molecule that binds to at

least two different binding sites or target molecules. The antibody of this disclosure may in fact be derivatized or linked to more than one other functional molecule to generate multispecific molecules that bind to more than two different binding sites and/or target molecules; such multispecific molecules are also intended to be encompassed by the term "bispecific molecule" as used herein. To create a bispecific molecule of this disclosure, an antibody of this disclosure can be functionally linked (*e.g.*, by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other binding molecules, such as another antibody, antibody fragment, peptide or binding mimetic, such that a bispecific molecule results.

Accordingly, the present disclosure includes bispecific molecules comprising at least one first binding specificity for IRTA family proteins and a second binding specificity for a second target epitope. In a particular embodiment of this disclosure, the second target epitope is an Fc receptor, *e.g.*, human FcγRI (CD64) or a human Fcα receptor (CD89). Therefore, this disclosure includes bispecific molecules capable of binding both to FcγR or FcαR expressing effector cells (*e.g.*, monocytes, macrophages or polymorphonuclear cells (PMNs)), and to target cells expressing IRTA proteins. These bispecific molecules target IRTA expressing cells to effector cell and trigger Fc receptor-mediated effector cell activities, such as phagocytosis of IRTA expressing cells, antibody dependent cell-mediated cytotoxicity (ADCC), cytokine release, or generation of superoxide anion.

In an embodiment of this disclosure in which the bispecific molecule is multispecific, the molecule can further include a third binding specificity, in addition to an anti-Fc binding specificity and an anti-IRTA binding specificity. In one embodiment, the third binding specificity is an anti-enhancement factor (EF) portion, *e.g.*, a molecule which binds to a surface protein involved in cytotoxic activity and thereby increases the immune response against the target cell. The "anti-enhancement factor portion" can be an antibody, functional antibody fragment or a ligand that binds to a given molecule, *e.g.*, an antigen or a receptor, and thereby results in an enhancement of the effect of the binding determinants for the Fc receptor or target cell antigen. The "anti-enhancement factor portion" can bind an Fc receptor or a target cell antigen. Alternatively, the anti-enhancement factor portion can bind to an entity that is different from the entity to which the first and second binding specificities bind. For example, the anti-enhancement factor portion can bind a cytotoxic T-cell

(*e.g.* via CD2, CD3, CD8, CD28, CD4, CD40, ICAM-1 or other immune cell that results in an increased immune response against the target cell).

In one embodiment, the bispecific molecules of this disclosure comprise as a binding specificity at least one antibody, or an antibody fragment thereof, including, *e.g.*, an Fab, Fab', $F(ab')_2$, Fv, Fd, dAb or a single chain Fv. The antibody may also be a light chain or heavy chain dimer, or any minimal fragment thereof such as a Fv or a single chain construct as described in U.S. Patent No. 4,946,778 to Ladner et al., the contents of which is expressly incorporated by reference.

In one embodiment, the binding specificity for an Fcγ receptor is provided by a monoclonal antibody, the binding of which is not blocked by human immunoglobulin G (IgG). As used herein, the term "IgG receptor" refers to any of the eight γ-chain genes located on chromosome 1. These genes encode a total of twelve transmembrane or soluble receptor isoforms which are grouped into three Fcγ receptor classes: FcγRI (CD64), FcγRII(CD32), and FcγRIII (CD16). In one preferred embodiment, the Fcγ receptor a human high affinity FcγRI. The human FcγRI is a 72 kDa molecule, which shows high affinity for monomeric IgG (10^8 - 10^9 M^{-1}).

The production and characterization of certain preferred anti-Fcγ monoclonal antibodies are described in PCT Publication WO 88/00052 and in U.S. Patent No. 4,954,617 to Fanger et al., the teachings of which are fully incorporated by reference herein. These antibodies bind to an epitope of FcγRI, FcγRII or FcγRIII at a site which is distinct from the Fcγ binding site of the receptor and, thus, their binding is not blocked substantially by physiological levels of IgG. Specific anti-FcγRI antibodies useful in this disclosure are mAb 22, mAb 32, mAb 44, mAb 62 and mAb 197. The hybridoma producing mAb 32 is available from the American Type Culture Collection, ATCC Accession No. HB9469. In other embodiments, the anti-Fcγ receptor antibody is a humanized form of monoclonal antibody 22 (H22). The production and characterization of the H22 antibody is described in Graziano, R.F. *et al.* (1995) *J. Immunol* 155 (10): 4996-5002 and PCT Publication WO 94/10332 to Tempest et al.. The H22 antibody producing cell line was deposited at the American Type Culture Collection under the designation HA022CL1 and has the accession no. CRL 11177.

In still other preferred embodiments, the binding specificity for an Fc receptor is provided by an antibody that binds to a human IgA receptor, *e.g.,* an Fc-alpha receptor (FcαRI (CD89)), the binding of which is preferably not blocked by human immunoglobulin A (IgA). The term "IgA receptor" is intended to include the gene product of one α-gene (FcαRI) located on chromosome 19. This gene is known to encode several alternatively spliced transmembrane isoforms of 55 to 110 kDa. Fcα RI (CD89) is constitutively expressed on monocytes/macrophages, eosinophilic and neutrophilic granulocytes, but not on non-effector cell populations. FcαRI has medium affinity ($\approx 5 \times 10^7\ M^{-1}$) for both IgA1 and IgA2, which is increased upon exposure to cytokines such as G-CSF or GM-CSF (Morton, H.C. *et al.* (1996) *Critical Reviews in Immunology* 16:423-440). Four FcαRI-specific monoclonal antibodies, identified as A3, A59, A62 and A77, which bind FcαRI outside the IgA ligand binding domain, have been described (Monteiro, R.C. *et al.* (1992) *J. Immunol.* 148:1764).

FcαRI and FcγRI are preferred trigger receptors for use in the bispecific molecules of this disclosure because they are (1) expressed primarily on immune effector cells, *e.g.,* monocytes, PMNs, macrophages and dendritic cells; (2) expressed at high levels (*e.g.,* 5,000-100,000 per cell); (3) mediators of cytotoxic activities (*e.g.,* ADCC, phagocytosis); and (4) mediate enhanced antigen presentation of antigens, including self-antigens, targeted to them.

While human monoclonal antibodies are preferred, other antibodies which can be employed in the bispecific molecules of this disclosure are murine, chimeric and humanized monoclonal antibodies.

The bispecific molecules of the present disclosure can be prepared by conjugating the constituent binding specificities, *e.g.,* the anti-FcR and anti-IRTA binding specificities, using methods known in the art. For example, each binding specificity of the bispecific molecule can be generated separately and then conjugated to one another. When the binding specificities are proteins or peptides, a variety of coupling or cross-linking agents can be used for covalent conjugation. Examples of cross-linking agents include protein A, carbodiimide, N-succinimidyl-S-acetyl-thioacetate (SATA), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), o-phenylenedimaleimide (oPDM), N-succinimidyl-3-(2-pyridyldithio)propionate

(SPDP), and sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohaxane-1-carboxylate (sulfo-SMCC) (see *e.g.,* Karpovsky *et al.* (1984) *J. Exp. Med.* 160:1686; Liu, MA *et al.* (1985) *Proc. Natl. Acad. Sci. USA* 82:8648). Other methods include those described in Paulus (1985) Behring Ins. Mitt. No. 78, 118-132; Brennan *et al.* (1985) *Science* 229:81-83, and Glennie *et al.* (1987) *J. Immunol.* 139: 2367-2375). Preferred conjugating agents are SATA and sulfo-SMCC, both available from Pierce Chemical Co. (Rockford, IL).

When the binding specificities are antibodies, they can be conjugated via sulfhydryl bonding of the C-terminus hinge regions of the two heavy chains. In a particularly preferred embodiment, the hinge region is modified to contain an odd number of sulfhydryl residues, preferably one, prior to conjugation.

Alternatively, both binding specificities can be encoded in the same vector and expressed and assembled in the same host cell. This method is particularly useful where the bispecific molecule is a mAb x mAb, mAb x Fab, Fab x $F(ab')_2$ or ligand x Fab fusion protein. A bispecific molecule of this disclosure can be a single chain molecule comprising one single chain antibody and a binding determinant, or a single chain bispecific molecule comprising two binding determinants. Bispecific molecules may comprise at least two single chain molecules. Methods for preparing bispecific molecules are described for example in U.S. Patent Numbers 5,260,203; 5,455,030; 4,881,175; 5,132,405; 5,091,513; 5,476,786; 5,013,653; 5,258,498; and 5,482,858, all of which are expressly incorporated herein by reference.

Binding of the bispecific molecules to their specific targets can be confirmed by, for example, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), FACS analysis, bioassay (*e.g.,* growth inhibition), or Western Blot assay. Each of these assays generally detects the presence of protein-antibody complexes of particular interest by employing a labeled reagent (*e.g.,* an antibody) specific for the complex of interest. For example, the FcR-antibody complexes can be detected using *e.g.,* an enzyme-linked antibody or antibody fragment which recognizes and specifically binds to the antibody-FcR complexes. Alternatively, the complexes can be detected using any of a variety of other immunoassays. For example, the antibody can be radioactively labeled and used in a radioimmunoassay (RIA) (see, for example, Weintraub, B., Principles of Radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March, 1986, which is

incorporated by reference herein). The radioactive isotope can be detected by such means as the use of a γcounter or a scintillation counter or by autoradiography.

Pharmaceutical Compositions

In another aspect, the present disclosure provides a composition, *e.g.*, a pharmaceutical composition, containing one or a combination of monoclonal antibodies, or antigen-binding portion(s) thereof, of the present disclosure, formulated together with a pharmaceutically acceptable carrier. Such compositions may include one or a combination of (*e.g.*, two or more different) antibodies, or immunoconjugates or bispecific molecules of this disclosure. For example, a pharmaceutical composition of this disclosure can comprise a combination of antibodies (or immunoconjugates or bispecifics) that bind to different epitopes on the target antigen or that have complementary activities.

Pharmaceutical compositions of this disclosure also can be administered in combination therapy, *i.e.*, combined with other agents. For example, the combination therapy can include an anti-IRTA antibody of the present disclosure combined with at least one other anti-cancer, anti-inflammatory or immunosuppressant agent. Examples of therapeutic agents that can be used in combination therapy are described in greater detail below in the section on uses of the antibodies of this disclosure.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (*e.g.*, by injection or infusion). Depending on the route of administration, the active compound, *i.e.*, antibody, immunoconjugate, or bispecific molecule, may be coated in a material to protect the compound from the action of acids and other natural conditions that may inactivate the compound.

The pharmaceutical compounds of this disclosure may include one or more pharmaceutically acceptable salts. A "pharmaceutically acceptable salt" refers to a salt that retains the desired biological activity of the parent compound and does not impart any undesired toxicological effects (see *e.g.*, Berge, S.M., *et al.* (1977) *J.*

Pharm. Sci. 66:1-19). Examples of such salts include acid addition salts and base addition salts. Acid addition salts include those derived from nontoxic inorganic acids, such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydroiodic, phosphorous and the like, as well as from nontoxic organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, aromatic acids, aliphatic and aromatic sulfonic acids and the like. Base addition salts include those derived from alkaline earth metals, such as sodium, potassium, magnesium, calcium and the like, as well as from nontoxic organic amines, such as N,N'-dibenzylethylenediamine, N-methylglucamine, chloroprocaine, choline, diethanolamine, ethylenediamine, procaine and the like.

A pharmaceutical composition of this disclosure also may include a pharmaceutically acceptable anti-oxidant. Examples of pharmaceutically acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Examples of suitable aqueous and nonaqueous carriers that may be employed in the pharmaceutical compositions of this disclosure include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms may be ensured both by sterilization procedures, *supra*, and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be

brought about by the inclusion of agents that delay absorption such as aluminum monostearate and gelatin.

Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of this disclosure is contemplated. Supplementary active compounds can also be incorporated into the compositions.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the subject being treated, and the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the composition which produces a therapeutic effect. Generally, out of one hundred per cent, this amount will range from about 0.01 per cent to about ninety-nine percent of active ingredient, preferably from about 0.1 per cent to about 70 per cent, most preferably from about 1 per cent to about 30 per cent of active ingredient in combination with a pharmaceutically acceptable carrier.

Dosage regimens are adjusted to provide the optimum desired response (*e.g.*, a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of this disclosure are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

For administration of the antibody, the dosage ranges from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg, of the host body weight. For example dosages can be 0.3 mg/kg body weight, 1 mg/kg body weight, 3 mg/kg body weight, 5 mg/kg body weight or 10 mg/kg body weight or within the range of 1-10 mg/kg. An exemplary treatment regime entails administration once per week, once every two weeks, once every three weeks, once every four weeks, once a month, once every 3 months or once every three to 6 months. Preferred dosage regimens for an anti-IRTA antibody of this disclosure include 1 mg/kg body weight or 3 mg/kg body weight via intravenous administration, with the antibody being given using one of the following dosing schedules: (i) every four weeks for six dosages, then every three months; (ii)

every three weeks; (iii) 3 mg/kg body weight once followed by 1 mg/kg body weight every three weeks.

In some methods, two or more monoclonal antibodies with different binding specificities are administered simultaneously, in which case the dosage of each antibody administered falls within the ranges indicated. Antibody is usually administered on multiple occasions. Intervals between single dosages can be, for example, weekly, monthly, every three months or yearly. Intervals can also be irregular as indicated by measuring blood levels of antibody to the target antigen in the patient. In some methods, dosage is adjusted to achieve a plasma antibody concentration of about 1-1000 μg /ml and in some methods about 25-300 μg /ml.

Alternatively, antibody can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the antibody in the patient. In general, human antibodies show the longest half life, followed by humanized antibodies, chimeric antibodies, and nonhuman antibodies. The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage is administered at relatively infrequent intervals over a long period of time. Some patients continue to receive treatment for the rest of their lives. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and preferably until the patient shows partial or complete amelioration of symptoms of disease. Thereafter, the patient can be administered a prophylactic regime.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present disclosure may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present disclosure employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight,

condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A "therapeutically effective dosage" of an anti-IRTA antibody of this disclosure preferably results in a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. For example, for the treatment of $IRTA^+$ tumors, a "therapeutically effective dosage" preferably inhibits cell growth or tumor growth by at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects. The ability of a compound to inhibit tumor growth can be evaluated in an animal model system predictive of efficacy in human tumors. Alternatively, this property of a composition can be evaluated by examining the ability of the compound to inhibit cell growth, such inhibition can be measured *in vitro* by assays known to the skilled practitioner. A therapeutically effective amount of a therapeutic compound can decrease tumor size, or otherwise ameliorate symptoms in a subject. One of ordinary skill in the art would be able to determine such amounts based on such factors as the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected.

A composition of the present disclosure can be administered via one or more routes of administration using one or more of a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. Preferred routes of administration for antibodies of this disclosure include intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

Alternatively, an antibody of this disclosure can be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically.

The active compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, *e.g., Sustained and Controlled Release Drug Delivery Systems*, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

Therapeutic compositions can be administered with medical devices known in the art. For example, in a preferred embodiment, a therapeutic composition of this disclosure can be administered with a needleless hypodermic injection device, such as the devices disclosed in U.S. Patent Nos. 5,399,163; 5,383,851; 5,312,335; 5,064,413; 4,941,880; 4,790,824; or 4,596,556. Examples of well-known implants and modules useful in the present disclosure include: U.S. Patent No. 4,487,603, which discloses an implantable micro-infusion pump for dispensing medication at a controlled rate; U.S. Patent No. 4,486,194, which discloses a therapeutic device for administering medicants through the skin; U.S. Patent No. 4,447,233, which discloses a medication infusion pump for delivering medication at a precise infusion rate; U.S. Patent No. 4,447,224, which discloses a variable flow implantable infusion apparatus for continuous drug delivery; U.S. Patent No. 4,439,196, which discloses an osmotic drug delivery system having multi-chamber compartments; and U.S. Patent No. 4,475,196, which discloses an osmotic drug delivery system. These patents are incorporated herein by reference. Many other such implants, delivery systems, and modules are known to those skilled in the art.

In certain embodiments, the human monoclonal antibodies of this disclosure can be formulated to ensure proper distribution *in vivo*. For example, the blood-brain barrier (BBB) excludes many highly hydrophilic compounds. To ensure that the therapeutic compounds of this disclosure cross the BBB (if desired), they can be formulated, for example, in liposomes. For methods of manufacturing liposomes, see, *e.g.*, U.S. Patents 4,522,811; 5,374,548; and 5,399,331. The liposomes may comprise one or more moieties which are selectively transported into specific cells or organs, thus enhance targeted drug delivery (see, *e.g.*, V.V. Ranade (1989) *J. Clin. Pharmacol.* 29:685). Exemplary targeting moieties include folate or biotin (see, *e.g.*,

U.S. Patent 5,416,016 to Low *et al.*); mannosides (Umezawa *et al.*, (1988) *Biochem. Biophys. Res. Commun.* 153:1038); antibodies (P.G. Bloeman *et al.* (1995) *FEBS Lett.* 357:140; M. Owais *et al.* (1995) *Antimicrob. Agents Chemother.* 39:180); surfactant protein A receptor (Briscoe *et al.* (1995) *Am. J. Physiol.* 1233:134); p120 (Schreier *et al.* (1994) *J. Biol. Chem.* 269:9090); see also K. Keinanen; M.L. Laukkanen (1994) *FEBS Lett.* 346:123; J.J. Killion; I.J. Fidler (1994) *Immunomethods* 4:273.

Uses and Methods

The antibodies, particularly the human antibodies, antibody compositions and methods of the present invention have numerous *in vitro* and *in vivo* diagnostic and therapeutic utilities involving the diagnosis and treatment of IRTA protein-mediated disorders. For example, these molecules can be administered to cells in culture, *in vitro* or *ex vivo*, or to human subjects, *e.g.*, in vivo, to treat, prevent and to diagnose a variety of disorders. As used herein, the term "subject" is intended to include human and non-human animals. Non-human animals includes all vertebrates, *e.g.*, mammals and non-mammals, such as non-human primates, sheep, dogs, cats, cows, horses, chickens, amphibians, and reptiles. Preferred subjects include human patients having disorders mediated by IRTA protein activity. The methods are particularly suitable for treating human patients having a disorder associated with aberrant IRTA protein expression or activity.. When antibodies to IRTA proteins are administered together with another agent, the two can be administered in either order or simultaneously.

Given the specific binding of the antibodies of the invention for IRTA proteins, compared to non-IRTA proteins, the antibodies of the invention can be used to specifically detect IRTA protein expression on the surface of cells and, moreover, can be used to purify IRTA proteins via immunoaffinity purification.

Furthermore, given the expression of IRTA proteins on various tumor cells, the human antibodies, antibody compositions and methods of the present invention can be used to treat a subject with a tumorigenic disorder, *e.g.*, a disorder characterized by the presence of tumor cells expressing IRTA proteins including, for example, Burkitt's lymphoma, non-Hodgkin's lymphoma, anaplastic large-cell lymphomas (ALCL), cutaneous T-cell lymphomas, nodular small cleaved-cell lymphomas, lymphocytic lymphomas, peripheral T-cell lymphomas, Lennert's

lymphomas, immunoblastic lymphomas, T-cell leukemia/lymphomas (ATLL), adult T-cell leukemia (T-ALL), entroblastic/centrocytic (cb/cc) follicular lymphomas cancers, diffuse large cell lymphomas of B lineage, angioimmunoblastic lymphadenopathy (AILD)-like T cell lymphoma, HIV associated body cavity based lymphomas, Embryonal Carcinomas, undifferentiated carcinomas of the rhino-pharynx (*e.g.*, Schmincke's tumor), Castleman's disease, Kaposi's Sarcoma and other B-cell lymphomas.

Still further, since the anti-IRTA antibodies of this disclosure bind to two or more different IRTA proteins, the antibodies have the additional benefit of being able to interact with more than one IRTA protein on the surface of cells that express multiple IRTA proteins (*e.g.*, tumor cells) and thus the antibodies may exhibit more efficacy (*e.g.*, anti-tumor activity) than an anti-IRTA antibody that binds only one IRTA subtype. Moreover, the cross-reactive binding specificity of the anti-IRTA antibodies of this disclosure means that these antibodies are more likely to be capable of interacting with a greater number of different target cells than anti-IRTA antibodies that bind only one IRTA subtype.

In one embodiment, the antibodies (*e.g.*, human monoclonal antibodies, multispecific and bispecific molecules and compositions) of the invention can be used to detect levels of IRTA proteins, or levels of cells which contain IRTA proteins on their membrane surface, which levels can then be linked to certain disease symptoms. Alternatively, the antibodies can be used to inhibit or block IRTA protein function which, in turn, can be linked to the prevention or amelioration of certain disease symptoms, thereby implicating IRTA proteins as mediators of the disease. This can be achieved by contacting a sample and a control sample with the anti-IRTA protein antibody under conditions that allow for the formation of a complex between the antibody and the IRTA protein. Any complexes formed between the antibody and the IRTA protein are detected and compared in the sample and the control.

In another embodiment, the antibodies (*e.g.*, human antibodies, multispecific and bispecific molecules and compositions) of the invention can be initially tested for binding activity associated with therapeutic or diagnostic use *in vitro*. For example, compositions of the invention can be tested using the flow cytometric assays described in the Examples below.

The antibodies (*e.g.*, human antibodies, multispecific and bispecific molecules, immunoconjugates and compositions) of the invention have additional utility in therapy and diagnosis of IRTA protein-related diseases. For example, the human monoclonal antibodies, the multispecific or bispecific molecules and the immunoconjugates can be used to elicit *in vivo* or *in vitro* one or more of the following biological activities: to inhibit the growth of and/or kill a cell expressing IRTA proteins; to mediate phagocytosis or ADCC of a cell expressing IRTA proteins in the presence of human effector cells, or to block IRTA protein ligand binding to IRTA protein.

In a particular embodiment, the antibodies (*e.g.*, human antibodies, multispecific and bispecific molecules and compositions) are used in vivo to treat, prevent or diagnose a variety of IRTA protein-related diseases. Examples of IRTA protein-related diseases include, among others, cancer, non-Hodgkin's lymphoma, Burkitt's lymphoma, anaplastic large-cell lymphomas (ALCL), cutaneous T-cell lymphomas, nodular small cleaved-cell lymphomas, lymphocytic lymphomas, peripheral T-cell lymphomas, Lennert's lymphomas, immunoblastic lymphomas, T-cell leukemia/lymphomas (ATLL), adult T-cell leukemia (T-ALL), entroblastic/centrocytic (cb/cc) follicular lymphomas cancers, diffuse large cell lymphomas of B lineage, angioimmunoblastic lymphadenopathy (AILD)-like T cell lymphoma, HIV associated body cavity based lymphomas, Embryonal Carcinomas, undifferentiated carcinomas of the rhino-pharynx (e.g., Schmincke's tumor), Castleman's disease, Kaposi's Sarcoma and other B-cell lymphomas.

Suitable routes of administering the antibody compositions (*e.g.*, human monoclonal antibodies, multispecific and bispecific molecules and immunoconjugates) of the invention *in vivo* and *in vitro* are well known in the art and can be selected by those of ordinary skill. For example, the antibody compositions can be administered by injection (*e.g.*, intravenous or subcutaneous). Suitable dosages of the molecules used will depend on the age and weight of the subject and the concentration and/or formulation of the antibody composition.

As previously described, human anti-IRTA protein antibodies of the invention can be co-administered with one or other more therapeutic agents, *e.g.*, a cytotoxic agent, a radiotoxic agent or an immunosuppressive agent. The antibody can be linked to the agent (as an immunocomplex) or can be administered separate from the agent.

In the latter case (separate administration), the antibody can be administered before, after or concurrently with the agent or can be co-administered with other known therapies, *e.g.*, an anti-cancer therapy, *e.g.*, radiation, surgery and the like. Such therapeutic agents include, among others, anti-neoplastic agents such as doxorubicin (adriamycin), cisplatin bleomycin sulfate, carmustine, chlorambucil, and cyclophosphamide hydroxyurea which, by themselves, are only effective at levels which are toxic or subtoxic to a patient. Cisplatin is intravenously administered as a 100 mg/dose once every four weeks and adriamycin is intravenously administered as a 60-75 mg/ml dose once every 21 days. Co-administration of the human anti-IRTA protein antibodies, or antigen binding fragments thereof, of the present invention with chemotherapeutic agents provides two anti-cancer agents which operate via different mechanisms which yield a cytotoxic effect to human tumor cells. Such co-administration can solve problems due to development of resistance to drugs or a change in the antigenicity of the tumor cells which would render them unreactive with the antibody.

Target-specific effector cells, *e.g.*, effector cells linked to compositions (*e.g.*, human antibodies, multispecific and bispecific molecules) of the invention can also be used as therapeutic agents. Effector cells for targeting can be human leukocytes such as macrophages, neutrophils or monocytes. Other cells include eosinophils, natural killer cells and other IgG- or IgA-receptor bearing cells. If desired, effector cells can be obtained from the subject to be treated. The target-specific effector cells can be administered as a suspension of cells in a physiologically acceptable solution. The number of cells administered can be in the order of 10^8-10^9 but will vary depending on the therapeutic purpose. In general, the amount will be sufficient to obtain localization at the target cell, *e.g.*, a tumor cell expressing IRTA proteins, and to effect cell killing by, *e.g.*, phagocytosis. Routes of administration can also vary.

Therapy with target-specific effector cells can be performed in conjunction with other techniques for removal of targeted cells. For example, anti-tumor therapy using the compositions (*e.g.*, human antibodies, multispecific and bispecific molecules) of the invention and/or effector cells armed with these compositions can be used in conjunction with chemotherapy. Additionally, combination immunotherapy may be used to direct two distinct cytotoxic effector populations toward tumor cell rejection. For example, anti-IRTA protein antibodies linked to anti-

Fc-gamma RI or anti-CD3 may be used in conjunction with IgG- or IgA-receptor specific binding agents.

Bispecific and multispecific molecules of the invention can also be used to modulate FcγR or FcγR levels on effector cells, such as by capping and elimination of receptors on the cell surface. Mixtures of anti-Fc receptors can also be used for this purpose.

The compositions (*e.g.*, human antibodies, multispecific and bispecific molecules and immunoconjugates) of the invention which have complement binding sites, such as portions from IgG1, -2, or -3 or IgM which bind complement, can also be used in the presence of complement. In one embodiment, *ex vivo* treatment of a population of cells comprising target cells with a binding agent of the invention and appropriate effector cells can be supplemented by the addition of complement or serum containing complement. Phagocytosis of target cells coated with a binding agent of the invention can be improved by binding of complement proteins. In another embodiment target cells coated with the compositions (*e.g.*, human antibodies, multispecific and bispecific molecules) of the invention can also be lysed by complement. In yet another embodiment, the compositions of the invention do not activate complement.

The compositions (*e.g.*, human antibodies, multispecific and bispecific molecules and immunoconjugates) of the invention can also be administered together with complement. Accordingly, within the scope of the invention are compositions comprising human antibodies, multispecific or bispecific molecules and serum or complement. These compositions are advantageous in that the complement is located in close proximity to the human antibodies, multispecific or bispecific molecules. Alternatively, the human antibodies, multispecific or bispecific molecules of the invention and the complement or serum can be administered separately.

The antibodies of this disclosure also can be used in combination with one or more additional therapeutic antibodies or other binding agents, such as Ig fusion proteins. Non-limiting examples of other antibodies or binding agents with which an anti-IRTA antibody of this disclosure can be administered in combination include antibodies or binding agents to CTLA-4, PSMA, CD30, IP-10, IFN-γ, CD70, PD-1, PD-L1, CD22. TNF, TNF-R, VEGF, VEGF-R, CCR5, IL-1, IL-18, IL-18R, CD19, Campath-1, EGFR, CD33, CD20, Her-2, CD25, gpIIb/IIIa, IgE, CD11a, α4 integrin.

Also within the scope of the present invention are kits comprising the antibody compositions of the invention (*e.g.*, human antibodies, bispecific or multispecific molecules, or immunoconjugates) and instructions for use. The kit can further contain one ore more additional reagents, such as an immunosuppressive reagent, a cytotoxic agent or a radiotoxic agent, or one or more additional human antibodies of the invention (*e.g.*, a human antibody having a complementary activity which binds to an epitope in the IRTA antigen distinct from the first human antibody).

Accordingly, patients treated with antibody compositions of the invention can be additionally administered (prior to, simultaneously with, or following administration of a human antibody of the invention) with another therapeutic agent, such as a cytotoxic or radiotoxic agent, which enhances or augments the therapeutic effect of the human antibodies.

In other embodiments, the subject can be additionally treated with an agent that modulates, *e.g.*, enhances or inhibits, the expression or activity of Fcγ or Fcγ receptors by, for example, treating the subject with a cytokine. Referred cytokines for administration during treatment with the multispecific molecule include of granulocyte colony-stimulating factor (G-CSF), granulocyte- macrophage colony-stimulating factor (GM-CSF), interferon-γ (IFN-γ), and tumor necrosis factor (TNF).

The compositions (*e.g.*, human antibodies, multispecific and bispecific molecules) of the invention can also be used to target cells expressing FcγR or IRTA protein, for example for labeling such cells. For such use, the binding agent can be linked to a molecule that can be detected. Thus, the invention provides methods for localizing *ex vivo* or *in vitro* cells expressing Fc receptors, such as FcγR, or IRTA protein. The detectable label can be, *e.g.*, a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor.

In a particular embodiment, the invention provides methods for detecting the presence of IRTA antigen in a sample, or measuring the amount of IRTA antigen, comprising contacting the sample, and a control sample, with a human monoclonal antibody, or an antigen binding portion thereof, which specifically binds to IRTA proteins, under conditions that allow for formation of a complex between the antibody or portion thereof and the IRTA protein. The formation of a complex is then detected, wherein a difference complex formation between the sample compared to the control sample is indicative the presence of IRTA antigen in the sample.

In other embodiments, the invention provides methods for treating an IRTA protein-mediated disorder in a subject, *e.g.*, cancer, non-Hodgkin's lymphoma, Burkitt's lymphoma, anaplastic large-cell lymphomas (ALCL), cutaneous T-cell lymphomas, nodular small cleaved-cell lymphomas, lymphocytic lymphomas, peripheral T-cell lymphomas, Lennert's lymphomas, immunoblastic lymphomas, T-cell leukemia/lymphomas (ATLL), adult T-cell leukemia (T-ALL), entroblastic/centrocytic (cb/cc) follicular lymphomas cancers, diffuse large cell lymphomas of B lineage, angioimmunoblastic lymphadenopathy (AILD)-like T cell lymphoma, HIV associated body cavity based lymphomas, Embryonal Carcinomas, undifferentiated carcinomas of the rhino-pharynx (e.g., Schmincke's tumor), Castleman's disease, Kaposi's Sarcoma and other B-cell lymphomas, by administering to the subject the human antibodies described above. Such antibodies and derivatives thereof are used to inhibit IRTA-induced activities associated with certain disorders, *e.g.*, proliferation and differentiation. By contacting the antibody with IRTA proteins (*e.g.*, by administering the antibody to a subject), the ability of IRTA protein to induce such activities is inhibited and, thus, the associated disorder is treated. The antibody composition can be administered alone or along with another therapeutic agent, such as a cytotoxic or a radiotoxic agent which acts in conjunction with or synergistically with the antibody composition to treat or prevent the IRTA-mediated disease.

In yet another embodiment, immunoconjugates of the invention can be used to target compounds (*e.g.*, therapeutic agents, labels, cytotoxins, radiotoxoins immunosuppressants, etc.) to cells which have IRTA cell surface receptors by linking such compounds to the antibody. Thus, the invention also provides methods for localizing *ex vivo* or *in vivo* cells expressing IRTA proteins (*e.g.*, with a detectable label, such as a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor). Alternatively, the immunoconjugates can be used to kill cells which have IRTA cell surface receptors by targeting cytotoxins or radiotoxins to IRTA proteins.

In another aspect, the invention provides a method of treating an autoimmune disorder in a subject. The method comprises administering to the subject an anti-IRTA antibody, or antigen-binding portion thereof, of the invention such that the autoimmune disorder in the subject is treated. The autoimmune disorder may be treated in the subject by, for example, using an unlabeled anti-IRTA antibody that inhibits B cell activity to thereby decrease an autoimmune reaction(s) mediated by the

B cell or, alternatively, using an anti-IRTA antibody labeled with a cytotoxic agent such that B cells are depleted from the patient to thereby inhibit autoimmune reactions. Non-limiting examples of preferred autoimmune disorders that can be treated include systemic lupus erythematosus and rheumatoid arthritis. Other examples of autoimmune disorders that can be treated include inflammatory bowel disease (including ulcerative colitis and Crohn's disease), Type I diabetes, multiple sclerosis, Sjogren's syndrome, autoimmune thyroiditis (including Grave's disease and Hashimoto's thyroiditis), psoriasis and glomerulonephritis. The antibody can be used alone or in combination with other anti-inflammatory or immunsuppresant agents, such as non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids (*e.g.*, prednisone, hydrocortisone), methotrexate, COX-2 inhibitors, TNF antagonists (*e.g.*, etanercept, infliximab, adalimumab) and immunosuppressants (such as 6-mercaptopurine, azathioprine and cyclosporine A).

The present disclosure is further illustrated by the following examples, which should not be construed as further limiting. The contents of all figures and all references, patents and published patent applications cited throughout this application are expressly incorporated herein by reference.

Example 1: Generation of Human Monoclonal Antibodies Against IRTA Proteins

Anti-IRTA human monoclonal antibodies were generated using transgenic mice that express human antibody genes, as follows.

Antigens

Two different fusion proteins, one composed of the extracellular domain of human IRTA2 linked to a heterologous polypeptide and the other composed of the extracellular domain of human IRTA4 linked to a heterologous polypeptide, were generated by standard recombinant methods and used as antigens for immunization.

Transgenic HuMab Mouse™ and KM Mouse™

Fully human monoclonal antibodies to IRTA proteins were prepared using either mice from the HCo7 strain of the transgenic HuMab Mouse™ or mice of the

transgenic transchromosomic KM Mouse™ strain, each of which express human antibody genes.

In the HCo7 mouse strain, the endogenous mouse kappa light chain gene has been homozygously disrupted as described in Chen *et al.* (1993) *EMBO J.* 12:811-820 and the endogenous mouse heavy chain gene has been homozygously disrupted as described in Example 1 of PCT Publication WO 01/09187. Furthermore, this mouse strain carries a human kappa light chain transgene, KCo5, as described in Fishwild *et al.* (1996) *Nature Biotechnology* 14:845-851, and a human heavy chain transgene, HCo7, as described in U.S. Pat. Nos. 5,545,806; 5,625,825; and 5,545,807.

In the KM Mouse™ strain, the endogenous mouse heavy chain and kappa light chain genes have been homozygously disrupted as described above and the mouse carries the KCo5 human kappa light chain transgene as described above. The strain also contains the SC20 transchromosome, which carries the human Ig heavy chain locus, as described in PCT Publication WO 02/43478. The KM Mouse™ strain is also described in detail in U.S. Application No. 20020199213.

HuMab Immunizations:

To generate fully human monoclonal antibodies to IRTA proteins, mice of the HCo7 HuMab Mouse™ strain or the KM Mouse™ strain were immunized with purified recombinant IRTA2 fusion protein or purified recombinant IRTA4 fusion protein, derived from mammalian cells that had been transfected with an expression vector containing the gene encoding the fusion protein. General immunization schemes are described in Lonberg, N. *et al* (1994) *Nature 368*(6474): 856-859; Fishwild, D. *et al.* (1996) *Nature Biotechnology* 14: 845-851 and PCT Publication WO 98/24884.

The mice were 6-16 weeks of age upon the first infusion of antigen. A purified recombinant IRTA2 or IRTA4 antigen preparation (5-50 μg purified from transfected mammalian cells expressing IRTA2 or IRTA4 fusion protein) was used to immunize the HuMab mice intraperitonealy (IP), subcutaneously (SC) or via footpad injection.

Generally, transgenic mice were immunized with antigen in complete Freund's adjuvant or Ribi adjuvant IP, SC or via footpad injection, followed by 2-21 days IP with the antigen in incomplete Freund's or Ribi adjuvant.

For immunization with the IRTA4 antigen, an initial high dose of the immunogen (50 μg) was used in the immunization protocol. The dosage was gradually lowered from 50 μg (administered one time) to 25 μg (administered four times) to 10 μg (administered one time) and then to 5 μg (administered one time). Finally, the dose was increased to 25 μg (administered two times) at the end of the immunization period. The immunization interval was two weeks apart, and the immunizations was carried out over four months, using IP and SC injection.

For immunization with the IRTA2 antigen, 25 μg was used. Mice were immunized with antigen via IP and SC injection, at 7-14 day intervals.

The immune response was monitored by retroorbital bleeds. The plasma was screened by ELISA (as described below), and mice with sufficient titers of anti-IRTA2 or anti-IRTA4 human immunoglobulin were used for fusions. Mice were boosted intravenously with antigen 2 or 3 days before sacrifice and removal of the spleen.

Selection of HuMab or KM Mice Producing Anti-IRTA Antibodies

To select HuMab or KM mice producing antibodies that bound an IRTA protein, sera from immunized mice was tested by a modified ELISA as originally described by Fishwild, D. *et al.* (1996). Briefly, microtiter plates were coated with purified recombinant IRTA2 or IRTA4 fusion protein at 1-2 μg/ml in PBS, 50 μl/wells incubated 4° C overnight, then blocked with 200 μl/well of 5% BSA in PBS. Dilutions of plasma from IRTA2- or IRTA4-immunized mice were added to each well and incubated for 1-2 hours at ambient temperature. The plates were washed with PBS/Tween and then incubated with a goat-anti-human kappa light chain polyclonal antibody conjugated with alkaline phosphatase for 1 hour at room temperature. After washing, the plates were developed with pNPP substrate and analyzed by spectrophotometer at OD 415-650. Mice that developed the highest titers of anti-IRTA2 or anti-IRTA4 antibodies were used for fusions. Fusions were performed as described below and hybridoma supernatants were tested for anti-IRTA2 or anti-IRTA4 activity by ELISA.

Generation of Hybridomas Producing Human Monoclonal Antibodies to IRTA Proteins

The mouse splenocytes, isolated from the HuMab or KM mice, were fused with PEG to a mouse myeloma cell line based upon standard protocols. The resulting hybridomas were then screened for the production of antigen-specific antibodies. Single cell suspensions of splenic lymphocytes from immunized mice were fused to one-fourth the number of P3X63 Ag8.6.53 (ATCC CRL 1580) nonsecreting mouse myeloma cells with 50% PEG (Sigma). Cells were plated at approximately 1 x 10^5/well in flat bottom microtiter plate, followed by about two week incubation in selective medium containing 10% fetal calf serum, supplemented with origen (IGEN) in RPMI, L-glutamine, sodium pyruvate, HEPES, penicillin, streptamycin, gentamycin, lx HAT, and beta-mercaptoethanol. After 1-2 weeks, cells were cultured in medium in which the HAT was replaced with HT. Individual wells were then screened by ELISA (described above) for human anti-IRTA2 or anti-IRTA4 monoclonal IgG antibodies. Once extensive hybridoma growth occurred, medium was monitored usually after 10-14 days. The antibody secreting hybridomas were replated, screened again and, if still positive for human IgG, anti-IRTA2 or anti-IRTA-4 monoclonal antibodies were subcloned at least twice by limiting dilution. The stable subclones were then cultured in vitro to generate small amounts of antibody in tissue culture medium for further characterization.

Hybridoma clones 4C1 (obtained from an HCo7 mouse immunized with an IRTA4 fusion protein) and 1B10 (obtained from a KM mouse immunized with an IRTA2 fusion protein) were selected for further analysis and sequencing.

Example 2: Structural Characterization of Human Anti-IRTA Monoclonal Antibodies 4C1 and 1B10

The cDNA sequences encoding the heavy and light chain variable regions of the mAbs expressed by the 4C1 and 1B10 clones, as described in Example 1, were sequenced using standard DNA sequencing techniques and the expressed proteins were characterized by standard protein chemistry analysis. Both clones were found to express an antibody comprising an IgG1 heavy chain and a kappa light chain.

The nucleotide and amino acid sequences of the heavy chain variable region of 4C1 are shown in Figure 1A and in SEQ ID NO: 17 and 13, respectively.

The nucleotide and amino acid sequences of the kappa light chain variable region of 4C1 are shown in Figure 1B and in SEQ ID NO: 19 and 15, respectively.

Comparison of the 4C1 heavy chain immunoglobulin sequence to the known human germline immunoglobulin heavy chain sequences demonstrated that the 4C1 heavy chain utilizes a V_H segment from human germline V_H 3-33, and a JH segment from human germline JH 4B. Further analysis of the 4C1 V_H sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CD3 regions as shown in Figure 1A and in SEQ ID NOs: 1, 3 and 5, respectively.

Comparison of the 4C1 light chain immunoglobulin sequence to the known human germline immunoglobulin light chain sequences demonstrated that the 4C1 kappa light chain utilizes a V_K segment from human germline V_K L18 and a J_K segment from human germline JK 4. Further analysis of the 4C1 V_K sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CD3 regions as shown in Figure 1B and in SEQ ID NOs: 7, 9 and 11, respectively.

The nucleotide and amino acid sequences of the heavy chain variable region of 1B10 are shown in Figure 2A and in SEQ ID NO: 18 and 14, respectively.

The nucleotide and amino acid sequences of the light chain variable region of 1B10 are shown in Figure 2B and in SEQ ID NO: 20 and 16, respectively.

Comparison of the 1B10 heavy chain immunoglobulin sequence to the known human germline immunoglobulin heavy chain sequences demonstrated that the 1B10 heavy chain utilizes a V_H segment from human germline V_H 3-7, a D segment from the human germline 3-10, and a JH segment from human germline JH 5B. Further analysis of the 1B10 V_H sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CD3 regions as shown in Figure 2A and in SEQ ID NOs: 2, 4 and 6, respectively.

Comparison of the 1B10 light chain immunoglobulin sequence to the known human germline immunoglobulin light chain sequences demonstrated that the 1B10 kappa light chain utilizes a V_k segment from human germline V_K A27 and a J_K segment from human germline JK 2. Further analysis of the 1B10 V_k sequence using the Kabat system of CDR region determination led to the delineation of the light chain

CDR1, CDR2 and CD3 regions as shown in Figure 2B and in SEQ ID NOs: 8, 10 and 12, respectively.

Figure 3 shows the alignment of the 4C1 heavy chain variable amino acid sequence (SEQ ID NO: 13) with the germline V_H 3-33 encoded amino acid sequence (SEQ ID NO: 21). The CDR1, CDR2 and CDR3 regions are delineated.

Figure 4 shows the alignment of the 4C1 kappa light chain variable amino acid sequence (SEQ ID NO: 15) with the germline V_K L18 encoded amino acid sequence (SEQ ID NO: 23). The CDR1, CDR2 and CDR3 regions are delineated.

Figure 5 shows the alignment of the 1B10 heavy chain variable amino acid sequence (SEQ ID NO: 14) with the germline V_H 3-7 encoded amino acid sequence (SEQ ID NO: 22). The CDR1, CDR2 and CDR3 regions are delineated.

Figure 6 shows the alignment of the 1B10 kappa light chain variable amino acid sequence (SEQ ID NO: 16) with the germline V_K A27 encoded amino acid sequence (SEQ ID NO: 24). The CDR1, CDR2 and CDR3 regions are delineated.

The 4C1 and 1B10 variable regions can be converted to full-length antibodies of any desired isotype using standard recombinant DNA techniques. For example, DNA encoding the V_H and V_L regions can be cloned into an expression vector that carries the heavy and light chain constant regions such that the variable regions are operatively linked to the constant regions. Alternatively, separate vectors can be used for expression of the full-length heavy chain and the full-length light chain. Non-limiting examples of expression vectors suitable for use in creating full-length antibodies include the pIE vectors described in U.S. Patent Application No. 20050153394 by Black.

Example 3: Binding of IRTA Monoclonal Antibodies to Cell Lines

In this example, the binding of mAbs 4C1 and 1B10 to various cell lines was examined by flow cytometry. The 4C1 and 1B10 monoclonal antibodies, as well as a control antibody, were serially diluted with cold 1x PBS + 0.1 % BSA. For the binding reaction, 50 µl of diluted antibody solution was added to a 50 µl cell suspension containing 4 x 10^5 cells and the mixture was incubated on ice for 30-60 minutes. The cells were then washed three times with 1x PBS + 0.1 % BSA. A 1:50 dilution of R-phycoerythrin-labeled goat anti-human IgG Fγ F(ab)2 fragment (Jackson

ImmunoResearch Labs, Cat. # 109-116-098) was added and the mixture was incubated on ice for 1 hour, followed by washing twice with cold 1x PBS + 0.1 % BSA and once with cold 1x PBS. After the final wash, 200 µl of cold 1x PBS was added to each solution and analysis of antibody binding was carried out by FACS.

To test the ability of the antibodies to bind to different IRTA proteins, the antibodies were incubated with a panel of CHO cell lines that had been transfected to express the IRTA1, IRTA2, IRTA3, IRTA4 or IRTA5 protein on the cell surface. Additionally, binding of the antibodies to the following B cell tumor lines was tested: Daudi (ATCC CCL-213), Raji (ATCC CCL-86), SU-DHL-6 (DSMZ ACC 572), Granta 519 (DSMZ ACC 342), JeKo (DSMZ ACC 553), P3HR1 (ATCC HTB-62). Binding was also tested to chronic lymphocytic leukemia (CLL) cells obtained from a CLL patient

The results of the flow cytometry analysis are summarized below in Table 1, which shows mean fluorescence intensity (MFI). For all cell lines except SU-DHL-6 and CLL cells, the results shown are for 5 µg/ml of antibody. For SU-DHL-6, the results shown are for 3 µg/ml of antibody. For CLL cells, the results shown are for 2 µg/ml of antibody.

Table 1: Mean Fluorescent Intensity of Antibody Binding

Cell Line	Control Antibody	4C1	1B10
CHO-IRTA1	6.1	6.2	7.4
CHO-IRTA2	5.5	7.6	4684
CHO-IRTA3	5.1	1068	40
CHO-IRTA4	5.6	2662	2247
CHO-IRTA5	4.3	7619	7410
Daudi	4.8	125	160
JeKo	7	9	15
Granta 519	7	19	97
P3HR1	6	19	29
Raji	4	72	20
SU-DHL-6	7.2	343	410
CLL	13	94	116

The flow cytometry results demonstrate that the 4C1 mAb (raised using an IRTA4 fusion protein as antigen) exhibits strong binding to the IRTA3, IRTA4 and IRTA5 proteins, while the 1B10 mAb (raised using an IRTA2 fusion protein as antigen) exhibits strong binding to the IRTA2, IRTA4 and IRTA5 proteins.

The flow cytometry results also demonstrate that the 4C1 antibody exhibits binding to a number of different B cell tumor cells and cell lines. In particular, binding to the Daudi, Raji and SU-DHL-6 cell lines and to CLL cells was readily detectable above background; lower level binding (although still above background) was observed with the Granta 519 and P3HR1 cell lines.

The flow cytometry results also demonstrate that the 1B10 antibody exhibits binding to a number of different B cell tumor cells and cell lines. In particular, binding to the Daudi, Granta 519 and SU-DHL-6 cell lines and to CLL cells was readily detectable above background; lower level binding (although still above background) was observed with the JeKo, Raji and P3HR1 cell lines.

Example 4: Binding Kinetics of Anti-IRTA Human Monoclonal Antibodies

In this example, binding affinity and binding kinetics of the anti-IRTA antibodies were examined by BIAcore analysis.

The binding of the 1B10 antibody to various IRTA family proteins was examined by BIAcore™ using a capture method. The 1B10 antibody was captured using either anti-CH1, a reagent antibody that is specific towards the heavy chain constant region1 of human antibody (Zymed, Clone HP6045, Stock conc. 1.0 mg/mL) or a goat anti-Hu IgG(Fc) (Kirkegaard & Perry Laboratory, Inc., Stock conc. 1.0 mg/ml), an antibody specific for the Fc region of the antibody. Anti-CH1 or anti-Hu IgG(Fc) was coated on a CM5 chip (BR-1000-14, Research Grade) at high density (9000-20000RUs). The coating was carried out based on the standard immobilization procedure recommended by the manufacturer. 1B10 purified antibody, with concentrations ranging from 0.8-10 μg/mL, was then captured on the anti-CH1 or anti-Hu IgG(Fc) coated surface at the flow-rate of 10uL/min for 0.5-1 minute. A single concentration of IRTA fusion protein IRTA2-C9 (200 nM) and multiple concentrations (25-500 nM) of other IRTA fusion proteins (IRTA3-His, IRTA4-C9 or IRTA5-Fc) were injected over captured antibody for 5 minutes at a flow rate of 25

μg/mL. The antigen was allowed to dissociate for 10-20 minutes. The chip surface was regenerated after each cycle with 10-15 μL of 10-50 mM NaOH (for the IRTA3, IRTA4 and IRTA5 experiments) or a combination of 50 mM NaOH + 100 mM NaCl and 20 mM HCl + 100 mM NaCl (for the IRTA2 experiment). Isotype controls were run on the chip, and the data used to subtract non-specific binding. All the experiments were carried out on a Biacore 3000 surface plasmon resonance instrument, using BIAcore Control software v 3.2. Data analysis was carried out using BiaEvaluation v3.2 software. The results are shown in Table 2 below. The BIAcore results for 1B10 confirm the flow cytometry results that 1B10 is capable of binding with high affinity to IRTA2, IRTA4 and IRTA5. Moreover, in the BIAcore assay, 1B10 also exhibited high affinity binding to IRTA3.

Table 2: Binding Kinetics of 1B10 Antibody to IRTA Family Proteins

Antigen	$K_D \times 10^{-9}$ (M)	$k_a \times 10^{4}$ (1/Ms)	$k_d \times 10^{-4}$ (1/s)
IRTA2*	2.07	8.78	1.81
IRTA3	110	4.25	46.6
IRTA4	158	5.98	94.6
IRTA5	1030	0.75	77.2

* Single concentration experiment

The binding of the 4C1 antibody to various IRTA family proteins was examined by BIAcore using a direct binding method. Three IRTA fusion proteins (IRTA2-C9, IRTA4-C9 and IRTA5-Fc) were coated on three different surfaces of CM5 chip at low density (approximately 200RUs), followed by standard immobilization procedures described in the BIAcore handbook. Multiple concentrations (67-667 nM) of purified 4C1 mAb were injected over immobilized antigen surface at the flow rate of 80 μl/min with an association time of three minutes and a dissociation time of 7.5 minutes. The chip surface was regenerated with 25 μl of 50 mM NaOH + 400 mM NaCl after each cycle. Appropriate isotype controls were run to check non-specific binding. All the experiments were carried out on a Biacore 3000, using BIAcore Control software v. 3.2. Data analysis was carried out using BiaEvaluation v. 3.2 software. The results for the binding of 4C1 to IRTA fusion proteins (IRTA2-C9, IRTA4-C9 and IRTA5-Fc) are summarized in Table 3 below. The BIAcore results

for 4C1 confirm the flow cytometry results that 4C1 is capable of binding to IRTA4 and IRTA5 with high affinity but does not specifically bind IRTA2.

Table 3: Binding Kinetics of 4C1 Antibody to IRTA Family Proteins

Antigen	$K_D \times 10^{-9}$ (M)	$k_a \times 10^{4}$ (1/Ms)	$k_d \times 10^{-4}$ (1/s)
IRTA2	ND	ND	ND
IRTA4	53.7	1.45	7.78
IRTA5	50.9	1.52	7.76

ND = no specific binding detected

Example 5: ADCC Activity of Anti-IRTA Human Monoclonal Antibodies

In this example, the activity of anti-IRTA human monoclonal antibodies was tested in an antibody dependent cellular cytotoxicity (ADCC) assay. Human peripheral blood mononuclear cells were isolated from normal volunteers and used as effector cells. The SU-DHL-6 cell line (DSMZ ACC 572), which expresses IRTA2, IRTA3, IRTA4 and IRTA5, was labeled and used as target cells in a standard ADCC assay. The ADCC activity of the human monoclonal antibodies 4C1 and 1B10 was tested at various concentrations (0.01 – 10 μg/ml). An antibody specific for the IRTA5 protein (referred to as 2G5; described in U.S. Application No. 20050266008) was used as a control.

To prepare the effector and target cells for the assay, human effector cells were prepared from whole blood as follows. Human peripheral blood mononuclear cells were purified from heparinized whole blood by standard Ficoll-paque separation. The cells were resuspended in RPMI1640 media containing 10% FBS (heat-inactivated) at 1 x 10^7 cells/ml. Target SU-DHL-6 cells (10^6 cells total) were incubated with 0.1 mCi ^{51}Cr in 5 % CO_2, 37° C incubator for 1 hour. The target cells were washed three times with RPMI1640 media, spun down and brought to a final volume of 1x10^5 cells/ml with RPMI1640 media containing 10 % FBS.

The SU-DHL-6 target cell line was tested for ADCC by the human 4C1, 1B10 and 2G5 monoclonal antibodies using ^{51}Cr releasing assay as follows. The target cell line (50 μl of labeled target cells, 5000 cells/well) was incubated with 50 μl of effector cells (5 x 10^5 cells/well) and 50 μl of antibody (0.01 – 10 μg/ml final

concentration). A target to effector ratio of 1:100 was used throughout the experiments. Cells were spun down at 2000 rpm and incubated for four hours incubation at 37° C. Fifty microliters of supernatants were then collected and counted in a Scintillation counter.

The % lysis was calculated as follows: (sample release – spontaneous release) / (maximum release – spontaneous release) x 100, where the spontaneous release is the counting value from wells which contain only target cells and maximum release is the counting value from wells containing only target cells and that have been treated with 20 % SDS.

The results are shown in the graph of Figure 7. The results demonstrate that the 1B10 and 4C1 antibodies were able to mediate dose-dependent ADCC of the SU-DHL-6 cell line. Moreover, the 1B10 and 4C1 antibodies, which are cross-reactive with multiple IRTA subtypes, exhibited higher ADCC activity than the 2G5 antibody, which is monospecific for IRTA5.

SUMMARY OF SEQUENCE LISTING

SEQ ID NO:	SEQUENCE		
1	V_H CDR1 a.a. 4C1		
2	V_H CDR1 a.a. 1B10		
3	V_H CDR2 a.a. 4C1		
4	V_H CDR2 a.a. 1B10		
5	V_H CDR3 a.a. 4C1		
6	V_H CDR3 a.a. 1B10		
7	V_K CDR1 a.a. 4C1		
8	V_K CDR1 a.a. 1B10		
9	V_K CDR2 a.a. 4C1		
10	V_K CDR2 a.a. 1B10		
11	V_K CDR3 a.a. 4C1		
12	V_K CDR3 a.a. 1B10		
13	V_H a.a. 4C1		
14	V_H a.a. 1B10		
15	V_K a.a. 4C1		
16	V_K a.a. 1B10		
17	V_H n.t. 4C1		
18	V_H n.t. 1B10		
19	V_K n.t. 4C1		
20	V_K n.t. 1B10		
21	V_H 3-33 germline a.a.		
22	V_H 3-7 germline a.a.		
23	V_k L18 germline a.a.		
24	V_k A27 germline a.a.		

What is claimed:

1. An isolated human monoclonal antibody, or an antigen-binding portion thereof, wherein the antibody specifically binds at least two IRTA family proteins.

2. The antibody of claim 1, which specifically binds an IRTA4 protein and at least one other IRTA family protein.

3. The antibody of claim 2, which specifically binds at lest an IRTA4 and an IRTA5 protein.

4. The antibody of claim 2, which specifically binds at least an IRTA3 protein, an IRTA4 protein and an IRTA5 protein.

5. The antibody of claim 4, wherein the IRTA3, IRTA4 and IRTA5 proteins are human IRTA3, IRTA4 and IRTA5 proteins.

6. The antibody of claim 2, which specifically binds at least an IRTA2 protein, an IRTA4 protein and an IRTA5 protein.

7. The antibody of claim 6, wherein the IRTA2, IRTA4 and IRTA5 proteins are human IRTA2, IRTA4 and IRTA5 proteins.

8. The antibody of claim 1, which exhibits antibody dependent cellular cytotoxicity (ADCC) activity.

9. The antibody of claim 1, which binds at least one B cell tumor cell or cell line.

10. The antibody of claim 9, which binds at least one B cell tumor cell or cell line selected from the group consisting of Daudi (ATCC CCL-213), SU-DHL-6 (DSMZ ACC 572) and chronic lymphocytic leukemia (CLL) cells.

11. The antibody of claim 9, which binds a Daudi B cell tumor cell line (ATCC CCL-213).

12. An isolated human monoclonal antibody, or antigen binding portion thereof, wherein the antibody cross-competes for binding to an IRTA family protein with a reference antibody, wherein the reference antibody comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 13 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 15; or

(b) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 14 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

13. The antibody of claim 12, wherein the reference antibody comprises: a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 13 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 15.

14. The antibody of claim 12, wherein the reference antibody comprises: a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 14 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

15. An isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a heavy chain variable region that is the product of or derived from a human V_H 3-33 gene or a human V_H 3-7 gene, wherein the antibody specifically binds at least two IRTA family proteins.

16. An isolated monoclonal antibody, or an antigen-binding portion thereof, comprising a light chain variable region that is the product of or derived from a human V_K L18 gene or a human V_K A27 gene, wherein the antibody specifically binds at least two IRTA family proteins.

17. An isolated monoclonal antibody, or an antigen-binding portion thereof, comprising:

(a) a heavy chain variable region that is the product of or derived from a human V_H 3-33 gene and a light chain variable region that is the product of or derived from a human V_K L18 gene; or

(b) a heavy chain variable region that is the product of or derived from a human V_H 3-7 gene and a light chain variable region that is the product of or derived from a human V_K A27 gene;

wherein the antibody specifically binds at least two IRTA family proteins.

18. The antibody of claim 1, which comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO: 1;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO: 3;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO: 5;

(d) a light chain variable region CDR1 comprising SEQ ID NO: 7;

(e) a light chain variable region CDR2 comprising SEQ ID NO: 9; and

(f) a light chain variable region CDR3 comprising SEQ ID NO: 11.

19. The antibody of claim 1, which comprises:

(a) a heavy chain variable region CDR1 comprising SEQ ID NO: 2;

(b) a heavy chain variable region CDR2 comprising SEQ ID NO: 4;

(c) a heavy chain variable region CDR3 comprising SEQ ID NO: 6;

(d) a light chain variable region CDR1 comprising SEQ ID NO: 8;

(e) a light chain variable region CDR2 comprising SEQ ID NO: 10; and

(f) a light chain variable region CDR3 comprising SEQ ID NO: 12.

20. An isolated monoclonal antibody, or antigen binding portion thereof, comprising:

(a) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 13-14; and

(b) a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 15-16;

wherein the antibody specifically binds at least two IRTA family proteins.

21. The antibody of claim 20, which comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 13; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 15.

22. The antibody of claim 20, which comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 14; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

23. A composition comprising the antibody, or antigen-binding portion thereof, of claim 1, and a pharmaceutically acceptable carrier.

24. An immunoconjugate comprising the antibody, or antigen-binding portion thereof, of claim 1, linked to a therapeutic agent.

25. A composition comprising the immunoconjugate of claim 24 and a pharmaceutically acceptable carrier.

26. The immunoconjugate of claim 24, wherein the therapeutic agent is a cytotoxin.

27. A composition comprising the immunoconjugate of claim 26 and a pharmaceutically acceptable carrier.

28. The immunoconjugate of claim 24, wherein the therapeutic agent is a radioactive isotope.

29. A composition comprising the immunoconjugate of claim 28 and a pharmaceutically acceptable carrier.

30. An isolated nucleic acid molecule encoding the antibody, or antigen-binding portion thereof, of claim 1.

31. An expression vector comprising the nucleic acid molecule of claim 30.

32. A host cell comprising the expression vector of claim 31.

33. A method for preparing an anti-IRTA antibody which comprises expressing the antibody in the host cell of claim 32 and isolating the antibody from the host cell.

34. A method of inhibiting growth of tumor cells expressing an IRTA protein, comprising contacting the cells with the antibody, or antigen-binding portion thereof, of claim 1 in an amount effective to inhibit growth of the tumor cells.

35. The method of claim 34, wherein the tumor cell is a B cell lymphoma.

36. The method of claim 35, wherein the B cell lymphoma is a non-Hodgkin's lymphoma or a chronic lymphocytic leukemia.

37. The method of claim 34, wherein the antibody, or antigen-binding portion thereof, is conjugated to a therapeutic agent.

38. The method of claim 37, wherein the therapeutic agent is a cytotoxin.

39. A method for preparing an anti-IRTA antibody comprising:

(a) providing: (i) a heavy chain variable region antibody sequence comprising a CDR1 sequence selected from the group consisting of SEQ ID NOs: 1-2, a CDR2 sequence selected from the group consisting of SEQ ID NOs: 3-4, and/or a CDR3 sequence selected from the group consisting of SEQ ID NOs: 5-6; and/or (ii) a light

chain variable region antibody sequence comprising a CDR1 sequence selected from the group consisting of SEQ ID NOs: 7-8, a CDR2 sequence selected from the group consisting of SEQ ID NOs: 9-10, and/or a CDR3 sequence selected from the group consisting of SEQ ID NOs: 11-12;

(b) altering at least one amino acid residue within the heavy chain variable region antibody sequence and/or the light chain variable region antibody sequence to create at least one altered antibody sequence; and

(c) expressing the altered antibody sequence as a protein.

Anti-IRTA 4C1 VH

```
V segment:    3-33
D segment:    ND
J segment:    JH4b

      Q   V   Q   L   V   E   S   G   G   G   V   V   Q   P   G   R   S   L
  1 CAG GTG CAG CTG GTG GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGG TCC CTG

                                                         CDR1
                                                  ~~~~~~~~~~~~~~~~~~~
      R   L   S   C   A   A   S   G   F   T   F   S   S   Y   G   M   H   W
 55 AGA CTC TCC TGT GCA GCG TCT GGA TTC ACC TTC AGT AGC TAT GGC ATG CAC TGG

                                                             CDR2
                                                      ~~~~~~~~~~~~~~~~~~~
      V   R   Q   A   P   G   K   G   L   E   W   V   A   V   I   W   Y   F
109 GTC CGC CAG GCT CCA GGC AAG GGT CTG GAG TGG GTG GCT GTT ATA TGG TAT TTT

                    CDR2
    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      G   S   N   T   Y   Y   T   D   S   V   K   G   R   F   T   I   S   R
163 GGA AGT AAT ACA TAC TAT ACA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC AGA

      D   N   S   K   N   T   L   Y   L   Q   M   N   S   L   R   A   E   D
217 GAC AAT TCC AAG AAC ACG CTG TAT CTG CAA ATG AAC AGC CTG AGA GCC GAG GAC

                                                  CDR3
                                      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      T   A   V   Y   Y   C   A   R   D   G   A   N   W   I   F   F   D   Y
271 ACG GCT GTG TAT TAC TGT GCG AGA GAT GGA GCT AAC TGG ATT TTC TTT GAC TAC

      W   G   Q   G   T   L   V   T   V   S   S
325 TGG GGC CAG GGA ACC CTG GTC ACC GTC TCC TCA
```

FIGURE 1A

Anti-IRTA 4C1 VK

V segment: L18
J segment: JK4

```
   A   I   Q   L   T   Q   S   P   S   S   L   S   A   S   V   G   D   R
 1 GCC ATC CAG TTG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT GTA GGA GAC AGA

                             CDR1
                             ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   V   T   I   T   C   R   A   S   Q   G   I   S   S   A   L   A   W   Y
55 GTC ACC ATC ACT TGC CGG GCA AGT CAG GGC ATT AGC AGT GCT TTA GCC TGG TAT

                                                         CDR2
                                                         ~~~~~~~~~~~~~~~~~~~
   Q   Q   K   P   G   K   A   P   K   L   L   I   Y   D   A   S   S   L
109 CAG CAG AAA CCA GGG AAA GCT CCT AAG CTC CTG ATC TAT GAT GCC TCC AGT TTG

    CDR2
    ~~~~~~~
   E   S   G   V   P   S   R   F   S   G   S   G   S   G   T   D   F   T
163 GAA AGT GGG GTC CCA TCA AGG TTC AGC GGC AGT GGA TCT GGG ACA GAT TTC ACT

                                                                 CDR3
                                                                 ~~~~~~~
   L   T   I   S   S   L   Q   P   E   D   F   A   T   Y   Y   C   Q   Q
217 CTC ACC ATC AGC AGC CTG CAG CCT GAA GAT TTT GCA ACT TAT TAC TGT CAA CAG

            CDR3
    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   F   K   S   Y   P   L   T   F   G   G   G   T   R   V   E   I   K
271 TTT AAA AGT TAC CCG CTC ACT TTC GGC GGA GGG ACC AGG GTG GAG ATC AAA
```

FIGURE 1B

Anti-IRTA 1B10 VH

V segment: 3-7
D segment: 3-10
J segment: JH5b

```
          E   V   Q   L   V   E   S   G   G   G   L   V   Q   P   G   G   S   L
1        GAG GTG CAG CTG GTG GAG TCT GGG GGA GGC TTG GTC CAG CCT GGG GGG TCC CTG

                                                         CDR1
                                                     ~~~~~~~~~~~~~~~~~~~~
          R   L   S   C   A   A   S   G   F   T   F   S   R   Y   W   M   S   W
55       AGA CTC TCC TGT GCA GCC TCT GGA TTC ACC TTT AGT CGC TAT TGG ATG AGC TGG

                                                             CDR2
                                                         ~~~~~~~~~~~~~~~~~~
          V   R   Q   A   P   G   K   G   L   E   W   V   A   N   I   K   Q   D
109      GTC CGC CAG GCT CCA GGG AAG GGG CTG GAG TGG GTG GCC AAC ATA AAG CAA GAT

                         CDR2
         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
          G   S   E   K   Y   Y   V   D   S   V   K   G   R   F   T   I   S   R
163      GGA AGT GAG AAA TAC TAT GTG GAC TCT GTG AAG GGC CGA TTC ACC ATC TCC AGA

          D   N   A   K   N   S   L   Y   L   Q   M   N   S   L   R   A   E   D
217      GAC AAC GCC AAG AAC TCA CTG TAT CTG CAA ATG AAC AGC CTG AGA GCC GAG GAC

                                                     CDR3
                                         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
          T   A   V   Y   Y   C   A   R   E   R   R   Y   Y   Y   G   S   G   S
271      ACG GCT GTG TAT TAC TGT GCG AGA GAG AGG AGG TAT TAC TAT GGT TCG GGG AGT

                     CDR3
         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
          D   Y   N   Y   W   F   D   P   W   G   Q   G   T   L   V   T   V   S
325      GAT TAT AAT TAC TGG TTC GAC CCC TGG GGC CAG GGA ACC CTG GTC ACC GTC TCC

          S
379      TCA
```

FIGURE 2A

Anti-IRTA 1B10 VK

V segment: A27
J segment: JK2

```
          E   I   V   L   T   Q   S   P   G   T   L   S   L   S   P   G   E   R
1         GAA ATT GTG TTG ACG CAG TCT CCA GGC ACC CTG TCT TTG TCT CCA GGG GAA AGA

                                  CDR1
                                  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
          A   T   L   S   C   R   A   S   Q   S   G   S   S   S   Y   L   A   W
55        GCC ACC CTC TCC TGC AGG GCC AGT CAG AGT GGT AGC AGC AGC TAC TTA GCC TGG

                                                                  CDR2
                                                                  ~~~~~~~~~~~~~~~~
          Y   Q   Q   K   P   G   Q   A   P   R   L   L   I   Y   G   A   S   N
109       TAC CAG CAG AAA CCT GGC CAG GCT CCC AGG CTC CTC ATC TAT GGT GCA TCC AAC

          CDR2
          ~~~~~~~~~~~~
          R   A   T   G   I   P   D   R   F   S   G   S   G   S   G   T   D   F
163       AGG GCC ACT GGC ATC CCA GAC AGG TTC AGT GGC AGT GGG TCT GGG ACA GAC TTC

                                                                          CDR3
                                                                          ~~~~
          T   L   T   I   S   R   L   E   P   E   D   F   A   V   Y   Y   C   Q
217       ACT CTC ACC ATC AGC AGA CTG GAG CCT GAA GAT TTT GCA GTG TAT TAC TGT CAG

                      CDR3
                      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
          Q   Y   G   S   S   P   P   Y   T   F   G   Q   G   T   K   L   E   I
271       CAG TAT GGT AGC TCA CCT CCG TAC ACT TTT GGC CAG GGG ACC AAG CTG GAG ATC

          K
325       AAA
```

FIGURE 2B

Anti-IRTA 4C1 VH

```
                                                                  _CDR1______
3-33 germline   Q V Q L V E S G G G V V Q P G R S L R L S C A A S G F T F S S Y G M H W
4C1 VH          - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                          _CDR2____________________________
3-33 germline   V R Q A P G K G L E W V A V I W Y D G S N K Y Y A D S V K G R F T I S R
4C1 VH          - - - - - - - - - - - - - - - - - F - - - T - - T - - - - - - - - - - -

                                                                    _CDR3______________
3-33 germline   D N S K N T L Y L Q M N S L R A E D T A V Y Y C A R
JH4b germline                                                                     F D Y
4C1 VH          - - - - - - - - - - - - - - - - - - - - - - - - - - D G A N W I F - - -

JH4b germline   W G Q G T L V T V S S
4C1 VH          - - - - - - - - - - - (JH4b)
```

FIGURE 3

Anti-IRTA 4C1 VK

```
                                                                  _CDR1__________
L18 germline      A I Q L T Q S P S S L S A S V G D R V T I T C R A S Q G I S S
4C1 VK1           - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                                  _CDR2_________
L18 germline      A L A W Y Q Q K P G K A P K L L I Y D A S S L E S G V P S R F
4C1 VK1           - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                                                  _CDR3____
L18 germline      S G S G S G T D F T L T I S S L Q P E D F A T Y Y C Q Q F N N
4C1 VK1           - - - - - - - - - - - - - - - - - - - - - - - - - - - - K S

L18 germline      Y P
JK4 germline        L T F G G G T K V E I K
4C1 VK1           - - - - - - - - - R - - - - (JK4)
```

FIGURE 4

Anti-IRTA 1B10 VH

```
                                                                  __CDR1___
3-7 germline     E V Q L V E S G G G L V Q P G G S L R L S C A A S G F T F S S Y W M S W
1B10 VH          - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - R - - - - -

                                             _____________CDR2________________
3-7 germline     V R Q A P G K G L E W V A N I K Q D G S E K Y Y V D S V K G R F T I S R
1B10 VH          - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                                                    _____CDR3__________
3-7 germline     D N A K N S L Y L Q M N S L R A E D T A V Y Y C A R
1B10 VH          - - - - - - - - - - - - - - - - - - - - - - - - - - E R R Y Y Y G S G S

                 _____CDR3______
JH5b germline            W F D P W G Q G T L V T V S S
1B10 VH          D Y N Y - - - - - - - - - - - - - - - (JH5b)
```

FIGURE 5

Anti-IRTA 1B10 VK

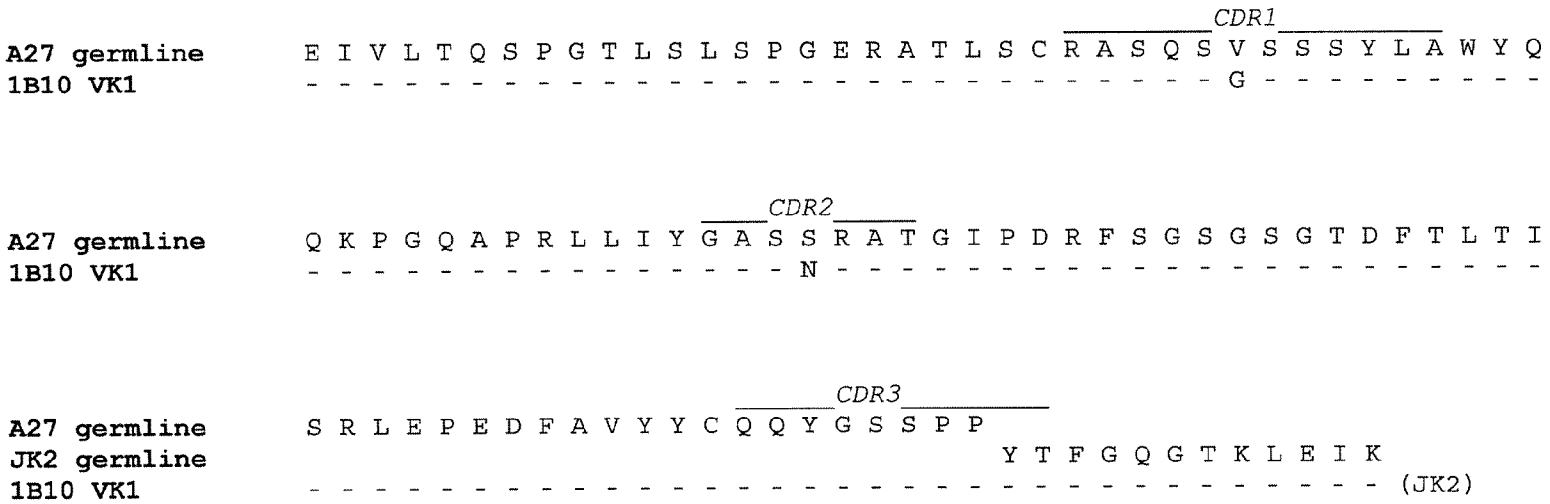

FIGURE 6

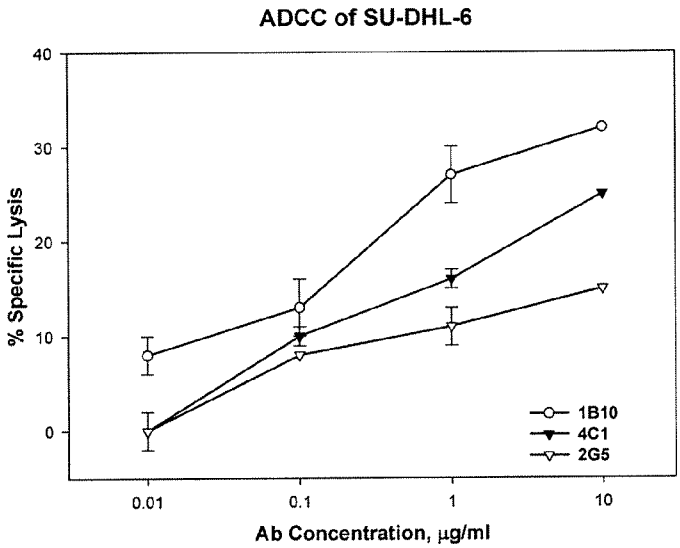
ADCC of SU-DHL-6
% Specific Lysis
40
30
20
10
0
0.01
0.1
1
10
Ab Concentration, μg/ml
1B10
4C1
2G5

FIGURE 7