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(54) Title: OPTIMIZED ANTIBODIES THAT TARGET CD19

(57) Abstract: The present invention describes antibodies that target CD19, wherein the antibodies comprise at least one modification relative to a parent antibody, wherein the modification alters affinity to an FcgγR or alters effector function as compared to the parent antibody. Also disclosed are methods of using the antibodies of the invention.

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OPTIMIZED ANTIBODIES THAT TARGET CD19

This application claims benefit under 35 U.S.C. §119(e) U.S. Provisional Patent Application No. 60/822,362 , filed August 14, 2006, incorporated herein by reference in its entiriety.

BACKGROUND

B cells

B cells are lymphocytes that play a large role in the humoral immune response. They are produced in the bone marrow of most mammals, and represent 5-15% of the circulating lymphoid pool. The principal function of B cells is to make antibodies against various antigens, and are an essential component of the adaptive immune system.

The human body makes millions of different types of B cells each day that circulate in the blood and lymph performing the role of immune surveillence. B cells, also referred to as B lymphocytes, do not produce antibodies until they become fully activated. Each B cell has a unique receptor protein (referred to as the B cell receptor (BCR)) on its surface that will bind to one particular antigen. The BCR is a membrane-bound immunoglobulin, and it is this molecule that allows the distinction of B cells from other types of lymphocytes, as well as being the main receptor involved in B-cell activation. Once a B cell encounters its cognate antigen and receives an additional signal from a T helper cell, it can further differentiate into various types of B cells listed below. The B cell may either become one of these cell types directly or it may undergo an intermediate differentiation step, the germinal center reaction, where the B cell will hypermutate the variable region of its immunoglobulin gene and possibly undergo class switching.

B-cell development occurs through several stages, each stage representing a change in the genome content at the antibody loci. The stages of B-cell development include Progenitor B cells, Early Pro-B cells, Late Pro-B cells, Large Pre-B cells, Small Pre-B cells, Immature B cells, and Mature B cells.

Mature B cells can be divided into four major types:

B-1 cells express CD5, a marker usually found on T cells. B-1 cells also express IgM in greater quantities than IgG. They secrete natural low affinity polyreactive antibodies found in the serum and often have specificities directed toward self-antigens, and common bacterial polysaccharides. B-1 cells are present in low numbers in the lymph nodes and spleen and are instead found predominantly in the peritoneal and pleural cavities.

B-2 cells are the conventional B cells to which most texts refer. They reside in bone marrow, spleen, and lymph nodes. They are short-lived, and when triggered by antigens may differentiate into IgG-producing memory B cells. In the course of these antibody responses IgG may undergo substantial affinity maturation.

Plasma B cells (also known as plasma cells) are large B cells that have been exposed to antigen and produce and secrete large amounts of antibodies, which assist in the destruction of microbes by binding and facilitating targeting by phagocytes, as well as activation of the complement system. Plasma cells are sometimes referred to as antibody factories.

Memory B cells are formed from activated B cells that are specific to the antigen encountered during the primary immune response. These cells live for a long time, and can respond quickly following a second exposure to the same antigen.

When a B cell fails in any step of the maturation process, it will die by a mechanism called apoptosis. If it recognizes self-antigen during the maturation process, the B cell will become suppressed (known as anergy) or undergo apoptosis. B cells are continuously produced in the bone marrow, but only a small portion of newly made B cells survive to participate in the long-lived peripheral B-cell pool.

In recent years, data have emerged suggesting that B lymphocytes play a broader role in immune responses and are not merely the passive recipients of signals that result in differentiation into antibody-producing plasma cells. Along with their traditional roles as antigen presenting cells and precursors of antibody-producing plasma cells, B cells have also been found to regulate antigen presenting cells (APCs) and T-cell functions, produce cytokines, and express receptor/ligand pairs that previously had been thought to be restricted to other cell types.

B-cell disorders

Because of their critical role in regulating the immune system, disregulation of B cells is associated with a variety of disorders. B-cell disorders, also referred to herein as B-cell related diseases, are divided into excessive or uncontrolled proliferation (lymphomas, leukemias), and defects of B-cell development/immunoglobulin production (immunodeficiencies). The majority (80%) of lymphoma cases are of B-cell origin. These include non-Hodgkin's lymphoma (NHL), acute lymphoblastic leukemia (ALL), and autoimmune related diseases.

NHL is a heterogeneous malignancy originating from lymphocytes. In the United States (U.S.), the incidence is estimated at 65,000/year with mortality of approximately 20,000 (American Cancer Society, 2006; and SEER Cancer Statistics Review). The disease can occur in all ages, the usual onset begins in adults over 40 years, with the incidence increasing with age.

NHL is characterized by a clonal proliferation of lymphocytes that accumulate in the lymph nodes, blood, bone marrow and spleen, although any major organ may be involved.

The diagnosis and histologic characterization of NHL is made using a combination of morphologic and immunophenotype criteria. The current classification system used by pathologists and clinicians is the World Health Organization (WHO) Classification of Tumours, which organizes NHL into precursor and mature B-cell or T-cell neoplasms. The PDQ is currently dividing NHL as indolent or aggressive for entry into clinical trials. For consistency the present document will also use a similar division. The indolent NHL group is comprised primarily of follicular subtypes, small lymphocytic lymphoma, MALT, and marginal zone; indolent encompasses approximately 50% of newly diagnosed B-cell NHL patients. Aggressive NHL includes patients with histologic diagnoses of primarily diffuse large B cell (40% of all newly diagnosed patients have diffuse large cell), Burkitt's, and mantle cell.

The clinical course of NHL is highly variable. A major determinant of clinical course is the histologic subtype. Most indolent types of NHL are considered to be incurable disease. Patients respond initially to either chemotherapy or antibody therapy and most will relapse. Studies to date have not demonstrated an improvement in survival with early intervention. In asymptomatic patients, it is acceptable to "watch and wait" until the patient becomes symptomatic or the disease pace appears to be accelerating. Over time, the disease may transform to a more aggressive histology. The median survival is 8 to 10 years, and indolent patients often receive 3 or more treatments during the treatment phase of their disease. Initial treatment of the symptomatic indolent NHL patient historically has been combination chemotherapy. The most commonly used agents include: cyclophosphamide, vincristine and prednisone (CVP); cyclophosphamide, adriamycin, vincristine, prednisone (CHOP); or the purine analog, fludarabine. Approximately 70% to 80% of patients will respond to their initial chemotherapy, duration of remissions last on the order of 2-3 years. Ultimately the majority of patients relapse. The discovery and clinical use of the anti-CD20 antibody, rituximab, has provided significant improvements in response and survival rate. The current standard of care for most patients is rituximab + CHOP (R-CHOP) or rituximab + CVP (R-CVP). Interferon is approved for initial treatment of NHL in combination with alkylating agents, but has limited use in the U.S.

Rituximab therapy has been shown to be efficacious in several types of NHL, and is currently approved as a first line treatment for both indolent (follicular lymphoma) and aggressive NHL (diffuse large B cell lymphoma). However, there are significant limitations of anti-CD20 monoclonal antibody (mAb), including primary resistance (50% response in relapsed indolent patients), acquired resistance (50% response rate upon re-treatment), rare complete

response (2% complete resonse rate in relapsed population), and a continued pattern of relapse. Finally, many B cells do not express CD20, and thus many B-cell disorders are not treatable using anti-CD20 antibody therapy. Antibodies against antigens other than CD20 may have anti-lymphoma effects that could overcome anti-CD20 resistance or augment the activity of anti-CD20 therapy.

In addition to NHL there are several types of leukemias that result from disregulation of B cells. Chronic lymphocytic leukemia (also known as "chronic lymphoid leukemia" or "CLL"), is a type of adult leukemia caused by an abnormal accumulation of B lymphocytes. In CLL, the malignant lymphocytes may look normal and mature, but they are not able to cope effectively with infection. CLL is the most common form of leukemia in adults. Men are twice as likely to develop CLL as women. However, the key risk factor is age. Over 75% of new cases are diagnosed in patients over age 50. More than 10,000 cases are diagnosed every year and the mortality is almost 5,000 a year (American Cancer Society, 2006; and SEER Cancer Statistics Review).

CLL is an incurable disease but progresses slowly in most cases. Many people with CLL lead normal and active lives for many years. Because of its slow onset, early-stage CLL is generally not treated since it is believed that early CLL intervention does not improve survival time or quality of life. Instead, the condition is monitored over time. Initial CLL treatments vary depending on the exact diagnosis and the progression of the disease. There are dozens of agents used for CLL therapy. Although the purine analogue fludarabine was shown to give superior response rates than chlorambucil as primary therapy, there is no evidence that early use of fludarabine improves overall survival. Combination chemotherapy regimens such as fludarabine with cyclophosphamide, FCR (fludarabine, cyclophosphamide and rituximab) and CHOP are effective in both newly-diagnosed and relapsed CLL. Allogeneic bone marrow (stem cell) transplantation is rarely used as a first-line treatment for CLL due to its risk.

"Refractory" CLL is a disease that no longer responds favorably to treatment. In this case more aggressive therapies, including bone marrow (stem cell) transplantation, are considered. The monoclonal antibody alemtuzumab, directed against CD52, may be used in patients with refractory, bone marrow-based disease.

Another type of leukemia is acute lymphoblastic leukemia (ALL), also known as acute lymphocytic leukemia. ALL is characterised by the overproduction and continuous multiplication of malignant and immature white blood cells (also known as lymphoblasts) in the bone marrow. 'Acute' refers to the undifferentiated, immature state of the circulating lymphocytes ("blasts"),

and that the disease progresses rapidly with life expectancy of weeks to months if left untreated. ALL is most common in childhood with a peak incidence of 4-5 years of age. Children of age 12-16 die more easily from it than others. Currently, at least 80% of childhood ALL are considered curable. Under 4,000 cases are diagnosed every year and the mortality is almost 1,500 a year (American Cancer Society, 2006; and SEER Cancer Statistics Review).

Autoimmunity results from a breakdown of self-tolerance involving humoral and/or cell-mediated immune mechanisms in. Among of the consequences of failure in central and/or peripheral tolerance, are survival and activation of self-reactive B cells and T cells. Examples of autoimmune diseases include, for example, rheumatoid arthritis (RA), systemic lupus erythematosus (SLE or lupus), multiple sclerosis, Sjogren's syndrome, and idiopathic thrombocytopenia purpura (ITP). The pathogenesis of most autoimmune diseases is coupled to the production of autoantibodies against self antigens, leading to a variety of associated pathologies. Autoantibodies are produced by terminally differentiated plasma cells that are derived from naïve or memory B cells. Furthermore, B cells can have other effects on autoimmune pathology, as antigen-presenting cells (APCs) that can interact with and stimulate helper T cells, further stimulating the cycle of anti-self immune response. Depletion of B cells can have direct impact on the production of autoantibodies. Indeed, treatment of RA and SLE with B-cell depletion therapies such as Rituxan has been demonstrated to have clinical benefit for both disease classes (Edwards & Cambridge, Nat. Rev. Immunol. 2006; Dass et al., Future Rheumatol. 2006; Martin & Chan, Annu. Rev. Immunol. 2006).

Unfortunately, it is not known a priori which mechanisms of action may be optimal for a given target antigen. Furthermore, it is not known which antibodies may be capable of mediating a given mechanism of action against a target cell. In some cases a lack of antibody activity, either Fv-mediated or Fc-mediated, may be due to the targeting of an epitope on the target antigen that is poor for mediating such activity. In other cases, the targeted epitope may be amenable to a desired Fv-mediated or Fc-mediated activity, yet the affinity (affinity of the Fv region for antigen or affinity of the Fc region for Fc receptors) may be insufficient. Towards addressing this problem, the present invention describes modifications to anti-CD19 antibodies that provide optimized Fv- and Fc- mediated activities. A broad array of applications of these optimized antibodies are contemplated.

SUMMARY OF THE INVENTION

In one aspect, the present invention is directed to an antibody binds CD19, wherein said antibody comprises at least one modification in the constant region relative to a parent antibody.

In a preferred embodiment, the antibody of the invention binds with altered affinity to an Fc receptor or alters effector function as compared to the parent antibody.

In one aspect, the invention is directed to antibody that binds CD19, including at least one modification in the constant region relative to a parent anti-CD19 antibody, wherein the antibody binds with increased affinity to the FcγRIIIa receptor as compared to the parent antibody.

In certain aspects, the modification is an amino acid. The modification can be at a position selected from the group consisting of 221, 222, 223, 224, 225, 227, 228, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 243, 244, 245, 246, 247, 249, 255, 258, 260, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 278, 280, 281, 282, 283, 284, 285, 286, 288, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 313, 317, 318, 320, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, and 337, wherein numbering is according to the EU index. The amino acid modification can be a substitution selected from the group consisting of 221K, 221Y, 222E, 222Y, 223E, 223K, 224E, 224Y, 225E, 225K, 225W, 227E, 227G, 227K, 227Y, 228E, 228G, 228K, 228Y, 230A, 230E, 230G, 230Y, 231E, 231G, 231K, 231P, 231Y, 232E, 232G, 232K, 232Y, 233A, 233D, 233F, 233G, 233H, 233I, 233K, 233L, 233M, 233N, 233Q, 233R, 233S, 233T, 233V, 233W, 233Y, 234A, 234D, 234E, 234F, 234G, 234H, 234I, 234K, 234M, 234N, 234P, 234Q, 234R, 234S, 234T, 234V, 234W, 234Y, 235A, 235D, 235E, 235F, 235G, 235H, 235I, 235K, 235M, 235N, 235P, 235Q, 235R, 235S, 235T, 235V, 235W, 235Y, 236A, 236D, 236E, 236F, 236H, 236I, 236K, 236L, 236M, 236N, 236P, 236Q, 236R, 236S, 236T, 236V, 236W, 236Y, 237D, 237E, 237F, 237H, 237I, 237K, 237L, 237M, 237N, 237P, 237Q, 237R, 237S, 237T, 237V, 237W, 237Y, 238D, 238E, 238F, 238G, 238H, 238I, 238K, 238L, 238M, 238N, 238Q, 238R, 238S, 238T, 238V, 238W, 238Y, 239D, 239E, 239F, 239G, 239H, 239I, 239K, 239L, 239M, 239N, 239P, 239Q, 239R, 239T, 239V, 239W, 239Y, 240A, 240I, 240M, 240T, 241D, 241E, 241L, 241R, 241S, 241W, 241Y, 243E, 243H, 243L, 243Q, 243R, 243W, 243Y, 244H, 245A, 246D, 246E, 246H, 246Y, 247G, 247V, 249H, 249Q, 249Y, 255E, 255Y, 258H, 258S, 258Y, 260D, 260E, 260H, 260Y, 262A, 262E, 262F, 262I, 262T, 263A, 263I, 263M, 263T, 264A, 264D, 264E, 264F, 264G, 264H, 264I, 264K, 264L, 264M, 264N, 264P, 264Q, 264R, 264S, 264T, 264W, 264Y, 265F, 265G, 265H, 265I, 265K, 265L, 265M, 265N, 265P, 265Q, 265R, 265S, 265T, 265V, 265W, 265Y, 266A, 266I, 266M, 266T, 267D, 267E, 267F, 267H, 267I, 267K, 267L, 267M, 267N, 267P, 267Q, 267R, 267T, 267V, 267W, 267Y, 268D, 268E, 268F, 268G, 268I, 268K, 268L, 268M, 268P, 268Q, 268R, 268T, 268V, 268W, 269F, 269G, 269H, 269I, 269K, 269L, 269M, 269N, 269P, 269R, 269S, 269T, 269V, 269W, 269Y, 270F, 270G, 270H, 270I, 270L, 270M, 270P, 270Q, 270R, 270S, 270T, 270W, 270Y, 271A, 271D,

271E, 271F, 271G, 271H, 271I, 271K, 271L, 271M, 271N, 271Q, 271R, 271S, 271T, 271V, 271W, 271Y, 272D, 272F, 272G, 272H, 272I, 272K, 272L, 272M, 272P, 272R, 272S, 272T, 272V, 272W, 272Y, 273I, 274D, 274E, 274F, 274G, 274H, 274I, 274L, 274M, 274N, 274P, 274R, 274T, 274V, 274W, 274Y, 275L, 275W, 276D, 276E, 276F, 276G, 276H, 276I, 276L, 276M, 276P, 276R, 276S, 276T, 276V, 276W, 276Y, 278D, 278E, 278G, 278H, 278I, 278K, 278L, 278M, 278N, 278P, 278Q, 278R, 278S, 278T, 278V, 278W, 280G, 280K, 280L, 280P, 280W, 281D, 281E, 281K, 281N, 281P, 281Q, 281Y, 282E, 282G, 282K, 282P, 282Y, 283G, 283H, 283K, 283L, 283P, 283R, 283Y, 284D, 284E, 284L, 284N, 284Q, 284T, 284Y, 285D, 285E, 285K, 285Q, 285W, 285Y, 286E, 286G, 286P, 286Y, 288D, 288E, 288Y, 290D, 290H, 290L, 290N, 290W, 291D, 291E, 291G, 291H, 291I, 291Q, 291T, 292D, 292E, 292T, 292Y, 293F, 293G, 293H, 293I, 293L, 293M, 293N, 293P, 293R, 293S, 293T, 293V, 293W, 293Y, 294F, 294G, 294H, 294I, 294K, 294L, 294M, 294P, 294R, 294S, 294T, 294V, 294W, 294Y, 295D, 295E, 295F, 295G, 295H, 295I, 295M, 295N, 295P, 295R, 295S, 295T, 295V, 295W, 295Y, 296A, 296D, 296E, 296G, 296H, 296I, 296K, 296L, 296M, 296N, 296Q, 296R, 296S, 296T, 296V, 297D, 297E, 297F, 297G, 297H, 297I, 297K, 297L, 297M, 297P, 297Q, 297R, 297S, 297T, 297V, 297W, 297Y, 298A, 298D, 298E, 298F, 298H, 298I, 298K, 298M, 298N, 298Q, 298R, 298T, 298W, 298Y, 299A, 299D, 299E, 299F, 299G, 299H, 299I, 299K, 299L, 299M, 299N, 299P, 299Q, 299R, 299S, 299V, 299W, 299Y, 300A, 300D, 300E, 300G, 300H, 300K, 300M, 300N, 300P, 300Q, 300R, 300S, 300T, 300V, 300W, 301D, 301E, 301H, 301Y, 302I, 303D, 303E, 303Y, 304D, 304H, 304L, 304N, 304T, 305E, 305T, 305Y, 313F, 317E, 317Q, 318H, 318L, 318Q, 318R, 318Y, 320D, 320F, 320G, 320H, 320I, 320L, 320N, 320P, 320S, 320T, 320V, 320W, 320Y, 322D, 322F, 322G, 322H, 322I, 322P, 322S, 322T, 322V, 322W, 322Y, 323I, 324D, 324F, 324G, 324H, 324I, 324L, 324M, 324P, 324R, 324T, 324V, 324W, 324Y, 325A, 325D, 325E, 325F, 325G, 325H, 325I, 325K, 325L, 325M, 325P, 325Q, 325R, 325S, 325T, 325V, 325W, 325Y, 326E, 326I, 326L, 326P, 326T, 327D, 327E, 327F, 327H, 327I, 327K, 327L, 327M, 327N, 327P, 327R, 327S, 327T, 327V, 327W, 327Y, 328A, 328D, 328E, 328F, 328G, 328H, 328I, 328K, 328M, 328N, 328P, 328Q, 328R, 328S, 328T, 328V, 328W, 328Y, 329D, 329E, 329F, 329G, 329H, 329I, 329K, 329L, 329M, 329N, 329Q, 329R, 329S, 329T, 329V, 329W, 329Y, 330E, 330F, 330G, 330H, 330I, 330L, 330M, 330N, 330P, 330R, 330S, 330T, 330V, 330W, 330Y, 331D, 331F, 331H, 331I, 331L, 331M, 331Q, 331R, 331T, 331V, 331W, 331Y, 332A, 332D, 332E, 332F, 332H, 332K, 332L, 332M, 332N, 332P, 332Q, 332R, 332S, 332T, 332V, 332W, 332Y, 333A, 333F, 333H, 333I, 333L, 333M, 333P, 333T, 333Y, 334A, 334F, 334I, 334L, 334P, 334T, 335D, 335F, 335G, 335H, 335I, 335L, 335M, 335N, 335P, 335R, 335S, 335V, 335W, 335Y, 336E, 336K, 336Y, 337E, 337H, and 337N, wherein numbering is according to the EU index.

In further aspects, the amino acid modification can be at a position selected from the group consisting of 221, 222, 223, 224, 225, 227, 228, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 243, 245, 246, 247, 249, 255, 258, 260, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 274, 275, 276, 278, 280, 281, 282, 283, 284, 285, 286, 288, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 313, 317, 318, 320, 322, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, and 337.In additional aspects, the substitution can be selected from the group consisting of 221K, 222Y, 223E, 223K, 224E, 224Y, 225E, 225W, 227E, 227G, 227K, 227Y, 228E, 228G, 228K, 228Y, 230A, 230E, 230G, 230Y, 231E, 231G, 231K, 231P, 231Y, 232E, 232G, 232K, 232Y, 233A, 233F, 233H, 233I, 233K, 233L, 233M, 233N, 233Q, 233R, 233S, 233T, 233V, 233W, 233Y, 234D, 234E, 234F, 234G, 234H, 234I, 234K, 234M, 234N, 234P, 234Q, 234R, 234S, 234T, 234W, 234Y, 235D, 235F, 235G, 235H, 235I, 235K, 235M, 235N, 235Q, 235R, 235S, 235T, 235V, 235W, 235Y, 236D, 236E, 236F, 236H, 236I, 236K, 236L, 236M, 236N, 236P, 236Q, 236R, 236S, 236T, 236V, 236W, 236Y, 237D, 237E, 237F, 237H, 237I, 237K, 237L, 237M, 237N, 237P, 237Q, 237R, 237S, 237T, 237V, 237W, 237Y, 238D, 238E, 238F, 238G, 238H, 238I, 238K, 238L, 238M, 238N, 238Q, 238R, 238S, 238T, 238V, 238W, 238Y, 239D, 239E, 239F, 239G, 239H, 239I, 239K, 239L, 239M, 239N, 239P, 239Q, 239R, 239T, 239V, 239W, 239Y, 240M, 240T, 241D, 241E, 241R, 241S, 241W, 241Y, 243E, 243H, 243Q, 243R, 243W, 243Y, 245A, 246D, 246H, 246Y, 247G, 247V, 249H, 249Q, 249Y, 255E, 255Y, 258H, 258S, 258Y, 260D, 260E, 260H, 260Y, 262A, 262E, 262F, 262I, 262T, 263A, 263I, 263M, 263T, 264D, 264E, 264F, 264G, 264H, 264I, 264K, 264L, 264M, 264N, 264P, 264Q, 264R, 264S, 264T, 264W, 264Y, 265F, 265G, 265H, 265I, 265K, 265L, 265M, 265P, 265Q, 265R, 265S, 265T, 265V, 265W, 265Y, 266A, 266I, 266M, 266T, 267D, 267E, 267F, 267H, 267I, 267K, 267L, 267M, 267N, 267P, 267Q, 267R, 267V, 267W, 267Y, 268F, 268G, 268I, 268M, 268P, 268T, 268V, 268W, 269F, 269G, 269H, 269I, 269L, 269M, 269N, 269P, 269R, 269S, 269T, 269V, 269W, 269Y, 270F, 270G, 270H, 270I, 270L, 270M, 270P, 270Q, 270R, 270S, 270T, 270W, 270Y, 271A, 271D, 271E, 271F, 271G, 271H, 271I, 271K, 271L, 271M, 271N, 271Q, 271R, 271S, 271T, 271V, 271W, 271Y, 272F, 272G, 272H, 272I, 272K, 272L, 272M, 272P, 272R, 272S, 272T, 272V, 272W, 272Y, 274D, 274E, 274F, 274G, 274H, 274I, 274L, 274M, 274P, 274R, 274T, 274V, 274W, 274Y, 275W, 276D, 276E, 276F, 276G, 276H, 276I, 276L, 276M, 276P, 276R, 276S, 276T, 276V, 276W, 278D, 278E, 278G, 278H, 278I, 278K, 278L, 278M, 278N, 278P, 278Q, 278R, 278S, 278T, 278V, 278W, 280G, 280P, 280W, 281E, 281K, 281N, 281P, 281Y, 282G, 282P, 282Y, 283G, 283H, 283K, 283L, 283P, 283R, 283Y, 284L, 284N, 284Q, 284T, 284Y, 285K, 285Q, 285W, 285Y, 286G, 286P, 286Y, 288Y, 290H, 290L, 290W, 291D, 291E, 291G, 291H, 291I, 291Q, 291T, 292D, 292E, 292T, 292Y, 293F, 293G, 293H, 293I, 293L, 293M,

293N, 293P, 293R, 293S, 293T, 293W, 293Y, 294F, 294G, 294H, 294I, 294K, 294L, 294M, 294P, 294R, 294S, 294T, 294V, 294W, 294Y, 295D, 295F, 295G, 295H, 295I, 295M, 295N, 295P, 295R, 295S, 295T, 295V, 295W, 295Y, 296A, 296D, 296E, 296G, 296I, 296K, 296L, 296M, 296N, 296Q, 296R, 296S, 296T, 296V, 297D, 297E, 297F, 297G, 297H, 297I, 297K, 297L, 297M, 297P, 297R, 297S, 297T, 297V, 297W, 297Y, 298E, 298F, 298H, 298I, 298K, 298M, 298Q, 298R, 298W, 298Y, 299A, 299D, 299E, 299F, 299G, 299H, 299I, 299K, 299L, 299M, 299N, 299P, 299Q, 299R, 299S, 299V, 299W, 299Y, 300A, 300D, 300E, 300G, 300H, 300K, 300M, 300N, 300P, 300Q, 300R, 300S, 300T, 300V, 300W, 301D, 301E, 301Y, 302I, 303D, 303E, 303Y, 304H, 304L, 304N, 304T, 305E, 305T, 305Y, 313F, 317E, 317Q, 318H, 318L, 318Q, 318R, 318Y, 320D, 320F, 320G, 320H, 320I, 320L, 320N, 320P, 320S, 320T, 320V, 320W, 320Y, 322D, 322F, 322G, 322H, 322I, 322P, 322S, 322T, 322V, 322W, 322Y, 324D, 324F, 324G, 324H, 324I, 324L, 324M, 324P, 324R, 324T, 324V, 324W, 324Y, 325A, 325D, 325E, 325F, 325G, 325H, 325I, 325K, 325L, 325M, 325P, 325Q, 325R, 325S, 325T, 325V, 325W, 325Y, 326L, 326P, 326T, 327D, 327E, 327F, 327H, 327I, 327K, 327L, 327M, 327P, 327R, 327V, 327W, 327Y, 328A, 328D, 328E, 328F, 328G, 328H, 328K, 328M, 328N, 328P, 328Q, 328R, 328S, 328T, 328V, 328W, 328Y, 329D, 329E, 329F, 329G, 329H, 329I, 329K, 329L, 329M, 329N, 329Q, 329R, 329S, 329T, 329V, 329W, 329Y, 330E, 330F, 330H, 330I, 330L, 330M, 330N, 330P, 330W, 330Y, 331D, 331F, 331H, 331I, 331L, 331M, 331Q, 331R, 331T, 331V, 331W, 331Y, 332A, 332F, 332H, 332L, 332M, 332N, 332P, 332Q, 332S, 332T, 332V, 332W, 332Y, 333F, 333H, 333I, 333L, 333M, 333P, 333T, 333Y, 334F, 334P, 334T, 335D, 335F, 335G, 335H, 335I, 335L, 335M, 335P, 335R, 335S, 335V, 335W, 335Y, 336E, 336K, 336Y, 337H, and 337N.

In further aspect, the modification is at a position selected from the group consisting of 221, 222, 223, 224, 225, 228, 230, 231, 232, 240, 244, 245, 247, 262, 263, 266, 271, 273, 275, 281, 284, 291, 299, 302, 304, 313, 323, 325, 328, 332, 336, wherein the positional numbering is according to the EU index. In additional aspects, the modification is selected from the group consisting of 221K, 221Y, 222E, 222Y, 223E, 223K, 224E, 224Y, 225E, 225K, 225W, 228E, 228G, 228K, 228Y, 230A, 230E, 230G, 230Y, 231E, 231G, 231K, 231P, 231Y, 232E, 232G, 232K, 232Y, 240A, 240I, 240M, 240T, 244H, 245A, 247G, 247V, 262A, 262E, 262F, 262I, 262T, 263A, 263I, 263M, 263T, 266A, 266I, 266M, 266T, 271A, 271D, 271E, 271F, 271G, 271H, 271I, 271K, 271L, 271M, 271N, 271Q, 271R, 271S, 271T, 271V, 271W, 271Y, 273I, 275L, 275W, 281D, 281E, 281K, 281N, 281P, 281Q, 281Y, 284D, 284E, 284L, 284N, 284Q, 284T, 284Y, 291D, 291E, 291G, 291H, 291I, 291Q, 291T, 299A, 299D, 299E, 299F, 299G, 299H, 299I, 299K, 299L, 299M, 299N, 299P, 299Q, 299R, 299S, 299V, 299W, 299Y, 304D,

304H, 304L, 304N, 304T, 313F, 323I, 325A, 325D, 325E, 325F, 325G, 325H, 325I, 325K, 325L, 325M, 325P, 325Q, 325R, 325S, 325T, 325V, 325W, 325Y, 328A, 328D, 328E, 328F, 328G, 328H, 328I, 328K, 328M, 328N, 328P, 328Q, 328R, 328S, 328T, 328V, 328W, 328Y, 332A, 332D, 332E, 332F, 332H, 332K, 332L, 332M, 332N, 332P, 332Q, 332R, 332S, 332T, 332V, 332W, 332Y, 336E, 336K, and 336Y.

The antibody can further include a second amino acid modification at a position selected from the group consisting of 221, 222, 223, 224, 225, 227, 228, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 243, 244, 245, 246, 247, 249, 255, 258, 260, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 278, 280, 281, 282, 283, 284, 285, 286, 288, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 313, 317, 318, 320, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, and 337, wherein numbering is according to the EU index. The second amino acid modification can be a substitution selected from the group consisting of 221K, 221Y, 222E, 222Y, 223E, 223K, 224E, 224Y, 225E, 225K, 225W, 227E, 227G, 227K, 227Y, 228E, 228G, 228K, 228Y, 230A, 230E, 230G, 230Y, 231E, 231G, 231K, 231P, 231Y, 232E, 232G, 232K, 232Y, 233A, 233D, 233F, 233G, 233H, 233I, 233K, 233L, 233M, 233N, 233Q, 233R, 233S, 233T, 233V, 233W, 233Y, 234A, 234D, 234E, 234F, 234G, 234H, 234I, 234K, 234M, 234N, 234P, 234Q, 234R, 234S, 234T, 234V, 234W, 234Y, 235A, 235D, 235E, 235F, 235G, 235H, 235I, 235K, 235M, 235N, 235P, 235Q, 235R, 235S, 235T, 235V, 235W, 235Y, 236A, 236D, 236E, 236F, 236H, 236I, 236K, 236L, 236M, 236N, 236P, 236Q, 236R, 236S, 236T, 236V, 236W, 236Y, 237D, 237E, 237F, 237H, 237I, 237K, 237L, 237M, 237N, 237P, 237Q, 237R, 237S, 237T, 237V, 237W, 237Y, 238D, 238E, 238F, 238G, 238H, 238I, 238K, 238L, 238M, 238N, 238Q, 238R, 238S, 238T, 238V, 238W, 238Y, 239D, 239E, 239F, 239G, 239H, 239I, 239K, 239L, 239M, 239N, 239P, 239Q, 239R, 239T, 239V, 239W, 239Y, 240A, 240I, 240M, 240T, 241D, 241E, 241L, 241R, 241S, 241W, 241Y, 243E, 243H, 243L, 243Q, 243R, 243W, 243Y, 244H, 245A, 246D, 246E, 246H, 246Y, 247G, 247V, 249H, 249Q, 249Y, 255E, 255Y, 258H, 258S, 258Y, 260D, 260E, 260H, 260Y, 262A, 262E, 262F, 262I, 262T, 263A, 263I, 263M, 263T, 264A, 264D, 264E, 264F, 264G, 264H, 264I, 264K, 264L, 264M, 264N, 264P, 264Q, 264R, 264S, 264T, 264W, 264Y, 265F, 265G, 265H, 265I, 265K, 265L, 265M, 265N, 265P, 265Q, 265R, 265S, 265T, 265V, 265W, 265Y, 266A, 266I, 266M, 266T, 267D, 267E, 267F, 267H, 267I, 267K, 267L, 267M, 267N, 267P, 267Q, 267R, 267T, 267V, 267W, 267Y, 268D, 268E, 268F, 268G, 268I, 268K, 268L, 268M, 268P, 268Q, 268R, 268T, 268V, 268W, 269F, 269G, 269H, 269I, 269K, 269L, 269M, 269N, 269P, 269R, 269S, 269T, 269V, 269W, 269Y, 270F, 270G, 270H, 270I, 270L, 270M, 270P, 270Q, 270R, 270S, 270T, 270W, 270Y, 271A, 271D, 271E, 271F,

271G, 271H, 271I, 271K, 271L, 271M, 271N, 271Q, 271R, 271S, 271T, 271V, 271W, 271Y, 272D, 272F, 272G, 272H, 272I, 272K, 272L, 272M, 272P, 272R, 272S, 272T, 272V, 272W, 272Y, 273I, 274D, 274E, 274F, 274G, 274H, 274I, 274L, 274M, 274N, 274P, 274R, 274T, 274V, 274W, 274Y, 275L, 275W, 276D, 276E, 276F, 276G, 276H, 276I, 276L, 276M, 276P, 276R, 276S, 276T, 276V, 276W, 276Y, 278D, 278E, 278G, 278H, 278I, 278K, 278L, 278M, 278N, 278P, 278Q, 278R, 278S, 278T, 278V, 278W, 280G, 280K, 280L, 280P, 280W, 281D, 281E, 281K, 281N, 281P, 281Q, 281Y, 282E, 282G, 282K, 282P, 282Y, 283G, 283H, 283K, 283L, 283P, 283R, 283Y, 284D, 284E, 284L, 284N, 284Q, 284T, 284Y, 285D, 285E, 285K, 285Q, 285W, 285Y, 286E, 286G, 286P, 286Y, 288D, 288E, 288Y, 290D, 290H, 290L, 290N, 290W, 291D, 291E, 291G, 291H, 291I, 291Q, 291T, 292D, 292E, 292T, 292Y, 293F, 293G, 293H, 293I, 293L, 293M, 293N, 293P, 293R, 293S, 293T, 293V, 293W, 293Y, 294F, 294G, 294H, 294I, 294K, 294L, 294M, 294P, 294R, 294S, 294T, 294V, 294W, 294Y, 295D, 295E, 295F, 295G, 295H, 295I, 295M, 295N, 295P, 295R, 295S, 295T, 295V, 295W, 295Y, 296A, 296D, 296E, 296G, 296H, 296I, 296K, 296L, 296M, 296N, 296Q, 296R, 296S, 296T, 296V, 297D, 297E, 297F, 297G, 297H, 297I, 297K, 297L, 297M, 297P, 297Q, 297R, 297S, 297T, 297V, 297W, 297Y, 298A, 298D, 298E, 298F, 298H, 298I, 298K, 298M, 298N, 298Q, 298R, 298T, 298W, 298Y, 299A, 299D, 299E, 299F, 299G, 299H, 299I, 299K, 299L, 299M, 299N, 299P, 299Q, 299R, 299S, 299V, 299W, 299Y, 300A, 300D, 300E, 300G, 300H, 300K, 300M, 300N, 300P, 300Q, 300R, 300S, 300T, 300V, 300W, 301D, 301E, 301H, 301Y, 302I, 303D, 303E, 303Y, 304D, 304H, 304L, 304N, 304T, 305E, 305T, 305Y, 313F, 317E, 317Q, 318H, 318L, 318Q, 318R, 318Y, 320D, 320F, 320G, 320H, 320I, 320L, 320N, 320P, 320S, 320T, 320V, 320W, 320Y, 322D, 322F, 322G, 322H, 322I, 322P, 322S, 322T, 322V, 322W, 322Y, 323I, 324D, 324F, 324G, 324H, 324I, 324L, 324M, 324P, 324R, 324T, 324V, 324W, 324Y, 325A, 325D, 325E, 325F, 325G, 325H, 325I, 325K, 325L, 325M, 325P, 325Q, 325R, 325S, 325T, 325V, 325W, 325Y, 326E, 326I, 326L, 326P, 326T, 327D, 327E, 327F, 327H, 327I, 327K, 327L, 327M, 327N, 327P, 327R, 327S, 327T, 327V, 327W, 327Y, 328A, 328D, 328E, 328F, 328G, 328H, 328I, 328K, 328M, 328N, 328P, 328Q, 328R, 328S, 328T, 328V, 328W, 328Y, 329D, 329E, 329F, 329G, 329H, 329I, 329K, 329L, 329M, 329N, 329Q, 329R, 329S, 329T, 329V, 329W, 329Y, 330E, 330F, 330G, 330H, 330I, 330L, 330M, 330N, 330P, 330R, 330S, 330T, 330V, 330W, 330Y, 331D, 331F, 331H, 331I, 331L, 331M, 331Q, 331R, 331T, 331V, 331W, 331Y, 332A, 332D, 332E, 332F, 332H, 332K, 332L, 332M, 332N, 332P, 332Q, 332R, 332S, 332T, 332V, 332W, 332Y, 333A, 333F, 333H, 333I, 333L, 333M, 333P, 333T, 333Y, 334A, 334F, 334I, 334L, 334P, 334T, 335D, 335F, 335G, 335H, 335I, 335L, 335M, 335N, 335P, 335R, 335S, 335V, 335W, 335Y, 336E, 336K, 336Y, 337E, 337H, and 337N, wherein numbering is according to the EU index.

In further aspects, the amino acid modification is 332E. The second amino acid modification can be selected from the group consisting of: 236A, 239D, 332E, 268D, 268E, 330Y, and 330L. In certain preferred embodiments, the second amino acid modification is 239D.

In other aspects, the modification is a glycoform modification that reduces the level of fucose relative to the parent antibody. In still other aspects, the invention is directed to a composition including plurality of glycosylated antibodies, wherein about 80-100% of the glycosylated antibodies in the composition comprise a mature core carbohydrate structure which lacks fucose.

In a further embodiment, the antibody reduces binding to FcγRIIb as compared to the parent anti-CD19 antibody.

In another aspect, the invention is directed to an antibody that binds CD19 and includes a heavy chain and/or a light chain. The heavy chain has a CDR1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 132 and 138, a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:111-115 and a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:116-118. The light chain has a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 119-128, a CDR2 comprising the amino acid sequence of SEQ ID NOs:129, and a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:130-131.

In further variations, the antibody has a variable heavy chain sequence selected from the group consisting of SEQ ID NOS: 13-16, 20-23, and 27-44, and/or a variable light chain sequence selected from the group consisting of SEQ ID NOS: 17-19, 24-26, and 45-79.

In still further variations, the antibody includes a heavy chain sequence selected from the group consisting of SEQ ID NOS: 86-95, and/or a light chain sequence selected from the group consisting of SEQ ID NOS: 96-110.

In various additional aspects, the invention is directed to a nucleic acid sequence encoding any of the antibodies disclosed herein.

In further aspects, the invention is directed to a method of treating a B-cell related disease by administering an antibody according to claim 1. In certain variations, the disease is selected from non-Hodgkin's lymphomas (NHL), chronic lymphocytic leukemia (CLL), B-cell acute lymphoblastic leukemia/lymphoma (B-ALL), and mantle cell lymphoma (MCL). In certain aspects, the disease is an autoimmune disease, such as rheumatoid arthritis (RA), systemic lupus

erythematosus (SLE or lupus), multiple sclerosis, Sjogren's syndrome, and idiopathic thrombocytopenia purpura (ITP).

In further aspects, the invention is directed to a composition comprising an antibody described herein and an acceptable carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings further illustrate aspects of the invention, and are not meant to constrain the invention to any particular application or theory of operation.

Figure 1: Amino acid sequence of homo sapiens CD19, as obtained from cDNA clone MGC:12802, IMAGE:4054919, GenBank Accession:BC006338.

Figure 2. Sequences of the natural antibody constant regions, including the kappa constant light chain, and the gamma constant heavy chains for IgG1, IgG2, IgG3, and IgG4. Also provided is the sequence of a Hybrid IgG constant chain, and a Hybrid IgG constant chain comprising the subsitutions 239D and I332E.

Figure 3. Alignment of the amino acid sequences of the human IgG immunoglobulins IgG1, IgG2, IgG3, and IgG4. Figure 3a provides the sequences of the CH1 (Cγ1) and hinge domains, and Figure 3b provides the sequences of the CH2 (Cγ2) and CH3 (Cγ3) domains. Positions are numbered according to the EU index of the IgG1 sequence, and differences between IgG1 and the other immunoglobulins IgG2, IgG3, and IgG4 are shown in gray. Allotypic polymorphisms exist at a number of positions, and thus slight differences between the presented sequences and sequences in the prior art may exist. The possible beginnings of the Fc region are labeled, defined herein as either EU position 226 or 230.

Figure 4. The common haplotypes of the gamma chain of human IgG1 (Figure 4a) and IgG2 (Figure 4b) showing the positions and the relevant amino acid substitutions.

Figure 5. Preferred embodiments of receptor binding profiles that include increases to, reductions to, or no effect to the binding to various receptors, where such changes may be beneficial in certain contexts.

Figure 6. Amino acid sequences of the heavy chain and light chain variable regions of the original 4G7 and HD37 antibodies (H0 and L0). Figure 6a provides the sequences of the VH and VL domains, and Figure 6b provides the sequences of the CDRs. CDR boundaries are determined according to the convention of Kabat (VH CDR1: 31-35b, VH CDR2: 50-65, VH CDR3: 95-102, VL CDR1: 24-34, VL CDR2: 50-56, and VL CDR3: 89-97).

Figure 7. The relative binding affinities of 4G7 Hybrid S239D/I332E and 4G7 IgG1

antibody to a panel of Fc receptors.

Figure 8. ADCC of 4G7 Hybrid S239D/I332E, HD37 Hybrid S239D/I332E, 4G7 IgG1, HD37 IgG1, and a negative control antibody on the Daudi cell line (Figure 8a) and ADCC of 4G7 Hybrid S239D/I332E, 4G7 IgG1, rituximab, and a negative control antibody on the SUP-B15 and Raji cell lines (Figure 8b).

Figure 9. A cell-surface binding assay of 4G7 Hybrid S239D/I332E to Raji cells.

Figure 10. Figure 10a shows ADCC assays of 4G7 Hybrid S239D/I332E, 4G7 IgG1, and rituximab on a panel of 14 cell lines representing various lymphomas and leukemias. Both parameters potency (EC50) and efficacy (% ADCC) are normalized to that of rituximab (anti-CD20). Figure 10b lists tested lymphoma and leukemia cell lines.

Figure 11. Heavy chain variable region sequences with reduced immunogenicity for anti-CD19 antibody 4G7.

Figure 12. Light chain variable region sequences with reduced immunogenicity for anti-CD19 antibody 4G7.

Figure 13. Heavy chain variable region sequences with reduced immunogenicity for anti-CD19 antibody HD37.

Figure 14. Light chain variable region sequences with reduced immunogenicity for anti-CD19 antibody HD37.

Figure 15. Results of a cell-surface binding assay of reduced immunogenicity 4G7 variants to Raji cells (Figure 15a) and ADCC of HD37_H2L1 Hybrid S239D/I332E and 4G7_H1L3 Hybrid S239D/I332E on MEC-1 cells (Figure 15b).

Figure 16. Cell-binding affinity on RS4;11 cells of affinity matured 4G7 relative to the H1L1 mAb.

Figure 17. Cell-binding data to RS4;11 cells of 4G7 variants incubated for 5 days at 37°C, pH 9.0 in 200 mM Tris-HCL showing the improvement in stability obtained.

Figure 18. Sequences for heavy chain variants of anti-CD19 that increase affinity and/or stability.

Figure 19. Sequences for light chain variants of anti-CD19 that increase affinity and/or stability.

Figure 20. Anti-proliferative properties of 4G7 Hybrid S239D/I332E on Raji cells.

Figure 21. Anti-proliferative properties of 4G7 stability and affinity improved Hybrid

S239D/I332E on SU-DHL-6 cells with and without cross-linking.

Figure 22. Phagocytosis of Raji and RS4;11 cells with 4G7 stability and affinity improved Hybrid S239D/I332E.

Figure 23. ADCC of 4G7 stability and affinity improved Hybrid S239D/I332E against multiple lymphoma cell lines using purified natural killer (NK) cells.

Figure 24. 4G7 stability and affinity improved Hybrid S239D/I332E binding to 293T cells transfected with human CD19.

Figure 25. Cross-reactivity of 4G7 stability and affinity improved Hybrid S239D/I332E to both cynomolgus and rhesus CD19.

Figure 26. ADCC on RS4;11 and MEC-1 cells using an enhanced effector function anti-CD19 antibody (4G7 H1L1 Hybrid S239D/I332E) with lower fucose content afforded by expression in the Lec13 system.

Figure 27. Single subsitutions made for enhanced stability and/or affinity. Variable region numbering is according to Kabat. An expanded set of positions is included in the CDRs. The canonical CDR boundaries defined by Kabat, as listed in Figure 6, are highlighted in gray.

Figure 28. Anti-CD19 variable region variants constructed to optimize affinity and stability.

Figure 29. Preferred variants and relative increase in binding affinity versus the parent H1L1 mAb.

Figure 30. B cell proliferation assay, showing capacity of variant anti-CD19 antibodies to inhibit viability of primary B cells. Figure 30a shows the dose-dependence of anti-mu antibody on B cell proliferation. Figure 30b shows B cell proliferation in the presence of fixed anti-mu (2 mg/ml) plus varying concentrations of anti-CD19 WT and Fc variant, and anti-CD30 Fc variant control antibodies. Anti-Anti-CD19_IgG1_WT = 4G7_H3_L1 IgG1_WT, Anti-CD19_Hybrid_S239D/I332E = 4G7_H3_L1_Hybrid_239D/332E, and Anti-CD30_S239D/I332E, used here as a negative control, = AC10_H3.69V2_L3.71_Hybrid_239D/332E (as disclosed in USSN 11/686,853, Lazar G.A. et al., filed March 15, 2007).

DETAILED DESCRIPTION OF THE INVENTION

The disclosure is directed to modified anti-CD19 antibodies and methods of using the same. In various aspects, the antibodies can have a having a modified Fc region, specific CDR

sequences, variable region sequences, and/or constant region modifications. In various embodiments, the antibodies are humanized. The disclosure is further directed to methods of using the antibodies in various disease indications, including those of B-cell origin such as B-cell origin non-Hodgkin's lymphoma (NHL), acute lymphoblastic leukemia (ALL), and autoimmune related diseases.

In order that the invention may be more completely understood, several definitions are set forth below. Such definitions are meant to encompass grammatical equivalents.

By "ADCC" or "antibody dependent cell-mediated cytotoxicity" as used herein is meant the cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause lysis of the target cell. In various aspects, the enhanced ADCC effector function can mean enhanced potency or enhanced efficacy. By "potency" as used in the experimental context is meant the concentration of antibody when a particular therapeutic effect is observed EC50 (half maximal effective concentration). By "efficacy" as used in the experimental context is meant the maximal possible effector function at saturating levels of antibody.

By "ADCP" or antibody dependent cell-mediated phagocytosis as used herein is meant the cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause phagocytosis of the target cell.

By "amino acid" and "amino acid identity" as used herein is meant one of the 20 naturally occurring amino acids or any non-natural analogues that may be present at a specific, defined position. Thus "amino acid" as used herein means both naturally occurring and synthetic amino acids. For example, homophenylalanine, citrulline and noreleucine are considered amino acids for the purposes of the invention. "Amino acid" also includes imino acid residues such as proline and hydroxyproline. The side chain may be in either the (R) or the (S) configuration. In the preferred embodiment, the amino acids are in the (S) or L-configuration. If non-naturally occurring side chains are used, non-amino acid substituents may be used, for example to prevent or retard *in vivo* degradation.

By "B cell" or "B lymphocyte" as used herein is meant a type of lymphocyte developed in bone marrow that circulates in the blood and lymph, and provides humoral immunity. B cells recognize free antigen molecules and differentiate or mature into plasma cells that secrete immunoglobulin (antibodies) that inactivate the antigens. Memory cells are also generated that make the specific Immunoglobulin (antibody) on subsequent encounters with such antigen. B cells are also known as "Beta cells" in the islet of Langerhans.

By "B-cell antigen" or "B-cell marker" as used herein is meant any protein that is expressed on B cells.

By "CD19" as used herein is meant the protein of SEQ ID NO:1 (depicted in Figure 1). CD19 is also known as B-cell surface antigen B4, B-cell antigen CD19, CD19 antigen, and Leu-12. Human CD19 is designated GeneID:930 by Entrez Gene, and HGNC:1633 by HGNC. CD19 can be encoded by the gene designated CD19. The use of "CD19" herein is meant to encompass all known or as yet undiscovered alleles and polymorphic forms of CD19.

By "CDC" or "complement dependent cytotoxicity" as used herein is meant the reaction wherein one or more complement protein components recognize bound antibody on a target cell and subsequently cause lysis of the target cell.

By "constant region" as used herein is meant the polypeptide including at least a portion of the first three constant regions of an antibody, having at least one effector function. Thus constant region thus refers to the last three constant region immunoglobulin domains of IgA, IgD, and IgG, and the last four constant region immunoglobulin domains of IgE and IgM, and the flexible hinge N-terminal to these domains. For IgA and IgM, Fc may include the J chain. For IgG, the constant region include immunoglobulin domains Cgamma 1, Cgamma2 and Cgamma3 (Cγ1, Cγ2 and Cγ3) and the hinge between Cgamma1 (Cγ1) and Cgamma2 (Cγ2). Although the boundaries of the constant region may vary, the human IgG heavy chain Fc region is usually defined to comprise residues to its carboxyl-terminus, wherein the numbering is according to the EU index as in Kabat. The constant light chain typically comprises a single domain, and as defined herein refers to positions 108-214 of Cκ or Cλ, wherein numbering is according to the EU index. For full length IgG antibodies, the constant heavy chain, as defined herein, refers to the N-terminus of the CH1 domain to the C-terminus of the CH3 domain, or positions 118-447, wherein numbering is according to the EU index."Constant region" may refer to this region in isolation, or a truncation or fusion include antibodies, Fc fusions, isolated Fcs, and Fc fragments. In various embodiments, the constant region may be the region of the antibody that is encoded by one of the light or heavy chain immunoglobulin constant region genes, i.e. the region of an antibody encoded by the kappa (Cκ) or lambda (Cλ) light chains. In various embodiments, the constant heavy chain or heavy chain constant region can be the the region of an antibody encoded by the mu, delta, gamma, alpha, or epsilon genes to define the antibody's isotype as IgM, IgD, IgG, IgA, or IgE, respectively.

By "effector function" as used herein is meant a biochemical event that results from the interaction of an antibody Fc region with an Fc receptor or ligand. Effector functions include

FcγR-mediated effector functions such as ADCC and ADCP, and complement-mediated effector functions such as CDC. By "effector cell" as used herein is meant a cell of the immune system that expresses one or more Fc receptors and mediates one or more effector functions. Effector cells include but are not limited to monocytes, macrophages, neutrophils, dendritic cells, eosinophils, mast cells, platelets, B cells, large granular lymphocytes, Langerhans' cells, natural killer (NK) cells, and γδ T cells, and may be from any organism including but not limited to humans, mice, rats, rabbits, and monkeys.

By "Fab" or "Fab region" as used herein is meant the polypeptides that comprise the V_H, CH1, V_H, and C_L immunoglobulin domains. Fab may refer to this region in isolation, or this region in the context of a full length antibody or antibody fragment.

By "Fc" or "Fc region", as used herein is meant the polypeptide comprising the constant region of an antibody excluding the first constant region immunoglobulin domain. Thus Fc refers to the last two constant region immunoglobulin domains of IgA, IgD, and IgG, and the last three constant region immunoglobulin domains of IgE and IgM, and the flexible hinge N-terminal to these domains. For IgA and IgM, Fc may include the J chain. For IgG, Fc comprises immunoglobulin domains Cgamma2 and Cgamma3 (Cγ2 and Cγ3) and the hinge between Cgamma1 (Cγ1) and Cgamma2 (Cγ2). Although the boundaries of the Fc region may vary, the human IgG heavy chain Fc region is usually defined to comprise residues C226 or P230 to its carboxyl-terminus, wherein the numbering is according to the EU index as in Kabat. Fc may refer to this region in isolation, or this region in the context of an Fc polypeptide, for example an antibody. By "Fc polypeptide" as used herein is meant a polypeptide that comprises all or part of an Fc region. Fc polypeptides include antibodies, Fc fusions, isolated Fcs, and Fc fragments.

By "Fc gamma receptor" or "FcγR" as used herein is meant any member of the family of proteins that bind the IgG antibody Fc region. In various embodiments, FcγR are substantially encoded by the FcγR genes. In humans this family includes but is not limited to FcγRI (CD64), including isoforms FcγRIa, FcγRIb, and FcγRIc; FcγRII (CD32), including isoforms FcγRIIa (including allotypes H131 and R131), FcγRIIb (including FcγRIIb-1 and FcγRIIb-2), and FcγRIIc; and FcγRIII (CD16), including isoforms FcγRIIIa (including allotypes V158 and F158) and FcγRIIIb (including allotypes FcγRIIIb-NA1 and FcγRIIIb-NA2) (Jefferis *et al.*, 2002, *Immunol Lett* 82:57-65, incorporated entirely by reference), as well as any undiscovered human FcγRs or FcγR isoforms or allotypes. Mouse FcγRs include but are not limited to FcγRI (CD64), FcγRII (CD32), FcγRIII (CD16), and FcγRIII-2 (CD16-2), as well as any undiscovered mouse FcγRs or FcγR isoforms or allotypes. An FcγR may be from any organism, including but not

limited to humans, mice, rats, rabbits, and monkeys.

By "Fc ligand" or "Fc receptor" as used herein is meant a molecule, preferably a polypeptide, from any organism that binds to the Fc region of an antibody to form an Fc-ligand complex. Fc ligands include but are not limited to FcγRs, FcRn, C1q, C3, mannan binding lectin, mannose receptor, *staphylococcal* protein A, *streptococcal* protein G, and viral FcγR. Fc ligands also include Fc receptor homologs (FcRH), which are a family of Fc receptors that are homologous to the FcγRs (Davis *et al.*, 2002, *Immunological Reviews* 190:123-136, incorporated entirely by reference). Fc ligands may include undiscovered molecules that bind Fc.

By "IgG" as used herein is meant a polypeptide belonging to the class of antibodies that are substantially encoded by a recognized immunoglobulin gamma gene. In humans this class comprises IgG1, IgG2, IgG3, and IgG4. In mice this class comprises IgG1, IgG2a, IgG2b, IgG3. By "immunoglobulin (Ig)" herein is meant a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes. Immunoglobulins include but are not limited to antibodies. Immunoglobulins may have a number of structural forms, including but not limited to full length antibodies, antibody fragments, and individual immunoglobulin domains. By "immunoglobulin (Ig) domain" herein is meant a region of an immunoglobulin that exists as a distinct structural entity as ascertained by one skilled in the art of protein structure. Ig domains typically have a characteristic β-sandwich folding topology. The known Ig domains in the IgG class of antibodies are V_H, Cγ1, Cγ2, Cγ3, V_L, and C_L.

By "modification" herein is meant an alteration in the physical, chemical, or sequence properties of a protein, polypeptide, antibody, or immunoglobulin. Preferred modifications of the invention are amino acid modifications and glycoform modifications.

By "amino acid modification" herein is meant an amino acid substitution, insertion, and/or deletion in a polypeptide sequence. By "amino acid substitution" or "substitution" herein is meant the replacement of an amino acid at a particular position in a parent polypeptide sequence with another amino acid. For example, the substitution I332E refers to a variant polypeptide, in this case a constant heavy chain variant, in which the isoleucine at position 332 is replaced with glutamic acid. The WT residue may or may not be designated. For the preceding example, 332E indicates the substitution of position 332 with a glutamic acid. For the purposes herein, multiple substitutions are typically separated by a slash. For example, 239D/332E refers to a double variant comprising the substitutions 239D and 332E. By "amino acid insertion" or "insertion" as used herein is meant the addition of an amino acid at a particular position in a parent polypeptide sequence. For example, insert -236 designates an insertion of glycine at position 236. By "amino

acid deletion" or "deletion" as used herein is meant the removal of an amino acid at a particular position in a parent polypeptide sequence. For example, G236- designates the deletion of glycine at position 236.

By "glycoform modification" or "modified glycoform" or "engineered glycoform" as used herein is meant a carbohydrate composition that is covalently attached to a protein, for example an antibody, wherein said carbohydrate composition differs chemically from that of a parent protein. Modified glycoform typically refers to the different carbohydrate or oligosaccharide; thus for example an antibody may comprise a modified glycoform. Alternatively, modified glycoform may refer to the antibody that comprises the different carbohydrate or oligosaccharide.

By "parent polypeptide", "parent protein", "precursor polypeptide", or "precursor protein" as used herein is meant an unmodified polypeptide that is subsequently modified to generate a variant. Said parent polypeptide may be a naturally occurring polypeptide, or a variant or engineered version of a naturally occurring polypeptide. Parent polypeptide may refer to the polypeptide itself, compositions that comprise the parent polypeptide, or the amino acid sequence that encodes it. Accordingly, by "parent antibody" or "parent immunoglobulin" as used herein is meant an antibody or immunoglobulin that is modified to generate a variant. By "parent anti-CD19 antibody" or "parent anti-CD19 immunoglobulin" as used herein is meant an antibody or immunoglobulin that binds CD19 and is modified to generate a variant.

By "protein" or "polypeptide" as used herein is meant at least two covalently attached amino acids, which includes proteins, polypeptides, oligopeptides and peptides. The protein may be made up of naturally occurring amino acids and peptide bonds, or synthetic peptidomimetic structures, i.e. "analogs", such as peptoids.

By "position" as used herein is meant a location in the sequence of a protein. Positions may be numbered sequentially, or according to an established format, for example the EU index as in Kabat. Corresponding positions are determined as outlined herein, generally through alignment with other parent sequences.

By "residue" as used herein is meant a position in a protein and its associated amino acid identity. For example, Asparagine 297 (also referred to as Asn297 and N297) is a residue at position 297 in the human antibody IgG1.

By "target antigen" or "target" or "antigen" as used herein is meant the molecule that is bound specifically by the variable region of a given antibody. A target antigen may be a protein, carbohydrate, lipid, or other chemical compound. By "target cell" as used herein is meant a cell that expresses a target antigen.

By "variable region" is meant the variable region of an antibody heavy chain or light chain. The heavy chain variable region (VH), as defined herein, refers to the N-terminus to the C-terminus of the VH domain, defined by residues 1-113 according to the numbering convention of Kabat. The light chain variable region (VL), as defined herein, refers to the N-terminus to the C-terminus of the VL domain, defined by residues 1-107 according to the numbering convention of Kabat. Those skilled in the art will recognize that the Kabat variable region numbering convention employs letters to account for the variable length of CDRs. Thus that a VH is defined by Kabat residues 1-113, and that a VL is defined by Kabat 1-107, does not necessarily mean that the VH domain contains exactly 113 residues, nor that VL contains exactly 107 residues. Rather, residues 1-113 of VH and 1-107 of VL according to Kabat are meant to encompass the structural domains that were determined by sequence alignments of a large set of variable length antibody variable regions of varying length ((Kabat *et al.*, 1991, Sequences of Proteins of Immunological Interest, 5th Ed., United States Public Health Service, National Institutes of Health, Bethesda, incorporated entirely by reference). In certain embodiments, the variable region can comprises one or more Ig domains substantially encoded by any of the Vκ, Vλ, and/or V_H genes that make up the kappa, lambda, and heavy chain immunoglobulin genetic loci respectively.

By "variant protein", "protein variant", "variant polypeptide", or "polypeptide variant" as used herein is meant a polypeptide sequence that differs from that of a parent polypeptide sequence by virtue of at least one amino acid modification. Variant polypeptide may refer to the polypeptide itself, a composition comprising the polypeptide, or the amino sequence that encodes it. Preferably, the variant polypeptide has at least one amino acid modification compared to the parent polypeptide, e.g. from about one to about ten amino acid modifications, and preferably from about one to about five amino acid modifications compared to the parent. The variant polypeptide sequence herein will preferably possess at least about 80% homology with a parent polypeptide sequence, and most preferably at least about 90% homology, more preferably at least about 95% homology. Accordingly, by "variant antibody" or "antibody variant" as used herein is meant an antibody sequence that differs from that of a parent antibody sequence by virtue of at least one amino acid modification. Antibody variant may refer to the antibody polypeptide itself, compositions comprising the antibody variant polypeptide, or the amino acid sequence that encodes it. Accordingly, by "variant antibody" or "antibody variant" as used herein is meant an antibody, as defined above, that differs in sequence from that of a parent antibody sequence by virtue of at least one amino acid modification. Variant antibody may refer to the protein itself, compositions comprising the protein, or the amino acid sequence that encodes it. Accordingly, by "constant heavy chain variant" or "constant light chain variant" or "Fc variant" as used herein is

meant a constant heavy chain, constant light chain, or Fc region polypeptide or sequence, respectively, that differs in sequence from that of a parent sequence by virtue of at least one amino acid modification.

By "wild type or WT" herein is meant an amino acid sequence or a nucleotide sequence that is found in nature, including allelic variations. A WT protein, polypeptide, antibody, immunoglobulin, IgG, etc., has an amino acid sequence or a nucleotide sequence that has not been intentionally modified.

For all immunoglobulin heavy chain constant region positions discussed in the present invention, numbering is according to the EU index as in Kabat (Kabat *et al.*, 1991, Sequences of Proteins of Immunological Interest, 5th Ed., United States Public Health Service, National Institutes of Health, Bethesda, incorporated entirely by reference). The "EU index as in Kabat" refers to the residue numbering of the human IgG1 EU antibody, as described in Edelman *et al.*, 1969, Biochemistry 63:78-85, incorporated entirely by reference.

Antibodies

As used herein, the term "antibody" refers to a monomeric or multimeric protein comprising one or more polypeptide chains. An antibody binds specifically to an antigen (e.g. CD19) and may be able to modulate the biological activity of the antigen. As used herein, the term "antibody" can include "full length antibody" and "Fc polypeptide."

By "full length antibody"herein is meant the structure that constitutes the natural biological form of an antibody, including variable and constant regions. For example, in most mammals, including humans and mice, the full length antibody of the IgG class is a tetramer and consists of two identical pairs of two immunoglobulin chains, each pair having one light and one heavy chain, each light chain comprising immunoglobulin domains V_L and C_L, and each heavy chain comprising immunoglobulin domains V_H, CH1 ($C\gamma1$), CH2 ($C\gamma2$), and CH3 ($C\gamma3$). In some mammals, for example in camels and llamas, IgG antibodies may consist of only two heavy chains, each heavy chain comprising a variable domain attached to the Fc region.

The term "antibody" also includes antibody fragments. Specific antibody fragments include, but are not limited to, (i) the Fab fragment consisting of VL, VH, CL and CH1 domains, (ii) the Fd fragment consisting of the VH and CH1 domains, (iii) the Fv fragment consisting of the VL and VH domains of a single antibody; (iv) the dAb fragment (Ward et al., 1989, Nature 341:544-546) which consists of a single variable, (v) isolated CDR regions, (vi) F(ab')2 fragments, a bivalent fragment comprising two linked Fab fragments (vii) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which

allows the two domains to associate to form an antigen binding site (Bird et al., 1988, Science 242:423-426, Huston et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:5879-5883), (viii) bispecific single chain Fv dimers (PCT/US92/09965) and (ix) "diabodies" or "triabodies", multivalent or multispecific fragments constructed by gene fusion (Tomlinson et. al., 2000, Methods Enzymol. 326:461-479; WO94/13804; Holliger et al., 1993, Proc. Natl. Acad. Sci. U.S.A. 90:6444-6448). In certain embodiments, antibodies are produced by recombinant DNA techniques. Other examples of antibody formats and architectures are described in Holliger & Hudson, 2006, Nature Biotechnology 23(9):1126-1136, and Carter 2006, Nature Reviews Immunology 6:343-357 and references cited therein, all expressly incorporated by reference. In additional embodiments, antibodies are produced by enzymatic or chemical cleavage of naturally occurring antibodies.

Natural antibody structural units typically comprise a tetramer. Each tetramer is typically composed of two identical pairs of polypeptide chains, each pair having one "light" (typically having a molecular weight of about 25 kDa) and one "heavy" chain (typically having a molecular weight of about 50-70 kDa). Each of the light and heavy chains are made up of two distinct regions, referred to as the variable and constant regions. For the IgG class of immunoglobulins, the heavy chain is composed of four immunoglobulin domains linked from N- to C-terminus in the order V_H-CH1-CH2-CH3, referring to the heavy chain variable domain, heavy chain constant domain 1, heavy chain constant domain 2, and heavy chain constant domain 3 respectively (also referred to as V_H-Cγ1-Cγ2-Cγ3, referring to the heavy chain variable domain, constant gamma 1 domain, constant gamma 2 domain, and constant gamma 3 domain respectively). The IgG light chain is composed of two immunoglobulin domains linked from N- to C-terminus in the order V_L-C_L, referring to the light chain variable domain and the light chain constant domain respectively. The constant regions show less sequence diversity, and are responsible for binding a number of natural proteins to elicit important biochemical events.

The variable region of an antibody contains the antigen binding determinants of the molecule, and thus determines the specificity of an antibody for its target antigen. The variable region is so named because it is the most distinct in sequence from other antibodies within the same class. In the variable region, three loops are gathered for each of the V domains of the heavy chain and light chain to form an antigen-binding site. Each of the loops is referred to as a complementarity-determining region (hereinafter referred to as a "CDR"), in which the variation in the amino acid sequence is most significant. There are 6 CDRs total, three each per heavy and light chain, designated V_H CDR1, V_H CDR2, V_H CDR3, V_L CDR1, V_L CDR2, and V_L CDR3. The variable region outside of the CDRs is referred to as the framework (FR) region. Although

not as diverse as the CDRs, sequence variability does occur in the FR region between different antibodies. Overall, this characteristic architecture of antibodies provides a stable scaffold (the FR region) upon which substantial antigen binding diversity (the CDRs) can be explored by the immune system to obtain specificity for a broad array of antigens. A number of high-resolution structures are available for a variety of variable region fragments from different organisms, some unbound and some in complex with antigen. Sequence and structural features of antibody variable regions are disclosed, for example, in Morea *et al.*, 1997, *Biophys Chem* 68:9-16; Morea *et al.*, 2000, *Methods* 20:267-279, and the conserved features of antibodies are disclosed, for example, in Maynard *et al.*, 2000, *Annu Rev Biomed Eng* 2:339-376, both incorporated entirely by reference.

Antibodies are grouped into classes, also referred to as isotypes, as determined genetically by the constant region. Human constant light chains are classified as kappa (Cκ) and lambda (Cλ) light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. The IgG class is the most commonly used for therapeutic purposes. In humans this class comprises subclasses IgG1, IgG2, IgG3, and IgG4. In mice this class comprises subclasses IgG1, IgG2a, IgG2b, IgG3. IgM has subclasses, including, but not limited to, IgM1 and IgM2. IgA has several subclasses, including but not limited to IgA1 and IgA2. Thus, "isotype" as used herein is meant any of the classes or subclasses of immunoglobulins defined by the chemical and antigenic characteristics of their constant regions. The known human immunoglobulin isotypes are IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgM1, IgM2, IgD, and IgE. Figure 2 provides the sequences of the human light chain kappa and heavy chain gamma constant chains. Figure 3 shows an alignment of the human IgG constant heavy chains.

Also useful for the invention may be IgGs that are hybrid compositions of the natural human IgG isotypes. Effector functions such as ADCC, ADCP, CDC, and serum half-life differ significantly between the different classes of antibodies, including for example human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, IgG, and IgM (Michaelsen et al., 1992, Molecular Immunology, 29(3): 319-326, entirely incorporated by reference). A number of studies have explored IgG1, IgG2, IgG3, and IgG4 variants in order to investigate the determinants of the effector function differences between them. See for example Canfield & Morrison, 1991, J. Exp. Med. 173: 1483-1491; Chappel et al., 1991, Proc. Natl. Acad. Sci. USA 88(20): 9036-9040; Chappel et al., 1993, Journal of Biological Chemistry 268:25124-25131; Tao et al., 1991, J . Exp. Med. 173: 1025-1028; Tao et al., 1993, J. Exp. Med. 178: 661-667; Redpath et al., 1998, Human Immunology, 59, 720-727, all entirely incorporated by reference.

As described in USSN 11/256,060, filed Oct. 21, 2005, entitled "IgG Immunoglobulin Variants with Optimized Effector Function", herein expressly incorporated by reference, it is possible to engineer amino acid modifications in an antibody that comprise constant regions from other immunoglobulin classes, for example as those illustrated in the alignments in Figure 3. Such engineered hybrid IgG compositions may provide improved effector function properties, including improved ADCC, phagocytosis, CDC, and serum half-life. For example, as illustrated by Figure 3, an IgG1/IgG3 hybrid variant may be constructed by substituting IgG1 positions in the CH2 and CH3 region with the amino acids from IgG3 at positions where the two isotypes differ. Thus a hybrid variant IgG antibody may be constructed that comprises one or more substitutions selected from the group consisting of: 274Q, 276K, 300F, 339T, 356E, 358M, 384S, 392N, 397M, 422I, 435R, and 436F, wherein numbering is according to the EU index. Such variant may provide alternate and/or improved effector function properties.

As another example, relatively poor effector function of IgG2 may be improved by replacing key FcγR binding residues with the corresponding amino acids in an IgG with better effector function. For example, key residue differences between IgG2 and IgG1 with respect to FcγR binding may include P233, V234, A235, -236 (referring to a deletion in IgG2 relative to IgG1), and G327. Thus one or more amino acid modifications in the parent IgG2 wherein one or more of these residues is replaced with the corresponding IgG1 amino acids, P233E, V234L, A235L, -236G (referring to an insertion of a glycine at position 236), and G327A, may provide enhanced effector function. The sequence of such an IgG, comprising a hybrid of residues from IgG1 and IgG2, refered to herein as "Hybrid" in the Examples and Figures, is provided in Figure 2.

As is well known in the art, immunoglobulin polymorphisms exist in the human population. Gm polymorphism is determined by the IGHG1, IGHG2 and IGHG3 genes which have alleles encoding allotypic antigenic determinants referred to as G1m, G2m, and G3m allotypes for markers of the human IgG1, IgG2 and IgG3 molecules (no Gm allotypes have been found on the gamma 4 chain). Markers may be classified into 'allotypes' and 'isoallotypes'. These are distinguished on different serological bases dependent upon the strong sequence homologies between isotypes. Allotypes are antigenic determinants specified by allelic forms of the Ig genes. Allotypes represent slight differences in the amino acid sequences of heavy or light chains of different individuals. Even a single amino acid difference can give rise to an allotypic determinant, although in many cases there are several amino acid substitutions that have occurred. Allotypes are sequence differences between alleles of a subclass whereby the antisera recognize only the allelic differences. An isoallotype is an allele in one isotype which produces

an epitope which is shared with a non-polymorphic homologous region of one or more other isotypes and because of this the antisera will react with both the relevant allotypes and the relevant homologous isotypes (Clark, 1997, IgG effector mechanisms, Chem Immunol. 65:88-110; Gorman & Clark, 1990, Semin Immunol 2(6):457-66, both incorporated entirely by reference).

Allelic forms of human immunoglobulins have been well-characterized (WHO Review of the notation for the allotypic and related markers of human immunoglobulins. J Immunogen 1976, 3: 357-362; WHO Review of the notation for the allotypic and related markers of human immunoglobulins. 1976, Eur. J. Immunol. 6, 599-601; E. van Loghem, 1986, Allotypic markers, Monogr Allergy 19: 40-51, all incorporated entirely by reference). Additionally, other polymorphisms have been characterized (Kim *et al.*, 2001, J. Mol. Evol. 54:1-9, incorporated entirely by reference). At present, 18 Gm allotypes are known: G1m (1, 2, 3, 17) or G1m (a, x, f, z), G2m (23) or G2m (n), G3m (5, 6, 10, 11, 13, 14, 15, 16, 21, 24, 26, 27, 28) or G3m (b1, c3, b5, b0, b3, b4, s, t, g1, c5, u, v, g5) (Lefranc, *et al.*, The human IgG subclasses: molecular analysis of structure, function and regulation. Pergamon, Oxford, pp. 43-78 (1990); Lefranc, G. *et al.*, 1979, Hum. Genet.: 50, 199-211, both incorporated entirely by reference). Allotypes that are inherited in fixed combinations are called Gm haplotypes. Figure 4 shows common haplotypes of the gamma chain of human IgG1 (Figure 4a) and IgG2 (Figure 4b) showing the positions and the relevant amino acid substitutions. Amino acid sequences of these allotypic versions of IgG1 and IgG2 are provided as SEQ IDs: 80-85. The antibodies of the present invention may be substantially encoded by any allotype, isoallotype, or haplotype of any immunoglobulin gene.

Allelic forms of human immunoglobulins have been well-characterized (WHO Review of the notation for the allotypic and related markers of human immunoglobulins. J Immunogen 1976, 3: 357-362; WHO Review of the notation for the allotypic and related markers of human immunoglobulins. 1976, Eur. J. Immunol. 6, 599-601; E. van Loghem, 1986, Allotypic markers, Monogr Allergy 19: 40-51, all incorporated entirely by reference). Additionally, other polymorphisms have been characterized (Kim *et al.*, 2001, J. Mol. Evol. 54:1-9, incorporated entirely by reference). At present, 18 Gm allotypes are known: G1m (1, 2, 3, 17) or G1m (a, x, f, z), G2m (23) or G2m (n), G3m (5, 6, 10, 11, 13, 14, 15, 16, 21, 24, 26, 27, 28) or G3m (b1, c3, b5, b0, b3, b4, s, t, g1, c5, u, v, g5) (Lefranc, *et al.*, The human IgG subclasses: molecular analysis of structure, function and regulation. Pergamon, Oxford, pp. 43-78 (1990); Lefranc, G. *et al.*, 1979, Hum. Genet.: 50, 199-211, both incorporated entirely by reference). Allotypes that are inherited in fixed combinations are called Gm haplotypes. Figure 4 shows common

haplotypes of the gamma chain of human IgG1 (Figure 4a) and IgG2 (Figure 4b) showing the positions and the relevant amino acid substitutions. The antibodies of the present invention may be substantially encoded by any allotype, isoallotype, or haplotype of any immunoglobulin gene.

Antibodies of the present invention may be substantially encoded by genes from any organism, preferably mammals, including but not limited to humans, rodents including but not limited to mice and rats, lagomorpha including but not limited to rabbits and hares, camelidae including but not limited to camels, llamas, and dromedaries, and non-human primates, including but not limited to Prosimians, Platyrrhini (New World monkeys), Cercopithecoidea (Old World monkeys), and Hominoidea including the Gibbons and Lesser and Great Apes. In a most preferred embodiment, the antibodies of the present invention are substantially human. The antibodies of the present invention may be substantially encoded by immunoglobulin genes belonging to any of the antibody classes. In a most preferred embodiment, the antibodies of the present invention comprise sequences belonging to the IgG class of antibodies, including human subclasses IgG1, IgG2, IgG3, and IgG4. In an alternate embodiment, the antibodies of the present invention comprise sequences belonging to the IgA (including human subclasses IgA1 and IgA2), IgD, IgE, IgG, or IgM classes of antibodies. The antibodies of the present invention may comprise more than one protein chain. That is, the present invention may find use in an antibody that is a monomer or an oligomer, including a homo- or hetero-oligomer.

In the most preferred embodiment, the antibodies of the invention are based on human IgG sequences, and thus human IgG sequences are used as the "base" sequences against which other sequences are compared, including but not limited to sequences from other organisms, for example rodent and primate sequences, as well as sequences from other immunoglobulin classes such as IgA, IgE, IgGD, IgGM, and the like. It is contemplated that, although the antibodies of the present invention are engineered in the context of one parent antibody, the variants may be engineered in or "transferred" to the context of another, second parent antibody. This is done by determining the "equivalent" or "corresponding" residues and substitutions between the first and second antibodies, typically based on sequence or structural homology between the sequences of the two antibodies. In order to establish homology, the amino acid sequence of a first antibody outlined herein is directly compared to the sequence of a second antibody. After aligning the sequences, using one or more of the homology alignment programs known in the art (for example using conserved residues as between species), allowing for necessary insertions and deletions in order to maintain alignment (i.e., avoiding the elimination of conserved residues through arbitrary deletion and insertion), the residues equivalent to particular amino acids in the primary sequence of the first antibody are defined. Alignment of conserved residues preferably should

conserve 100% of such residues. However, alignment of greater than 75% or as little as 50% of conserved residues is also adequate to define equivalent residues. Equivalent residues may also be defined by determining structural homology between a first and second antibody that is at the level of tertiary structure for antibodies whose structures have been determined. In this case, equivalent residues are defined as those for which the atomic coordinates of two or more of the main chain atoms of a particular amino acid residue of the parent or precursor (N on N, CA on CA, C on C and O on O) are within 0.13 nm and preferably 0.1 nm after alignment. Alignment is achieved after the best model has been oriented and positioned to give the maximum overlap of atomic coordinates of non-hydrogen protein atoms of the proteins. Regardless of how equivalent or corresponding residues are determined, and regardless of the identity of the parent antibody in which the antibodies are made, what is meant to be conveyed is that the antibodies discovered by the present invention may be engineered into any second parent antibody that has significant sequence or structural homology with said antibody. Thus for example, if a variant antibody is generated wherein the parent antibody is human IgG1, by using the methods described above or other methods for determining equivalent residues, said variant antibody may be engineered in a human IgG2 parent antibody, a human IgA parent antibody, a mouse IgG2a or IgG2b parent antibody, and the like. Again, as described above, the context of the parent antibody does not affect the ability to transfer the antibodies of the present invention to other parent antibodies. For example, the variant antibodies that are engineered in a human IgG1 antibody that targets one antigen epitope may be transferred into a human IgG2 antibody that targets a different antigen epitope, and so forth.

In the IgG class of immunoglobulins, there are several immunoglobulin domains in the heavy chain. By "immunoglobulin (Ig) domain"herein is meant a region of an immunoglobulin having a distinct tertiary structure. Of interest in the present invention are the domains of the constant heavy chain, including, the constant heavy (CH) domains and the hinge. In the context of IgG antibodies, the IgG isotypes each have three CH regions: "CH1" refers to positions 118-220, "CH2" refers to positions 237-340, and "CH3" refers to positions 341-447 according to the EU index as in Kabat. By "hinge" or "hinge region" or "antibody hinge region" or "immunoglobulin hinge region" herein is meant the flexible polypeptide comprising the amino acids between the first and second constant domains of an antibody. Structurally, the IgG CH1 domain ends at EU position 220, and the IgG CH2 domain begins at residue EU position 237. Thus for IgG the hinge is herein defined to include positions 221 (D221 in IgG1) to 236 (G236 in IgG1), wherein the numbering is according to the EU index as in Kabat. In some embodiments, for example in the context of an Fc region, the lower hinge is included, with the "lower hinge"

generally referring to positions 226 or 230. The constant heavy chain, as defined herein, refers to the N-terminus of the CH1 domain to the C-terminus of the CH3 domain, thus comprising positions 118-447, wherein numbering is according to the EU index. The constant light chain comprises a single domain, and as defined herein refers to positions 108-214 of Cκ or Cλ, wherein numbering is according to the EU index.

Antibodies of the invention may include multispecific antibodies, notably bispecific antibodies, also sometimes referred to as "diabodies". These are antibodies that bind to two (or more) different antigens. Diabodies can be manufactured in a variety of ways known in the art, e.g., prepared chemically or from hybrid hybridomas. In one embodiment, the antibody is a minibody. Minibodies are minimized antibody-like proteins comprising a scFv joined to a CH3 domain. In some cases, the scFv can be joined to the Fc region, and may include some or all of the hinge region. For a description of multispecific antibodies see Holliger & Hudson, 2006, Nature Biotechnology 23(9):1126-1136 and references cited therein, all expressly incorporated by reference.

In one embodiment, the antibody of the invention is an antibody fragment. Of particular interest are antibodies that comprise Fc regions, Fc fusions, and the constant region of the heavy chain (CH1-hinge-CH2-CH3). Antibodies of the present invention may comprise Fc fragments. An Fc fragment of the present invention may comprise from 1 - 90% of the Fc region, with 10 - 90% being preferred, and 30 - 90% being most preferred. Thus for example, an Fc fragment of the present invention may comprise an IgG1 Cγ2 domain, an IgG1 Cγ2 domain and hinge region, an IgG1 Cγ3 domain, and so forth. In one embodiment, an Fc fragment of the present invention additionally comprises a fusion partner, effectively making it an Fc fragment fusion. Fc fragments may or may not contian extra polypeptide sequence.

Chimeric, humanized, and fully human antibodies

Immunogenicity is the result of a complex series of responses to a substance that is perceived as foreign, and may include production of neutralizing and non-neutralizing antibodies, formation of immune complexes, complement activation, mast cell activation, inflammation, hypersensitivity responses, and anaphylaxis. Several factors can contribute to protein immunogenicity, including but not limited to protein sequence, route and frequency of administration, and patient population. Immunogenicity may limit the efficacy and safety of a protein therapeutic in multiple ways. Efficacy can be reduced directly by the formation of neutralizing antibodies. Efficacy may also be reduced indirectly, as binding to either neutralizing or non-neutralizing antibodies typically leads to rapid clearance from serum. Severe side effects

and even death may occur when an immune reaction is raised. Thus in a preferred embodiment, protein engineering is used to reduce the immunogenicity of the antibodies of the present invention.

In some embodiments, the scaffold components can be a mixture from different species. Such antibody may be a chimeric antibody and/or a humanized antibody. In general, both "chimeric antibodies" and "humanized antibodies" refer to antibodies that combine regions from more than one species. "Chimeric antibodies" traditionally comprise variable region(s) from a mouse (or rat, in some cases) and the constant region(s) from a human (Morrison *et al.*, 1984, *Proc Natl Acad Sci USA* 81: 6851-6855, incorporated entirely by reference).

By "humanized" antibody as used herein is meant an antibody comprising a human framework region (FR) and one or more complementarity determining regions (CDR's) from a non-human (usually mouse or rat) antibody. The non-human antibody providing the CDR's is called the "donor" and the human immunoglobulin providing the framework is called the "acceptor". In certain embodiments, humanization relies principally on the grafting of donor CDRs onto acceptor (human) VL and VH frameworks (Winter US 5225539, incorporated entirely by reference). This strategy is referred to as "CDR grafting". "Backmutation" of selected acceptor framework residues to the corresponding donor residues is often required to regain affinity that is lost in the initial grafted construct (US 5693762, incorporated entirely by reference). The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region, typically that of a human immunoglobulin, and thus will typically comprise a human Fc region. A variety of techniques and methods for humanizing and reshaping non-human antibodies are well known in the art (See Tsurushita & Vasquez, 2004, Humanization of Monoclonal Antibodies, Molecular Biology of B Cells, 533-545, Elsevier Science (USA), and references cited therein, all incorporated entirely by reference). Humanization or other methods of reducing the immunogenicity of nonhuman antibody variable regions may include resurfacing methods, as described for example in Roguska *et al.*, 1994, Proc. Natl. Acad. Sci. USA 91:969-973, incorporated entirely by reference. In one embodiment, selection based methods may be employed to humanize and/or affinity mature antibody variable regions, that is, to increase the affinity of the variable region for its target antigen. Other humanization methods may involve the grafting of only parts of the CDRs, including but not limited to methods described in USSN 09/810,502; Tan *et al.*, 2002, J. Immunol. 169:1119-1125; De Pascalis *et al.*, 2002, J. Immunol. 169:3076-3084, incorporated entirely by reference. Structure-based methods may be employed for humanization and affinity maturation, for example as described in USSN 10/153,159 and related applications, all incorporated entirely by

reference.

In certain variations, the immunogenicity of the antibody is reduced using a method described in USSN 11/004,590, entitled "Methods of Generating Variant Proteins with Increased Host String Content and Compositions Thereof", filed on December 3, 2004, incorporated entirely by reference.

Modifications to reduce immunogenicity may include modifications that reduce binding of processed peptides derived from the parent sequence to MHC proteins. For example, amino acid modifications would be engineered such that there are no or a minimal number of immune epitopes that are predicted to bind, with high affinity, to any prevalent MHC alleles. Several methods of identifying MHC-binding epitopes in protein sequences are known in the art and may be used to score epitopes in an antibody of the present invention. See for example USSN 09/903,378, USSN 10/754,296, USSN 11/249,692, and references cited therein, all expressly incorporated by reference.

In an alternate embodiment, the antibodies of the present invention may be fully human, that is the sequences of the antibodies are completely or substantially human. "Fully human antibody " or "complete human antibody" refers to a human antibody having the gene sequence of an antibody derived from a human chromosome with the modifications outlined herein. A number of methods are known in the art for generating fully human antibodies, including the use of transgenic mice (Bruggemann *et al.*, 1997, *Curr Opin Biotechnol* 8:455-458,) or human antibody libraries coupled with selection methods (Griffiths *et al.*, 1998, *Curr Opin Biotechnol* 9:102-108,) both incorporated entirely by reference.

Antibodies that target CD19

The antibodies of the present invention may be virtually any antibody that binds to CD19. The variable regions of any known or undiscovered anti-CD19 antibodies may find use in the present invention. Antibodies of the invention may display selectivity for CD19 versus alternative targets, or selectivity for a specific form of the target versus alternative forms. Examples include full-length versus splice variants, cell-surface vs. soluble forms, selectivity for various polymorphic variants, or selectivity for specific conformational forms of a target. An antibody of the present invention may bind any epitope or region on CD19, and may be specific for fragments, mutant forms, splice forms, or aberrent forms of said antigens. A number of useful antibodies have been discovered that target CD19 that may find use in the present invention. Suitable antibodies or immunoadhesins include the CD19 antibodies or immunoadhesins in MT-103 (a single-chain bispecific CD19/CD3 antibody; Hoffman, P. et al. 2005. Int. J. Cancer. 115:

98-104; Schlereth, B. et al. 2006. Cancer Immunol. Immunother. 55: 503-514), a CD19/CD16 diabody (Schlenzka, J. et al. 2004. Anti-cancer Drugs. 15: 915-919; Kipriyanov, S.M. et al. 2002. J. Immunol. 169: 137-144), BU12-saporin (Flavell, D.J. et al. 1995. Br. J. Cancer. 72: 1373-1379), and anti-CD19-idarubicin (Rowland, A.J. et al. 1993. Cancer Immunol. Immunother. 55: 503-514); Olson, US Pub. No. 2004/0136908A1, filed Mar. 4, 2004; US 5,686,072; Olson, WO 02/080987A1, filed Mar. 29, 2002; Tedder, WO 06/133450A1, filed Jun. 8, 2006; Tedder, WO 06/121852A2, filed Apr. 5, 2006; Tedder, WO 06/089133A2, filed Feb. 15, 2006; Tedderm US Pub. No. 2006/280738A1, filed Jun. 8, 2006; US 7,109,304; Hansen, US Pub. No. 2005/070693A1, filed Aug. 2, 2004; Hansen, US Pub. No. 2006/257398A1, filed Jun. 1, 2006; Hansen, WO 05/012493A2, filed Aug. 2, 2004; Rao-Naik, WO 07/002223A2, filed Jun. 20, 2006; Page, US Pub. 2002/182208A1, filed May 16, 2002; US 5,686,072; Page, EP00481790B1, filed Oct. 17, 1991; Hariharan, US Pub. No. 2003/103971A1, filed Sep. 12, 2002; Goldenberg, US Pub. No. 2003/133930A1, filed Jan. 24, 2003; Goldenberg, US Pub. No. 2004/219156A1, filed Dec. 30, 2002; Hariharan, US Pub. No. 2007/0009519A1, filed Jul. 21, 2006; Curd, WO 00/067796A1, filed May 4, 2000; Kipriyanov, WO 03/088998A1, filed Apr. 15, 2003; US 7,112,324, US 7,129,330, Olson, US Pub. No. 2004/0136908A1, filed Mar. 4, 2004; Dorken, US Pub. No. 2006/0193852A1, filed May 5, 2006; Amphlett, US Pub. No. 2007/0009541A1, filed Sep. 14, 2006; Kersey, WO 96/36360A1, filed May 15, 1996; Kufer, WO 04/106381A1, filed May 26, 2004; Little, US Pub. No. 2007/031436A1, filed Oct. 10, 2006; Kufer, US Pub. No. 2007/123479A1, filed May 26, 2004; Baeuerle, WO 07/068354A1, filed Nov. 29, 2006; Le Gall, EP 01314741B1, filed Nov. 14, 2001; Pesando, WO 91/13974A1, filed Mar. 12, 1991; Allen et al, Clin. Cancer. Res 2005;11(9) May 1, 2005; Barbin et al, J. Immunother, Vol. 29, No. 2, March/April 2006; Bruenke et al, Brit. J. Haem, 130, 218-2228 (2005); Callard, J. Immunol. Vol. 148, 2983-2987, No. 10, May 15, 1992; Carter et al, Immunol. Res. 2002; 26/1-3:45-54; Carter & Barrington, Curr. Dir. Autoimmun. Basel, Karger, 2004, vol 7, pp 4-32; WWWK Cheng et al, Biochim. Biophys. Acta 1768 (2007) 21-29; Cochlovius, Cancer Res. 60, 4336-4341, Aug. 15, 2000; LJN Cooper et al, Blood Cells, Molecules & Diseases, 33 (2004) 83-89; LJN Cooper et al, Blood, 15 Feb. 2005, Vol. 105, No. 4, pp 1622-1631; Culton et al, J. Clin. Immunol., Vol. 27, No. 1, Jan. 2007; Daniel et al, Blood, Vol. 92, No. 12 (Dec. 15), 1998: pp 4750-4757; Doody et al, Curr. Opin. Immun., 1996, pp 378-382; Dreier et al, Int. J. Cancer, 100, 690-697 (2002); Dreier et al, J. Immunol., 2003, pp. 4397-4402; Fearon & Carter, Annu. Rev. Immunol. 1995. 13:127-149; Fearon & Carroll, Annu. Rev. Immunol. 2000. 18:393-422; Fujimoto & Sato, J. Dermatol. Sci. (2007) in press; Le Gall et al, Prot. Engr, Des. & Select., vol. 17, no. 4, pp.357-366, 2004; Ghetie et al, Blood, 1 Jul 2004, Vol. 104, No. 1, pp.178-183; Ghetie et al, Blood, Vol. 83, No. 5 (Mar 1), 1994: pp 1329-1336; Ghetie et al, Clin. Cancer Res., Vol. 5, 3920-2927, Dec.

1999; Ginaldi et al, J. Clin. Pathol, 1998; 51:364-369; Grossbard et al, Clin. Cancer Res., Vol. 5, 2392-2398, Sept. 1999; Grossbard et al, Brit. J. Haematol., 1998, 102, 509-515; Grossbard et al, Blood, Vol. 80, No. 4 (Aug 15), 1992: pp 863-878; Grossbard & Fidias, Clin. Immunol. & Immunolpath., Vol. 76, No. 2, Aug., pp. 107-114, 1995; M. Green, Cancer Immunol. Immunother. (2004) 53: 625-632; Harata et al, Blood, 1 Sept. 2004, Vol. 104, No. 5, pp1442-1449; Hekman et al, Cancer Immunol. Immunother. (1991) 32: 364-372; Hoffmann et al, Int. J. Cancer: 115, 98-104 (2005); Kipriyanov et al, J. Immunol. (2002), pp. 138-144; Kipriyanov et al, Int. J. Cancer: 77, 763-772 (1998); Kipriyanov et al, J. Immunol. Meth 196 (1996) 51-62; Lang et al, Blood, 15 May 2004, Vol. 103, No. 10, pp 3982-3985; Lankester et al, J. Biol. Chem., Vol, 271, No. 37, Sept. 13, pp. 22326-22330, 1996; Loeffler et al, Blood, 15 Mar 2000, Vol. 95, No. 6, pp 2098-2103; Masir, et al Histopathol., 2006, 48, pps. 239-246; Bargou et al, MT103 (MEDI-538) Poster; Mitchell et al, J. Nucl. Med. 2003; 44:1106-1112; Molhoj et al, Molec. Immunol., 44 (2007) 1935-1943; Pietersz et al, Cancer Immunol. Immunother (1995) 41: 53-60; Sapra et al, Clin. Cancer Res. Vol. 10, 1100-1111, Feb. 1, 2004; Schlereth et al, Cancer Immunol. Immunother. (2006) 55: 503-514; Schwemmlein et al, Leukemia (2007) 21, 1405-1412; Sieber et al, Brit. J. Haematology, 2003, 121, 458-461; Stone et al, Blood, Vol, 88, No. 4 (Aug 15), 1996: 1188-1197; Sun et al, Molec. Immunolog. 41 (2004) 929-938; Tedder & Isaacs, J. Immunolog. Vol. 143, 712-717, No. 2 Jul 15, 1989; Tedder et al, Curr. Dir. Autoimmun. Basel, Karger, 2005, vol 8, pp 55-90; Tedder et al, Springer Semin. Immun. (2006) 28: 351-364; Tiroch et al, J. Immunol., 2002, 168: 3275-3282; Uckun et al, Blood, Vol 71, No 1 (Jan), 1988: pp 13-29; Uckun et al, J. Immunol., Vol 134, No 3, Mar 1985, pp 2010-2016; Vallera et al, Clin. Cancer Res. 2005; 11(10) May 15, 2005; Vlasveld et al, Cancer Immunol. Immunother (1995) 40: 37-47; Vuist et al, Cancer Res, 49, 3783-3788, July 15, 1989; Vuis et al, Cancer Res, 50, 5767-5772, Sept. 15, 1990; Yan et al, Int. Immunol. Vol 17, No. 7, pp 869-877 (2005); Yazawa, et la, PNAS 2005; 102; 15178-15183, all hereby incorporated entirely by reference. The molecules described in US 5,686,072, WO 02/080987A1 and US Pub. No. 2004/0136908A1 and identified as 4G7, the molecules described in WO 1007/002223A2 and Tedder, are preferred.

The antibodies of the present invention may find use in a wide range of products. In one embodiment the antibody of the invention is a therapeutic, a diagnostic, or a research reagent, preferably a therapeutic. Alternatively, the antibody of the present invention may be used for agricultural or industrial uses. An antibody of the present invention may find use in an antibody composition that is monoclonal or polyclonal. The antibodies of the present invention may be agonists, antagonists, neutralizing, inhibitory, or stimulatory. In a preferred embodiment, the antibodies of the present invention are used to kill target cells that bear the target antigen, for

example cancer cells. In an alternate embodiment, the antibodies of the present invention are used to block, antagonize, or agonize the target antigen. In an alternately preferred embodiment, the antibodies of the present invention are used to block, antagonize, or agonize the target antigen and kill the target cells that bear the target antigen.

Anti-CD19 antibodies as therapeutics to treat B-cell disorders

Antibodies are a class of therapeutic proteins that may be used to treat B-cell disorders. A number of favorable properties of antibodies, including but not limited to specificity for target, ability to mediate immune effector mechanisms, and long half-life in serum, make antibodies powerful therapeutics. The present invention describes antibodies against the B-cell antigen CD19.

B-cell antigen CD19 (CD19, also known as B-cell surface antigen B4, Leu-12) is a human pan-B-cell surface marker that is expressed from early stages of pre-B cell development through terminal differentiation into plasma cells. CD19 promotes the proliferation and survival of mature B cells. It associates in a complex with CD21 on the cell surface. It also associates with CD81 and Leu-13 and potentiates B cell receptor (BCR) signaling. Together with the BCR, CD19 modulates intrinsic and antigen receptor-induced signaling thresholds critical for clonal expansion of B cells and humoral immunity. In collaboration with CD21 it links the adaptive and the innate immune system. Upon activation, the cytoplasmic tail of CD19 becomes phosphorylated which leads to binding by Src-family kinases and recruitment of PI-3 kinase. It is an attractive immunotherapy target for cancers of lymphoid origin since it is also expressed on the vast majority of NHL cells as well as some leukemias.

A number of antibodies or antibody conjugates that target CD19 have been evaluated in pre-clinical studies or in clinical trials for the treatment of cancers. These anti-CD19 antibodies or antibody conjugates include but are not limited to MT-103 (a single-chain bispecific CD19/CD3 antibody; Hoffman et al, 2005 Int J Cancer 115:98-104; Schlereth et al, 2006 Cancer Immunol Immunother 55:503-514), a CD19/CD16 diabody (Schlenzka et al, 2004 Anti-cancer Drugs 15:915-919; Kipriyanov et al, 2002 J Immunol 169:137-144), BU12-saporin (Flavell et al, 1995 Br J Cancer 72:1373-1379), and anti-CD19-idarubicin (Rowland et al, 1993 Cancer Immunol Immunother 55:503-514); all expressly incorporated by reference.

Fc optimization of anti-CD19 antibodies

There are a number of characterized mechanisms by which antibodies mediate cellular effects, including anti-proliferation via blockage of needed growth pathways, intracellular signaling leading to apoptosis, enhanced down regulation and/or turnover of receptors,

complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cell-mediated phagocytosis (ADCP) and promotion of an adaptive immune response (Cragg *et al.*, 1999, *Curr Opin Immunol* 11:541-547; Glennie *et al.*, 2000, *Immunol Today* 21:403-410, both incorporated entirely by reference). Antibody efficacy may be due to a combination of these mechanisms, and their relative importance in clinical therapy for oncology appears to be cancer dependent.

The importance of FcγR-mediated effector functions for the activity of some antibodies has been demonstrated in mice (Clynes *et al.*, 1998, *Proc Natl Acad Sci U S A* 95:652-656; Clynes *et al.*, 2000, *Nat Med* 6:443-446, both incorporated entirely by reference), and from observed correlations between clinical efficacy in humans and their allotype of high (V158) or low (F158) affinity polymorphic forms of FcγRIIIa (Cartron *et al.*, 2002, Blood 99:754-758; Weng & Levy, 2003, Journal of Clinical Oncology, 21:3940-3947, both incorporated entirely by reference). Together these data suggest that an antibody that is optimized for binding to certain FcγRs may better mediate effector functions, and thereby destroy target cells more effectively in patients. Thus a promising means for enhancing the anti-tumor potency of antibodies is via enhancement of their ability to mediate cytotoxic effector functions such as ADCC, ADCP, and CDC. Additionally, antibodies can mediate anti-tumor mechanism via growth inhibitory or apoptotic signaling that may occur when an antibody binds to its target on tumor cells. Such signaling may be potentiated when antibodies are presented to tumor cells bound to immune cells via FcγR. Therefore increased affinity of antibodies to FcγRs may result in enhanced anti-proliferative effects.

Antibody engineering for optimized effector function has been achieved using amino acid modifcations (see for example USSN 10/672,280 and USSN 11/124,620 and references cited therein, all incorporated entirely by reference), and engineered glycoforms (see for example Umaña et al., 1999, Nat Biotechnol 17:176-180; Shinkawa et al., 2003, J Biol Chem 278:3466-3473, Yamane-Ohnuki et al., 2004, Biotechnology and Bioengineering 87(5):614-621, all incorporated entirely by reference).

Modifications for optimizing effector function

The present invention is directed to antibodies comprising modifications, wherein said modifications alter affinity to one or more Fc receptors, and/or alter the ability of the antibody to mediate one or more effector functions. Modifications of the invention include amino acid modifications and glycoform modifications.

Amino acid modifications

As described in USSN 11/124,620, filed May 5, 2005, entitled "Optimized Fc Variants", and incorporated entirely by reference, amino acid modifications at heavy chain constant region positions positions 221, 222, 223, 224, 225, 227, 228, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 243, 244, 245, 246, 247, 249, 255, 258, 260, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 278, 280, 281, 282, 283, 284, 285, 286, 288, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 313, 317, 318, 320, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, and 337, allow modification of FcγR binding properties, effector function, and potentially clinical properties of antibodies.

In particular, variants that alter binding to one or more human Fc receptors may comprise an amino acid modification in the heavy chain constant region, as described herein, selected from the group consisting of 221K, 221Y, 222E, 222Y, 223E, 223K, 224E, 224Y, 225E, 225K, 225W, 227E, 227G, 227K, 227Y, 228E, 228G, 228K, 228Y, 230A, 230E, 230G, 230Y, 231E, 231G, 231K, 231P, 231Y, 232E, 232G, 232K, 232Y, 233A, 233D, 233F, 233G, 233H, 233I, 233K, 233L, 233M, 233N, 233Q, 233R, 233S, 233T, 233V, 233W, 233Y, 234A, 234D, 234E, 234F, 234G, 234H, 234I, 234K, 234M, 234N, 234P, 234Q, 234R, 234S, 234T, 234V, 234W, 234Y, 235A, 235D, 235E, 235F, 235G, 235H, 235I, 235K, 235M, 235N, 235P, 235Q, 235R, 235S, 235T, 235V, 235W, 235Y, 236A, 236D, 236E, 236F, 236H, 236I, 236K, 236L, 236M, 236N, 236P, 236Q, 236R, 236S, 236T, 236V, 236W, 236Y, 237D, 237E, 237F, 237H, 237I, 237K, 237L, 237M, 237N, 237P, 237Q, 237R, 237S, 237T, 237V, 237W, 237Y, 238D, 238E, 238F, 238G, 238H, 238I, 238K, 238L, 238M, 238N, 238Q, 238R, 238S, 238T, 238V, 238W, 238Y, 239D, 239E, 239F, 239G, 239H, 239I, 239K, 239L, 239M, 239N, 239P, 239Q, 239R, 239T, 239V, 239W, 239Y, 240A, 240I, 240M, 240T, 241D, 241E, 241L, 241R, 241S, 241W, 241Y, 243E, 243H, 243L, 243Q, 243R, 243W, 243Y, 244H, 245A, 246D, 246E, 246H, 246Y, 247G, 247V, 249H, 249Q, 249Y, 255E, 255Y, 258H, 258S, 258Y, 260D, 260E, 260H, 260Y, 262A, 262E, 262F, 262I, 262T, 263A, 263I, 263M, 263T, 264A, 264D, 264E, 264F, 264G, 264H, 264I, 264K, 264L, 264M, 264N, 264P, 264Q, 264R, 264S, 264T, 264W, 264Y, 265F, 265G, 265H, 265I, 265K, 265L, 265M, 265N, 265P, 265Q, 265R, 265S, 265T, 265V, 265W, 265Y, 266A, 266I, 266M, 266T, 267D, 267E, 267F, 267H, 267I, 267K, 267L, 267M, 267N, 267P, 267Q, 267R, 267T, 267V, 267W, 267Y, 268D, 268E, 268F, 268G, 268I, 268K, 268L, 268M, 268P, 268Q, 268R, 268T, 268V, 268W, 269F, 269G, 269H, 269I, 269K, 269L, 269M, 269N, 269P, 269R, 269S, 269T, 269V, 269W, 269Y, 270F, 270G, 270H, 270I, 270L, 270M, 270P, 270Q, 270R, 270S, 270T, 270W, 270Y, 271A, 271D, 271E, 271F, 271G, 271H, 271I, 271K, 271L,

271M, 271N, 271Q, 271R, 271S, 271T, 271V, 271W, 271Y, 272D, 272F, 272G, 272H, 272I, 272K, 272L, 272M, 272P, 272R, 272S, 272T, 272V, 272W, 272Y, 273I, 274D, 274E, 274F, 274G, 274H, 274I, 274L, 274M, 274N, 274P, 274R, 274T, 274V, 274W, 274Y, 275L, 275W, 276D, 276E, 276F, 276G, 276H, 276I, 276L, 276M, 276P, 276R, 276S, 276T, 276V, 276W, 276Y, 278D, 278E, 278G, 278H, 278I, 278K, 278L, 278M, 278N, 278P, 278Q, 278R, 278S, 278T, 278V, 278W, 280G, 280K, 280L, 280P, 280W, 281D, 281E, 281K, 281N, 281P, 281Q, 281Y, 282E, 282G, 282K, 282P, 282Y, 283G, 283H, 283K, 283L, 283P, 283R, 283Y, 284D, 284E, 284L, 284N, 284Q, 284T, 284Y, 285D, 285E, 285K, 285Q, 285W, 285Y, 286E, 286G, 286P, 286Y, 288D, 288E, 288Y, 290D, 290H, 290L, 290N, 290W, 291D, 291E, 291G, 291H, 291I, 291Q, 291T, 292D, 292E, 292T, 292Y, 293F, 293G, 293H, 293I, 293L, 293M, 293N, 293P, 293R, 293S, 293T, 293V, 293W, 293Y, 294F, 294G, 294H, 294I, 294K, 294L, 294M, 294P, 294R, 294S, 294T, 294V, 294W, 294Y, 295D, 295E, 295F, 295G, 295H, 295I, 295M, 295N, 295P, 295R, 295S, 295T, 295V, 295W, 295Y, 296A, 296D, 296E, 296G, 296H, 296I, 296K, 296L, 296M, 296N, 296Q, 296R, 296S, 296T, 296V, 297D, 297E, 297F, 297G, 297H, 297I, 297K, 297L, 297M, 297P, 297Q, 297R, 297S, 297T, 297V, 297W, 297Y, 298A, 298D, 298E, 298F, 298H, 298I, 298K, 298M, 298N, 298Q, 298R, 298T, 298W, 298Y, 299A, 299D, 299E, 299F, 299G, 299H, 299I, 299K, 299L, 299M, 299N, 299P, 299Q, 299R, 299S, 299V, 299W, 299Y, 300A, 300D, 300E, 300G, 300H, 300K, 300M, 300N, 300P, 300Q, 300R, 300S, 300T, 300V, 300W, 301D, 301E, 301H, 301Y, 302I, 303D, 303E, 303Y, 304D, 304H, 304L, 304N, 304T, 305E, 305T, 305Y, 313F, 317E, 317Q, 318H, 318L, 318Q, 318R, 318Y, 320D, 320F, 320G, 320H, 320I, 320L, 320N, 320P, 320S, 320T, 320V, 320W, 320Y, 322D, 322F, 322G, 322H, 322I, 322P, 322S, 322T, 322V, 322W, 322Y, 323I, 324D, 324F, 324G, 324H, 324I, 324L, 324M, 324P, 324R, 324T, 324V, 324W, 324Y, 325A, 325D, 325E, 325F, 325G, 325H, 325I, 325K, 325L, 325M, 325P, 325Q, 325R, 325S, 325T, 325V, 325W, 325Y, 326E, 326I, 326L, 326P, 326T, 327D, 327E, 327F, 327H, 327I, 327K, 327L, 327M, 327N, 327P, 327R, 327S, 327T, 327V, 327W, 327Y, 328A, 328D, 328E, 328F, 328G, 328H, 328I, 328K, 328M, 328N, 328P, 328Q, 328R, 328S, 328T, 328V, 328W, 328Y, 329D, 329E, 329F, 329G, 329H, 329I, 329K, 329L, 329M, 329N, 329Q, 329R, 329S, 329T, 329V, 329W, 329Y, 330E, 330F, 330G, 330H, 330I, 330L, 330M, 330N, 330P, 330R, 330S, 330T, 330V, 330W, 330Y, 331D, 331F, 331H, 331I, 331L, 331M, 331Q, 331R, 331T, 331V, 331W, 331Y, 332A, 332D, 332E, 332F, 332H, 332K, 332L, 332M, 332N, 332P, 332Q, 332R, 332S, 332T, 332V, 332W, 332Y, 333A, 333F, 333H, 333I, 333L, 333M, 333P, 333T, 333Y, 334A, 334F, 334I, 334L, 334P, 334T, 335D, 335F, 335G, 335H, 335I, 335L, 335M, 335N, 335P, 335R, 335S, 335V, 335W, 335Y, 336E, 336K, 336Y, 337E, 337H, and 337N, wherein numbering is according to the EU index.

As described in USSN 11/090,981, filed March 24, 2005, entitled "Immunoglobulin variants outside the Fc region", and incorporated entirely by reference, amino acid modifications at heavy chain constant region positions 118, 119, 120, 121, 122, 124, 126, 129, 131, 132, 133, 135, 136, 137, 138, 139, 147, 148, 150, 151, 152, 153, 155, 157, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 183, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 201, 203, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 216, 217, 218, 219, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, and 236, allow modification of FcγR binding properties, effector function, and potentially clinical properties of antibodies.

As described in USSN 11/090,981, filed March 24, 2005, entitled "Immunoglobulin variants outside the Fc region", and incorporated entirely by reference, amino acid modifications at light chain constant region positions 108, 109, 110, 111, 112, 114, 116, 121, 122, 123, 124, 125, 126, 127, 128, 129, 131, 137, 138, 140, 141, 142, 143, 145, 147, 149, 150, 151, 152, 153, 154, 155, 156, 157, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 176, 180, 181, 182, 183, 184, 185, 187, 188, 189, 190, 191, 193, 195, 197, 199, 200, 202, 203, 204, 205, 206, 207, 208, 210, 211, 212, 213, allow modification of FcγR binding properties, effector function, and potentially clinical properties of antibodies.

In particular, variants that alter binding to one or more human Fc receptors may comprise an amino acid modification in the heavy chain constant region, as described herein, selected from the group consisting of 118K, 118E, 118Y, 119R, 119E, 119Y, 120R, 120E, 120I, 121E, 121Y, 121H, 122E, 122R, 124K, 124E, 124Y, 126K, 126D, 129L, 129D, 131G, 131T, 132D, 132R, 132L, 133R, 133E, 133L, 135I, 135E, 135K, 136E, 136K, 136I, 137E, 138S, 138R, 138D, 139I, 139E, 139K, 147A, 147E, 148Y, 148K, 150L, 150K, 150E, 151A, 151D, 152L, 152K, 153L, 153D, 155E, 155K, 155I, 157E, 157K, 157Y, 159K, 159D, 159L, 160K, 160E, 160Y, 161D, 162D, 162K, 162Y, 163R, 164R, 164E, 164Y, 165D, 165R, 165Y, 166D, 167A, 168L, 169E, 171G, 171H, 172K, 172L, 172E, 173T, 173D, 174E, 174K, 174Y, 175D, 175L, 176D, 176R, 176L, 177R, 177E, 177Y, 178D, 179K, 179Y, 179E, 180K, 180L, 180E, 183T, 187I, 187K, 187E, 188I, 189D, 189G, 190I, 190K, 190E, 191D, 191R, 191Y, 192N, 192R, 192L, 193F, 193E, 194R, 194D, 195R, 195D, 195Y, 196K, 196D, 196L, 197R, 197E, 197Y, 198L, 199T, 199D, 199K, 201E, 201K, 201L, 203D, 203L, 203K, 205D, 205L, 206A, 206E, 207K, 207D, 208R, 208E, 208Y, 209E, 209K, 209Y, 210L, 210E, 210Y, 211R, 211E, 211Y, 212Q, 212K, 212H, 212L, 212Y, 213N, 213E, 213H, 213L, 213Y, 214N, 214E, 214H, 214L, 214Y, 216N, 216K, 216H, 216L, 216Y, 217D, 217H, 217A, 217V, 217G, 218D, 218E, 218Q, 218T, 218H, 218L, 218Y, 219D, 219E, 219Q, 219K, 219T, 219H, 219L, 219I, 219Y, 205A, 210A, 213A, 214A,

218A, 221K, 221Y, 221E, 221N, 221Q, 221R, 221S, 221T, 221H, 221A, 221V, 221L, 221I, 221F, 221M, 221W, 221P, 221G, 222E, 222Y, 222D, 222N, 222Q, 222R, 222S, 222T, 222H, 222V, 222L, 222I, 222F, 222M, 222W, 222P, 222G, 222A, 223D, 223N, 223Q, 223R, 223S, 223H, 223A, 223V, 223L, 223I, 223F, 223M, 223Y, 223W, 223P, 223G, 223E, 223K, 224D, 224N, 224Q, 224K, 224R, 224S, 224T, 224V, 224L, 224I, 224F, 224M, 224W, 224P, 224G, 224E, 224Y, 224A, 225D, 225N, 225Q, 225R, 225S, 225H, 225A, 225V, 225L, 225I, 225F, 225M, 225Y, 225P, 225G, 225E, 225K, 225W, 226S, 227E, 227K, 227Y, 227G, 227D, 227N, 227Q, 227R, 227S, 227T, 227H, 227A, 227V, 227L, 227I, 227F, 227M, 227W, 228K, 228Y, 228G, 228D, 228N, 228Q, 228R, 228T, 228H, 228A, 228V, 228L, 228I, 228F, 228M, 228W, 229S, 230A, 230E, 230Y, 230G, 230D, 230N, 230Q, 230K, 230R, 230S, 230T, 230H, 230V, 230L, 230I, 230F, 230M, 230W, 231K, 231P, 231D, 231N, 231Q, 231R, 231S, 231T, 231H, 231V, 231L, 231I, 231F, 231M, 231W, 232E, 232K, 232Y, 232G, 232D, 232N, 232Q, 232R, 232S, 232T, 232H, 232A, 232V, 232L, 232I, 232F, 232M, 232W, 233D, 233N, 233Q, 233R, 233S, 233T, 233H, 233A, 233V, 233L, 233I, 233F, 233M, 233Y, 233W, 233G, 234D, 234E, 234N, 234Q, 234T, 234H, 234Y, 234I, 234V, 234F, 234K, 234R, 234S, 234A, 234M, 234G, 235D, 235S, 235N, 235Q, 235T, 235H, 235Y, 235I, 235V, 235F, 235E, 235K, 235R, 235A, 235M, 235W, 235P, 235G, 236D, 236E, 236N, 236Q, 236K, 236R, 236S, 236T, 236H, 236A, 236V, 236L, 236I, 236F, 236M, 236Y, 236W, and 236P, wherein numbering is according to the EU index.

In particular, variants that alter binding to one or more human Fc receptors may comprise an amino acid modification in the light chain constant region, as described herein, selected from the group consisting of 108D, 108I, 108Q, 109D, 109P, 109R, 110E, 110I, 110K, 111E, 111K, 111L, 112E, 112R, 112Y, 114D, 114I, 114K, 116T, 121D, 122R, 122S, 122Y, 123L, 123R, 124E, 125E, 125K, 126D, 126L, 126Q, 127A, 127D, 127K, 128N, 129E, 129I, 129K, 131T, 137K, 137S, 138D, 138K, 138L, 140E, 140H, 140K, 141E, 141K, 142D, 142G, 142L, 143A, 143L, 143R, 145D, 145T, 145Y, 147A, 147E, 147K, 149D, 149Y, 150A, 151I, 151K, 152L, 152R, 152S, 153D, 153H, 153S, 154E, 154R, 154V, 155E, 155I, 155K, 156A, 156D, 156R, 157N, 158D, 158L, 158R, 159E, 159K, 159L, 160K, 160V, 161K, 161L, 162T, 163E, 163K, 163T, 164Q, 165K, 165P, 165Y, 166E, 166M, 166S, 167K, 167L, 168K, 168Q, 168Y, 169D, 169H, 169S, 170I, 170N, 170R, 171A, 171N, 171V, 172E, 172I, 172K, 173K, 173L, 173Q, 174A, 176T, 180E, 180K, 180S, 181K, 182E, 182R, 182T, 183D, 183L, 183P, 184E, 184K, 184Y, 185I, 185Q, 185R, 187K, 187Y, 188E, 188S, 188Y, 189D, 189K, 189Y, 190E, 190L, 190R, 191E, 191R, 191S, 193E, 193K, 193S, 195I, 195K, 195Q, 197E, 197K, 197L, 199E, 199K, 199Y, 200S, 202D, 202R, 202Y, 203D, 203L, 203R, 204T, 205E, 205K, 206E, 206I,

206K, 207A, 207E, 207L, 208E, 208K, 208T, 210A, 210E, 210K, 211A, 211E, 211P, 212E, 212K, 212T, 213L, 213R, wherein numbering is according to the EU index.

Additional substitutions that may also be used in the present invention include other substitutions that modulate Fc receptor affinity, FcγR-mediated effector function, and/or complement mediated effector function include but are not limited to 298A, 298T, 326A, 326D, 326E, 326W, 326Y, 333A, 333S, 334L, and 334A (US 6,737,056; Shields *et al*, Journal of Biological Chemistry, 2001, 276(9):6591-6604; US 6,528,624; Idusogie *et al.*, 2001, J. Immunology 166:2571-2572), 247L, 255L, 270E, 392T, 396L, and 421K (USSN 10/754,922; USSN 10/902,588), and 280H, 280Q, and 280Y (USSN 10/370,749), all incorporated entirely by reference.

In other embodiments, antibodies of the present invention may be combined with constant heavy chain variants that alter FcRn binding. These include modifications that modify FcRn affinity in a pH-specific manner. In particular, variants that increase Fc binding to FcRn include but are not limited to: 250E, 250Q, 428L, 428F, 250Q/428L (Hinton *et al.*, 2004, J. Biol. Chem. 279(8): 6213-6216, Hinton *et al.* 2006 Journal of Immunology 176:346-356, USSN 11/102621, PCT/US2003/033037, PCT/US2004/011213, USSN 10/822300, USSN 10/687118, PCT/US2004/034440, USSN 10/966673, all incorporated entirely by reference), 256A, 272A, 286A, 305A, 307A, 311A, 312A, 376A, 378Q, 380A, 382A, 434A (Shields *et al*, Journal of Biological Chemistry, 2001, 276(9):6591-6604, USSN 10/982470, US6737056, USSN 11/429793, USSN 11/429786, PCT/US2005/029511, USSN 11/208422, all incorporated entirely by reference), 252F, 252T, 252Y, 252W, 254T, 256S, 256R, 256Q, 256E, 256D, 256T, 309P, 311S, 433R, 433S, 433I, 433P, 433Q, 434H, 434F, 434Y, 252Y/254T/256E, 433K/434F/436H, 308T/309P/311S (Dall Acqua *et al.* Journal of Immunology, 2002, 169:5171-5180, US7083784, PCT/US97/03321, US6821505, PCT/US01/48432, USSN 11/397328, all incorporated entirely by reference), 257C, 257M, 257L, 257N, 257Y, 279E, 279Q, 279Y, insertion of Ser after 281, 283F, 284E, 306Y, 307V, 308F, 308Y 311V, 385H, 385N, (PCT/US2005/041220, USSN 11/274065, USSN 11/436,266, all incorporated entirely by reference) 204D, 284E, 285E, 286D, and 290E (PCT/US2004/037929 incorporated entirely by reference).

In some embodiments of the invention, antibodies may comprise isotypic modifications, that is modifications in a parent IgG to the amino acid type in an alternate IgG. For example as illustrated in Figure 3, an IgG1/IgG3 hybrid variant may be constructed by substituting IgG1 positions in the CH2 and/or CH3 region with the amino acids from IgG3 at positions where the two isotypes differ. Thus a hybrid variant IgG antibody may be constructed that comprises one or more substitutions selected from the group consisting of: 274Q, 276K, 300F, 339T, 356E, 358M,

384S, 392N, 397M, 422I, 435R, and 436F. In other embodiments of the invention, an IgG1/IgG2 hybrid variant may be constructed by substituting IgG2 positions in the CH2 and/or CH3 region with amino acids from IgG1 at positions where the two isotypes differ. Thus a hybrid variant IgG antibody may be constructed that comprises one or more modifications selected from the group consisting of 233E, 234L, 235L, -236G (referring to an insertion of a glycine at position 236), and 327A.

Glycoform modifications

Many polypeptides, including antibodies, are subjected to a variety of post-translational modifications involving carbohydrate moieties, such as glycosylation with oligosaccharides. There are several factors that can influence glycosylation. The species, tissue and cell type have all been shown to be important in the way that glycosylation occurs. In addition, the extracellular environment, through altered culture conditions such as serum concentration, may have a direct effect on glycosylation. (Lifely *et al.*, 1995, Glycobiology 5(8): 813-822), incorporated entirely by reference.

All antibodies contain carbohydrate at conserved positions in the constant regions of the heavy chain. Each antibody isotype has a distinct variety of N-linked carbohydrate structures. Aside from the carbohydrate attached to the heavy chain, up to 30% of human IgGs have a glycosylated Fab region. IgG has a single N-linked biantennary carbohydrate at Asn297 of the CH2 domain. For IgG from either serum or produced ex vivo in hybridomas or engineered cells, the IgG are heterogeneous with respect to the Asn297 linked carbohydrate (Jefferis *et al.*, 1998, Immunol. Rev. 163:59-76; Wright *et al.*, 1997, Trends Biotech 15:26-32, both incorporated entirely by reference). For human IgG, the core oligosaccharide normally consists of $GlcNAc_2Man_3GlcNAc$, with differing numbers of outer residues.

The carbohydrate moieties of the present invention will be described with reference to commonly used nomenclature for the description of oligosaccharides. A review of carbohydrate chemistry which uses this nomenclature is found in Hubbard *et al.* 1981, Ann. Rev. Biochem. 50:555-583, incorporated entirely by reference. This nomenclature includes, for instance, Man, which represents mannose; GlcNAc, which represents 2-N-acetylglucosamine; Gal which represents galactose; Fuc for fucose; and Glc, which represents glucose. Sialic acids are described by the shorthand notation NeuNAc, for 5-N-acetylneuraminic acid, and NeuNGc for 5-glycolylneuraminic.

The term "glycosylation" means the attachment of oligosaccharides (carbohydrates containing two or more simple sugars linked together e.g. from two to about twelve simple

sugars linked together) to a glycoprotein. The oligosaccharide side chains are typically linked to the backbone of the glycoprotein through either N- or O-linkages. The oligosaccharides of the present invention occur generally are attached to a CH2 domain of an Fc region as N-linked oligosaccharides. "N-linked glycosylation" refers to the attachment of the carbohydrate moiety to an asparagine residue in a glycoprotein chain. The skilled artisan will recognize that, for example, each of murine IgG1, IgG2a, IgG2b and IgG3 as well as human IgG1, IgG2, IgG3, IgG4, IgA and IgD CH2 domains have a single site for N-linked glycosylation at amino acid residue 297 (Kabat *et al.* Sequences of Proteins of Immunological Interest, 1991, incorporated entirely by reference).

For the purposes herein, a "mature core carbohydrate structure" refers to a processed core carbohydrate structure attached to an Fc region which generally consists of the following carbohydrate structure GlcNAc(Fucose)-GlcNAc-Man-(Man-GlcNAc)$_2$ typical of biantennary oligosaccharides. The mature core carbohydrate structure is attached to the Fc region of the glycoprotein, generally via N-linkage to Asn297 of a CH2 domain of the Fc region. A "bisecting GlcNAc" is a GlcNAc residue attached to the β1,4 mannose of the mature core carbohydrate structure. The bisecting GlcNAc can be enzymatically attached to the mature core carbohydrate structure by a β(1,4)-N-acetylglucosaminyltransferase III enzyme (GnTIII). CHO cells do not normally express GnTIII (Stanley *et al.*, 1984, J. Biol. Chem. 261:13370-13378), but may be engineered to do so (Umana *et al.*, 1999, Nature Biotech. 17:176-180).

The present invention contemplates antibodies that comprise modified glycoforms or engineered glycoforms. By "modified glycoform" or "engineered glycoform" as used herein is meant a carbohydrate composition that is covalently attached to a protein, for example an antibody, wherein said carbohydrate composition differs chemically from that of a parent protein. Engineered glycoforms may be useful for a variety of purposes, including but not limited to enhancing or reducing FcγR-mediated effector function. In a preferred embodiment, the antibodies of the present invention are modified to control the level of fucosylated and/or bisecting oligosaccharides that are covalently attached to the Fc region.

Historically, antibodies produced in Chinese Hamster Ovary Cells (CHO), one of the most commonly used industrial hosts, contain about 2 to 6% in the population that are nonfucosylated. YB2/0 (rat myeloma) and Lec13 cell line (a lectin mutant of CHO line which has a deficient GDP-mannose 4,6 dehydratase leading to the deficiency of GDP-fucose or GDP-sugar intermediates that are the substrate of α1,6-fucosyltransferase (Ripka et al., 1986), however, can produce antibodies with 78% to 98% nonfucosylated species. Unfortunately, the yield of

antibody from these cells is extremely poor and therefore these cell lines are not useful to make therapeutic antibody products commercially. The FUT8 gene encodes the α1,6-fucosyltransferase enzyme that catalyzes the tranfer of a fucosyl residue from GDP-fucose to position 6 of Asn-linked (N-linked) GlcNac of an N-glycan (Yanagidani et al., 1997, J Biochem 121:626-632). It is known that the α1,6 fucosyltransferase is the only enzyme responsible for adding fucose to the N-linked biantennary carbohydrate at Asn297 in the CH2 domain of the IgG antibody.

A variety of methods are well known in the art for generating modified glycoforms (Umaña *et al.*, 1999, Nat Biotechnol 17:176-180; Davies *et al.*, 2001, Biotechnol Bioeng 74:288-294; Shields *et al.*, 2002, J Biol Chem 277:26733-26740; Shinkawa *et al.*, 2003, J Biol Chem 278:3466-3473); (US 6,602,684; USSN 10/277,370; USSN 10/113,929; PCT WO 00/61739A1; PCT WO 01/29246A1; PCT WO 02/31140A1; PCT WO 02/30954A1); Yamane-Ohnuki et al., 2004, Biotechnology and Bioengineering 87(5):614-621; (Potelligent™ technology [Biowa, Inc., Princeton, NJ]; GlycoMAb™ glycosylation engineering technology [GLYCART biotechnology AG, Zürich, Switzerland]; all of which are expressly incorporated by reference). These techniques control the level of fucosylated and/or bisecting oligosaccharides that are covalently attached to the Fc region, for example by expressing an IgG in various organisms or cell lines, engineered or otherwise (for example Lec-13 CHO cells or rat hybridoma YB2/0 cells), by regulating enzymes involved in the glycosylation pathway (for example FUT8 [α1,6-fucosyltranserase] and/or β1-4- N-acetylglucosaminyltransferase III [GnTIII]), or by modifying carbohydrate(s) after the IgG has been expressed.

Other methods for modifying glycoforms of the antibodies of the invention include using glycoengineered strains of yeast (Li *et al.*, 2006, Nature Biotechnology 24(2):210-215), moss (Nechansky *et al.*, 2007, Mol Immunjol 44(7):1826-8), and plants (Cox *et al.*, 2006, Nat Biotechnol 24(12):1591-7). Methods for modying glycoforms include but are not limited to using a glycoengineered strain of yeast *Pichia pastoris* (Li *et al.*, 2006, Nature Biotechnology 24(2):210-215), a glycoengineered strain of the moss *Physcomitrella patens* wherein the enzymes β1,2-xylosyltransferase and/or α1,3-fucosyltransferase are knocked out in (Nechansky *et al.*, 2007, Mol Immunjol 44(7):1826-8), and the use of RNA interference to inhibit endogenous alpha-1,3-fucosyltransferase and/or beta-1,2-xylosyltransferase in the aquatic plant Lemna minor (Cox *et al.*, 2006, Nat Biotechnol 24(12):1591-7).

Modified or engineered glycoform typically refers to the different carbohydrate or oligosaccharide; thus for example an antibody may comprise an engineered glycoform. Alternatively, engineered glycoform may refer to the antibody that comprises the different

carbohydrate or oligosaccharide. For the purposes of modified glycoforms described herein, a "parent antibody" is a glycosylated antibody having the same amino acid sequence and mature core carbohydrate structure as an engineered glycoform of the present invention, except that fucose is attached to the mature core carbohydrate structure of the parent antibody. For instance, in a composition comprising the parent glycoprotein about 50-100% or about 70-100% of the parent glycoprotein comprises a mature core carbohydrate structure having fucose attached thereto.

The present invention provides a composition comprising a glycosylated antibody having an Fc region, wherein about 51-100% of the glycosylated antibody in the composition comprises a mature core carbohydrate structure which lacks fucose, attached to the Fc region of the antibody. More preferably, about 80-100% of the antibody in the composition comprises a mature core carbohydrate structure which lacks fucose and most preferably about 90-99% of the antibody in the composition lacks fucose attached to the mature core carbohydrate structure. In a most preferred embodiment, the antibody in the composition both comprises a mature core carbohydrate structure that lacks fucose and additionally comprises at least one amino acid modification in the Fc region. In the most preferred embodiment, the combination of engineered glycoform and amino acid modification provides optimal Fc receptor binding properties to the antibody.

Optimized properties of antibodies

The present invention provides variant antibodies that are optimized for a number of therapeutically relevant properties. A variant antibody comprises one or more amino acid modifications relative to a parent antibody, wherein said amino acid modification(s) provide one or more optimized properties. Thus the antibodies of the present invention are variants antibodies. An antibody of the present invention differs in amino acid sequence from its parent antibody by virtue of at least one amino acid modification. Thus variant antibodies of the present invention have at least one amino acid modification compared to the parent. Alternatively, the variant antibodies of the present invention may have more than one amino acid modification as compared to the parent, for example from about one to fifty amino acid modifications, preferably from about one to ten amino acid modifications, and most preferably from about one to about five amino acid modifications compared to the parent. Thus the sequences of the variant antibodies and those of the parent antibodies are substantially homologous. For example, the variant antibody sequences herein will possess about 80% homology with the parent antibody sequence, preferably at least about 90% homology, and most preferably at least about 95% homology.

In a most preferred embodiment, the antibodies of the present invention comprise amino acid modifications that provide optimized effector function properties relative to the parent. Most preferred substitutions and optimized effector function properties are described in USSN 10/672,280, PCT US03/30249, and USSN 10/822,231, and USSN 60/627,774, filed 11/12/2004 and entitled "Optimized Fc Variants". Properties that may be optimized include but are not limited to enhanced or reduced affinity for an FcγR. In a preferred embodiment, the antibodies of the present invention are optimized to possess enhanced affinity for a human activating FcγR, preferably FcγRI, FcγRIIa, FcγRIIc, FcγRIIIa, and FcγRIIIb, most preferably FcγRIIIa. In an alternately preferred embodiment, the antibodies are optimized to possess reduced affinity for the human inhibitory receptor FcγRIIb. These preferred embodiments are anticipated to provide antibodies with enhanced therapeutic properties in humans, for example enhanced effector function and greater anti-cancer potency. In an alternate embodiment, the antibodies of the present invention are optimized to have reduced or ablated affinity for a human FcγR, including but not limited to FcγRI, FcγRIIa, FcγRIIb, FcγRIIc, FcγRIIIa, and FcγRIIIb. These embodiments are anticipated to provide antibodies with enhanced therapeutic properties in humans, for example reduced effector function and reduced toxicity. In other embodiments, antibodies of the present invention provide enhanced affinity for one or more FcγRs, yet reduced affinity for one or more other FcγRs. For example, an antibody of the present invention may have enhanced binding to FcγRIIIa, yet reduced binding to FcγRIIb. Alternately, an antibody of the present invention may have enhanced binding to FcγRIIa and FcγRI, yet reduced binding to FcγRIIb. In yet another embodiment, an antibody of the present invention may have enhanced affinity for FcγRIIb, yet reduced affinity to one or more activating FcγRs.

The modification of the invention preferably enhance binding affinity for one or more FcγRs. By "greater affinity" or "improved affinity" or "enhanced affinity" or "better affinity" than a parent immunoglobulin, as used herein is meant that an Fc variant binds to an Fc receptor with a significantly higher equilibrium constant of association (KA) or lower equilibrium constant of dissociation (KD) than the parent polypeptide when the amounts of variant and parent polypeptide in the binding assay are essentially the same. For example, the Fc variant with improved FcγR binding affinity may display from about 5 fold to about 1000 fold, e.g. from about 10 fold to about 500 fold improvement in Fc receptor binding affinity compared to the parent polypeptide, where Fc receptor binding affinity is determined, for example, as disclosed in the Examples herein. Accordingly, by "reduced affinity" as compared to a parent Fc polypeptide as used herein is meant that an Fc variant binds an Fc receptor with significantly lower KA or

higher KD than the parent polypeptide.

Data in the present study indicate that human WT IgG1 binds to human V158 FcγRIIIa with an affinity of approximately 240 nM (Example 1). This is consistent with the literature which indicate that binding is approximately 200-500 nM, as determined by Biacore (210 nM as shown in Okazaki et al, 2004, J Mol Bio 336:1239-49; 250 nM as shown in Lazar et al, Proc Natl Acad Sci USA 103(11):4005-4010) and calorimetry (530 nM, Okazaki et al, 2004, J Mol Bio 336:1239-49). However affinity as low as 750 nM was measured in one study (Ferrara et al., 2006, J Biol Chem 281(8):5032-5036). Although binding to F158 FcγRIIIa was lower than the 5 uM cutoff applied in the present study, the literature indicates that human WT IgG1 binds to human F158 FcγRIIIa with an affinity of approximately 3-5 uM, as indicated by calorimetry (2.7 uM, in Okazaki et al, 2004, J Mol Bio 336:1239-49) and Biacore (5.0 uM, Ferrara et al., 2006, J Biol Chem 281(8):5032-5036).

Preferred embodiments comprise optimization of Fc binding to a human FcγR, however in alternate embodiments the antibodies of the present invention possess enhanced or reduced affinity for FcγRs from nonhuman organisms, including but not limited to rodents and non-human primates. antibodies that are optimized for binding to a nonhuman FcγR may find use in experimentation. For example, mouse models are available for a variety of diseases that enable testing of properties such as efficacy, toxicity, and pharmacokinetics for a given drug candidate. As is known in the art, cancer cells can be grafted or injected into mice to mimic a human cancer, a process referred to as xenografting. Testing of antibodies that comprise antibodies that are optimized for one or more mouse FcγRs, may provide valuable information with regard to the efficacy of the protein, its mechanism of action, and the like. The antibodies of the present invention may also be optimized for enhanced functionality and/or solution properties in aglycosylated form. In a preferred embodiment, the aglycosylated antibodies of the present invention bind an Fc ligand with greater affinity than the aglycosylated form of the parent antibody. Said Fc ligands include but are not limited to FcγRs, C1q, FcRn, and proteins A and G, and may be from any source including but not limited to human, mouse, rat, rabbit, or monkey, preferably human. In an alternately preferred embodiment, the antibodies are optimized to be more stable and/or more soluble than the aglycosylated form of the parent antibody.

Antibodies of the invention may comprise modifications that modulate interaction with Fc ligands other than FcγRs, including but not limited to complement proteins, FcRn, and Fc receptor homologs (FcRHs). FcRHs include but are not limited to FcRH1, FcRH2, FcRH3, FcRH4, FcRH5, and FcRH6 (Davis *et al.*, 2002, Immunol. Reviews 190:123-136, incorporated

entirely by reference).

Preferably, the Fc ligand specificity of the antibody of the present invention will determine its therapeutic utility. The utility of a given antibody for therapeutic purposes will depend on the epitope or form of the target antigen and the disease or indication being treated. For some targets and indications, enhanced FcγR-mediated effector functions may be preferable. This may be particularly favorable for anti-cancer antibodies. Thus antibodies may be used that comprise antibodies that provide enhanced affinity for activating FcγRs and/or reduced affinity for inhibitory FcγRs. For some targets and indications, it may be further beneficial to utilize antibodies that provide differential selectivity for different activating FcγRs; for example, in some cases enhanced binding to FcγRIIa and FcγRIIIa may be desired, but not FcγRI, whereas in other cases, enhanced binding only to FcγRIIa may be preferred. For certain targets and indications, it may be preferable to utilize antibodies that enhance both FcγR-mediated and complement-mediated effector functions, whereas for other cases it may be advantageous to utilize antibodies that enhance either FcγR-mediated or complement-mediated effector functions. For some targets or cancer indications, it may be advantageous to reduce or ablate one or more effector functions, for example by knocking out binding to C1q, one or more FcγR's, FcRn, or one or more other Fc ligands. For other targets and indications, it may be preferable to utilize antibodies that provide enhanced binding to the inhibitory FcγRIIb, yet WT level, reduced, or ablated binding to activating FcγRs. This may be particularly useful, for example, when the goal of an antibody is to inhibit inflammation or auto-immune disease, or modulate the immune system in some way.

Clearly an important parameter that determines the most beneficial selectivity of a given antibody to treat a given disease is the context of the antibody, that is what type of antibody is being used. Thus the Fc ligand selectivity or specificity of a given antibody will provide different properties depending on whether it composes an antibody or an antibodies with a coupled fusion or conjugate partner. For example, toxin, radionucleotide, or other conjugates may be less toxic to normal cells if the antibody that comprises them has reduced or ablated binding to one or more Fc ligands. As another example, in order to inhibit inflammation or auto-immune disease, it may be preferable to utilize an antibody with enhanced affinity for activating FcγRs, such as to bind these FcγRs and prevent their activation. Conversely, an antibody that comprises two or more Fc regions with enhanced FcγRIIb affinity may co-engage this receptor on the surface of immune cells, thereby inhibiting proliferation of these cells. Whereas in some cases an antibodies may engage its target antigen on one cell type yet engage FcγRs on separate cells from the target

antigen, in other cases it may be advantageous to engage FcγRs on the surface of the same cells as the target antigen. For example, if an antibody targets an antigen on a cell that also expresses one or more FcγRs, it may be beneficial to utilize an antibody that enhances or reduces binding to the FcγRs on the surface of that cell. This may be the case, for example when the antibody is being used as an anti-cancer agent, and co-engagement of target antigen and FcγR on the surface of the same cell promote signaling events within the cell that result in growth inhibition, apoptosis, or other anti-proliferative effect. Alternatively, antigen and FcγR co-engagement on the same cell may be advantageous when the antibody is being used to modulate the immune system in some way, wherein co-engagement of target antigen and FcγR provides some proliferative or anti-proliferative effect. Likewise, antibodies that comprise two or more Fc regions may benefit from antibodies that modulate FcγR selectivity or specificity to co-engage FcγRs on the surface of the same cell.

The Fc ligand specificity of the antibodies of the present invention can be modulated to create different effector function profiles that may be suited for particular antigen epitopes, indications or patient populations. Figure 5 describes several preferred embodiments of receptor binding profiles that include improvements to, reductions to or no effect to the binding to various receptors, where such changes may be beneficial in certain contexts. The receptor binding profiles in Figure 5 could be varied by degree of increase or decrease to the specified receptors. Additionally, the binding changes specified could be in the context of additional binding changes to other receptors such as C1q or FcRn, for example by combining with ablation of binding to C1q to shut off complement activation, or by combining with enhanced binding to C1q to increase complement activation. Other embodiments with other receptor binding profiles are possible, the listed receptor binding profiles are exemplary.

The presence of different polymorphic forms of FcγRs provides yet another parameter that impacts the therapeutic utility of the antibodies of the present invention. Whereas the specificity and selectivity of a given antibody for the different classes of FcγRs significantly affects the capacity of an antibody to target a given antigen for treatment of a given disease, the specificity or selectivity of an antibody for different polymorphic forms of these receptors may in part determine which research or pre-clinical experiments may be appropriate for testing, and ultimately which patient populations may or may not respond to treatment. Thus the specificity or selectivity of antibodies of the present invention to Fc ligand polymorphisms, including but not limited to FcγR, C1q, FcRn, and FcRH polymorphisms, may be used to guide the selection of valid research and pre-clinical experiments, clinical trial design, patient selection, dosing

dependence, and/or other aspects concerning clinical trials.

Other modifications

Antibodies of the present invention may comprise one or more modifications that provide optimized properties that are not specifically related to effector function per se. Said modifications may be amino acid modifications, or may be modifications that are made enzymatically or chemically. Such modification(s) likely provide some improvement in the antibody, for example an enhancement in its stability, solubility, function, or clinical use. The present invention contemplates a variety of improvements that made be made by coupling the antibodies of the present invention with additional modifications.

In one embodiment, the variable region of an antibody of the present invention may be affinity matured, that is to say that amino acid modifications have been made in the VH and/or VL domains of the antibody to enhance binding of the antibody to its target antigen. Such types of modifications may improve the association and/or the dissociation kinetics for binding to the target antigen. Other modifications include those that improve selectivity for target antigen vs. alternative targets. These include modifications that improve selectivity for antigen expressed on target vs. non-target cells. Other improvements to the target recognition properties may be provided by additional modifications. Such properties may include, but are not limited to, specific kinetic properties (i.e. association and dissociation kinetics), selectivity for the particular target versus alternative targets, and selectivity for a specific form of target versus alternative forms. Examples include full-length versus splice variants, cell-surface vs. soluble forms, selectivity for various polymorphic variants, or selectivity for specific conformational forms of the target antigen.

Antibodies of the invention may comprise one or more modifications that provide reduced or enhanced internalization of an antibody. In one embodiment, antibodies of the present invention can be utilized or combined with additional modifications in order to reduce the cellular internalization of an antibody that occurs via interaction with one or more Fc ligands. This property might be expected to enhance effector function, and potentially reduce immunogenicity of the antibodies of the invention. Alternatively, antibodies of the present antibodies of the present invention can be utilized directly or combined with additional modifications in order to enhance the cellular internalization of an antibody that occurs via interaction with one or more Fc ligands. For example, in a preferred embodiment, an antibody is used that provides enhanced binding to FcγRI, which is expressed on dendritic cells and active early in immune response. This strategy could be further enhanced by combination with

additional modifications, either within the antibody or in an attached fusion or conjugate partner, that promote recognition and presentation of Fc peptide fragments by MHC molecules. These strategies are expected to enhance target antigen processing and thereby improve antigenicity of the target antigen (Bonnerot and Amigorena, 1999, *Immunol Rev.* 172:279-84, incorporated entirely by reference), promoting an adaptive immune response and greater target cell killing by the human immune system. These strategies may be particularly advantageous when the targeted antigen is shed from the cellular surface. An additional application of these concepts arises with idiotype vaccine immunotherapies, in which clone-specific antibodies produced by a patient's lymphoma cells are used to vaccinate the patient.

In a preferred embodiment, modifications are made to improve biophysical properties of the antibodies of the present invention, including but not limited to stability, solubility, and oligomeric state. Modifications can include, for example, substitutions that provide more favorable intramolecular interactions in the antibody such as to provide greater stability, or substitution of exposed nonpolar amino acids with polar amino acids for higher solubility. A number of optimization goals and methods are described in USSN 10/379,392, incorporated entirely by reference, that may find use for engineering additional modifications to further optimize the antibodies of the present invention. The antibodies of the present invention can also be combined with additional modifications that reduce oligomeric state or size, such that tumor penetration is enhanced, or *in vivo* clearance rates are increased as desired.

Other modifications to the antibodies of the present invention include those that enable the specific formation or homodimeric or homomultimeric molecules. Such modifications include but are not limited to engineered disulfides, as well as chemical modifications or aggregation methods which may provide a mechanism for generating covalent homodimeric or homomultimers. For example, methods of engineering and compositions of such molecules are described in Kan *et al.*, 2001, *J. Immunol.*, 2001, 166: 1320–1326; Stevenson *et al.*, 2002, *Recent Results Cancer Res.* 159: 104-12; US 5,681,566; Caron *et al.*, 1992, J. Exp. Med. 176:1191-1195, and Shopes, 1992, J. Immunol. 148(9):2918-22, all incorporated entirely by reference. Additional modifications to the variants of the present invention include those that enable the specific formation or heterodimeric, heteromultimeric, bifunctional, and/or multifunctional molecules. Such modifications include, but are not limited to, one or more amino acid substitutions in the CH3 domain, in which the substitutions reduce homodimer formation and increase heterodimer formation. For example, methods of engineering and compositions of such molecules are described in Atwell *et al.*, 1997, J. Mol. Biol. 270(1):26-35, and Carter *et al.*, 2001, J. Immunol. Methods 248:7-15, both incorporated entirely by reference. Additional modifications include

modifications in the hinge and CH3 domains, in which the modifications reduce the propensity to form dimers.

In further embodiments, the antibodies of the present invention comprise modifications that remove proteolytic degradation sites. These may include, for example, protease sites that reduce production yields, as well as protease sites that degrade the administered protein *in vivo*. In a preferred embodiment, additional modifications are made to remove covalent degradation sites such as deamidation (i.e. deamidation of glutaminyl and asparaginyl residues to the corresponding glutamyl and aspartyl residues), oxidation, and proteolytic degradation sites. Deamidation sites that are particular useful to remove are those that have enhance propensity for deamidation, including, but not limited to asparaginyl and gltuamyl residues followed by glycines (NG and QG motifs, respectively). In such cases, substitution of either residue can significantly reduce the tendency for deamidation. Common oxidation sites include methionine and cysteine residues. Other covalent modifications, that can either be introduced or removed, include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the "-amino groups of lysine, arginine, and histidine side chains (T.E. Creighton, Proteins: Structure and Molecular Properties, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983), incorporated entirely by reference), acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group. Additional modifications also may include but are not limited to posttranslational modifications such as N-linked or O-linked glycosylation and phosphorylation.

Modifications may include those that improve expression and/or purification yields from hosts or host cells commonly used for production of biologics. These include, but are not limited to various mammalian cell lines (e.g. CHO), yeast cell lines, bacterial cell lines, and plants. Additional modifications include modifications that remove or reduce the ability of heavy chains to form inter-chain disulfide linkages. Additional modifications include modifications that remove or reduce the ability of heavy chains to form intra-chain disulfide linkages.

The antibodies of the present invention may comprise modifications that include the use of unnatural amino acids incorporated using, for example, the technologies developed by Schultz and colleagues, including but not limited to methods described by Cropp & Shultz, 2004, Trends Genet. 20(12):625-30, Anderson *et al.*, 2004, Proc. Natl. Acad. Sci. U.S.A. 101(2):7566-71, Zhang *et al.*, 2003, 303(5656):371-3, and Chin *et al.*, 2003, Science 301(5635):964-7, all incorporated entirely by reference. In some embodiments, these modifications enable manipulation of various functional, biophysical, immunological, or manufacturing properties discussed above. In additional embodiments, these modifications enable additional chemical

modification for other purposes. Other modifications are contemplated herein. For example, the antibody may be linked to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol (PEG), polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol. Additional amino acid modifications may be made to enable specific or non-specific chemical or posttranslational modification of the antibodies. Such modifications, include, but are not limited to PEGylation and glycosylation. Specific substitutions that can be utilized to enable PEGylation include, but are not limited to, introduction of novel cysteine residues or unnatural amino acids such that efficient and specific coupling chemistries can be used to attach a PEG or otherwise polymeric moiety. Introduction of specific glycosylation sites can be achieved by introducing novel N-X-T/S sequences into the antibodies of the present invention.

Covalent modifications of antibodies are included within the scope of this invention, and are generally, but not always, done post-translationally. For example, several types of covalent modifications of the antibody are introduced into the molecule by reacting specific amino acid residues of the antibody with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues.

In some embodiments, the covalent modification of the antibodies of the invention comprises the addition of one or more labels. The term "labeling group" means any detectable label. In some embodiments, the labeling group is coupled to the antibody via spacer arms of various lengths to reduce potential steric hindrance. Various methods for labeling proteins are known in the art and may be used in performing the present invention. In general, labels fall into a variety of classes, depending on the assay in which they are to be detected: a) isotopic labels, which may be radioactive or heavy isotopes; b) magnetic labels (e.g., magnetic particles); c) redox active moieties; d) optical dyes; enzymatic groups (e.g. horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase); e) biotinylated groups; and f) predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags, etc.). In some embodiments, the labeling group is coupled to the antibody via spacer arms of various lengths to reduce potential steric hindrance. Various methods for labeling proteins are known in the art and may be used in performing the present invention. Specific labels include optical dyes, including, but not limited to, chromophores, phosphors and fluorophores, with the latter being specific in many instances. Fluorophores can be either “small molecule” fluores, or proteinaceous fluores. By “fluorescent label” is meant any molecule that may be detected via its inherent fluorescent properties.

Antibody conjugates and fusions

In one embodiment, the antibodies of the invention are antibody "fusion proteins", sometimes referred to herein as "antibody conjugates". The fusion partner or conjugate partner can be proteinaceous or non-proteinaceous; the latter generally being generated using functional groups on the antibody and on the conjugate partner. Conjugate and fusion partners may be any molecule, including small molecule chemical compounds and polypeptides. For example, a variety of antibody conjugates and methods are described in Trail *et al.*, 1999, Curr. Opin. Immunol. 11:584-588, incorporated entirely by reference. Possible conjugate partners include but are not limited to cytokines, cytotoxic agents, toxins, radioisotopes, chemotherapeutic agent, anti-angiogenic agents, a tyrosine kinase inhibitors, and other therapeutically active agents. In some embodiments, conjugate partners may be thought of more as payloads, that is to say that the goal of a conjugate is targeted delivery of the conjugate partner to a targeted cell, for example a cancer cell or immune cell, by the antibody. Thus, for example, the conjugation of a toxin to an antibody targets the delivery of said toxin to cells expressing the target antigen. As will be appreciated by one skilled in the art, in reality the concepts and definitions of fusion and conjugate are overlapping. The designation of an antibody as a fusion or conjugate is not meant to constrain it to any particular embodiment of the present invention. Rather, these terms are used loosely to convey the broad concept that any antibody of the present invention may be linked genetically, chemically, or otherwise, to one or more polypeptides or molecules to provide some desirable property.

Suitable conjugates include, but are not limited to, labels as described below, drugs and cytotoxic agents including, but not limited to, cytotoxic drugs (e.g., chemotherapeutic agents) or toxins or active fragments of such toxins. Suitable toxins and their corresponding fragments include diptheria A chain, exotoxin A chain, ricin A chain, abrin A chain, curcin, crotin, phenomycin, enomycin and the like. Cytotoxic agents also include radiochemicals made by conjugating radioisotopes to antibodies, or binding of a radionuclide to a chelating agent that has been covalently attached to the antibody. Additional embodiments utilize calicheamicin, auristatins, geldanamycin, maytansine, and duocarmycins and analogs; for the latter, see U.S. 2003/0050331, incorporated entirely by reference.

In one embodiment, the antibodies of the present invention are fused or conjugated to a cytokine. By "cytokine" as used herein is meant a generic term for proteins released by one cell population that act on another cell as intercellular mediators. For example, as described in Penichet *et al.*, 2001, J. Immunol. Methods 248:91-101, incorporated entirely by reference, cytokines may be fused to antibody to provide an array of desirable properties. Examples of such

cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor-alpha and -beta; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF-beta; platelet-growth factor; transforming growth factors (TGFs) such as TGF-alpha and TGF-beta; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-alpha, beta, and -gamma; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1alpha, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12; IL-15, a tumor necrosis factor such as TNF-alpha or TNF-beta; C5a; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture, and biologically active equivalents of the native sequence cytokines.

In an alternate embodiment, the antibodies of the present invention are fused, conjugated, or operably linked to a toxin, including but not limited to small molecule toxins and enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof. For example, a variety of immunotoxins and immunotoxin methods are described in Thrush *et al.*, 1996, Ann. Rev. Immunol. 14:49-71, incorporated entirely by reference. Small molecule toxins include but are not limited to calicheamicin, maytansine (US 5,208,020, incorporated entirely by reference), trichothene, and CC1065. In one embodiment of the invention, the antibody is conjugated to one or more maytansine molecules (e.g. about 1 to about 10 maytansine molecules per antibody molecule). Maytansine may, for example, be converted to May-SS-Me which may be reduced to May-SH3 and reacted with modified antibody (Chari *et al.*, 1992, *Cancer Research* 52: 127-131, incorporated entirely by reference) to generate a maytansinoid-antibody conjugate. Another conjugate of interest comprises an antibody conjugated to one or more calicheamicin molecules. The calicheamicin family of antibiotics are capable of producing double-stranded DNA breaks at sub-picomolar concentrations. Structural analogues of calicheamicin that may be used include but are not limited to γ_1^1, α_2^1, α_3, N-acetyl-γ_1^1, PSAG, and Θ^1_1, (Hinman *et al.*, 1993, Cancer Research 53:3336-3342; Lode *et al.*, 1998, Cancer Research 58:2925-2928) (US 5,714,586; US 5,712,374;

US 5,264,586; US 5,773,001, all incorporated entirely by reference). Dolastatin 10 analogs such as auristatin E (AE) and monomethylauristatin E (MMAE) may find use as conjugates for the antibodies of the present invention (Doronina *et al.*, 2003, Nat Biotechnol 21(7):778-84; Francisco *et al.*, 2003 Blood 102(4):1458-65, both incorporated entirely by reference). Useful enyzmatically active toxins include but are not limited to diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. See, for example, PCT WO 93/21232, incorporated entirely by reference. The present invention further contemplates a conjugate between an antibody of the present invention and a compound with nucleolytic activity, for example a ribonuclease or DNA endonuclease such as a deoxyribonuclease (Dnase).

In an alternate embodiment, an antibody of the present invention may be fused, conjugated, or operably linked to a radioisotope to form a radioconjugate. A variety of radioactive isotopes are available for the production of radioconjugate antibodies. Examples include, but are not limited to, At211, I131, I125, Y90, Re186, Re188, Sm153, Bi212, P32, and radioactive isotopes of Lu.

In yet another embodiment, an antibody of the present invention may be conjugated to a "receptor" (such streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (e.g. avidin) which is conjugated to a cytotoxic agent (e.g. a radionucleotide). In an alternate embodiment, the antibody is conjugated or operably linked to an enzyme in order to employ Antibody Dependent Enzyme Mediated Prodrug Therapy (ADEPT). ADEPT may be used by conjugating or operably linking the antibody to a prodrug-activating enzyme that converts a prodrug (e.g. a peptidyl chemotherapeutic agent, see PCT WO 81/01145, incorporated entirely by reference) to an active anti-cancer drug. See, for example, PCT WO 88/07378 and US 4,975,278, both incorporated entirely by reference. The enzyme component of the immunoconjugate useful for ADEPT includes any enzyme capable of acting on a prodrug in such a way so as to covert it into its more active, cytotoxic form. Enzymes that are useful in the method of this invention include but are not limited to alkaline phosphatase useful for converting phosphate-containing prodrugs into free drugs; arylsulfatase useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-

fluorouracil; proteases, such as serratia protease, thermolysin, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), that are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as .beta.-galactosidase and neuramimidase useful for converting glycosylated prodrugs into free drugs; beta-lactamase useful for converting drugs derivatized with .alpha.-lactams into free drugs; and penicillin amidases, such as penicillin V amidase or penicillin G amidase, useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. Alternatively, antibodies with enzymatic activity, also known in the art as "abzymes", can be used to convert the prodrugs of the invention into free active drugs (see, for example, Massey, 1987, *Nature* 328: 457-458, incorporated entirely by reference). Antibody-abzyme conjugates can be prepared for delivery of the abzyme to a tumor cell population. A variety of additional conjugates are contemplated for the antibodies of the present invention. A variety of chemotherapeutic agents, anti-angiogenic agents, tyrosine kinase inhibitors, and other therapeutic agents are described below, which may find use as antibody conjugates.

Also contemplated as fusion and conjugate partners are Fc polypeptides. Thus an antibody may be a multimeric Fc polypeptide, comprising two or more Fc regions. The advantage of such a molecule is that it provides multiple binding sites for Fc receptors with a single protein molecule. In one embodiment, Fc regions may be linked using a chemical engineering approach. For example, Fab's and Fc's may be linked by thioether bonds originating at cysteine residues in the hinges, generating molecules such as $FabFc_2$. Fc regions may be linked using disulfide engineering and/or chemical cross-linking. In a preferred embodiment, Fc regions may be linked genetically. . In a preferred embodiment, Fc regions in an antibody are linked genetically to generated tandemly linked Fc regions as described in USSN 11/022,289, filed 12/21/2004, entitled "Fc polypeptides with novel Fc ligand binding sites," incorporated entirely by reference. Tandemly linked Fc polypeptides may comprise two or more Fc regions, preferably one to three, most preferably two Fc regions. It may be advantageous to explore a number of engineering constructs in order to obtain homo- or hetero- tandemly linked antibodies with the most favorable structural and functional properties. Tandemly linked antibodies may be homo- tandemly linked antibodies, that is an antibody of one isotype is fused genetically to another antibody of the same isotype. It is anticipated that because there are multiple Fc□R, C1q, and/or FcRn binding sites on tandemly linked Fc polypeptides, effector functions and/or pharmacokinetics may be enhanced. In an alternate embodiment, antibodies from different isotypes may be tandemly linked, referred to as hetero- tandemly linked antibodies. For example, because of the capacity to target FcγR and

FcαRI receptors, an antibody that binds both FcγRs and FcαRI may provide a significant clinical improvement.

In addition to antibodies, an antibody-like protein that is finding an expanding role in research and therapy is the Fc fusion (Chamow *et al.*, 1996, *Trends Biotechnol* 14:52-60; Ashkenazi *et al.*, 1997, *Curr Opin Immunol* 9:195-200, both incorporated entirely by reference). "Fc fusion" is herein meant to be synonymous with the terms "immunoadhesin", "Ig fusion", "Ig chimera", and "receptor globulin" (sometimes with dashes) as used in the prior art (Chamow *et al.*, 1996, Trends Biotechnol 14:52-60; Ashkenazi *et al.*, 1997, Curr Opin Immunol 9:195-200). An Fc fusion is a protein wherein one or more polypeptides is operably linked to Fc. An Fc fusion combines the Fc region of an antibody, and thus its favorable effector functions and pharmacokinetics, with the target-binding region of a receptor, ligand, or some other protein or protein domain. The role of the latter is to mediate target recognition, and thus it is functionally analogous to the antibody variable region. Because of the structural and functional overlap of Fc fusions with antibodies, the discussion on antibodies in the present invention extends also to Fc.

Virtually any protein or small molecule may be linked to Fc to generate an Fc fusion. Protein fusion partners may include, but are not limited to, the variable region of any antibody, the target-binding region of a receptor, an adhesion molecule, a ligand, an enzyme, a cytokine, a chemokine, or some other protein or protein domain. Small molecule fusion partners may include any therapeutic agent that directs the Fc fusion to a therapeutic target. Such targets may be any molecule, preferably an extracellular receptor, that is implicated in disease.

Fusion and conjugate partners may be linked to any region of an antibody of the present invention, including at the N- or C- termini, or at some residue in-between the termini. In a preferred embodiment, a fusion or conjugate partner is linked at the N- or C-terminus of the antibody, most preferably the N-terminus. A variety of linkers may find use in the present invention to covalently link antibodies to a fusion or conjugate partner. By "linker", "linker sequence", "spacer", "tethering sequence" or grammatical equivalents thereof, herein is meant a molecule or group of molecules (such as a monomer or polymer) that connects two molecules and often serves to place the two molecules in a preferred configuration. Linkers are known in the art; for example, homo-or hetero-bifunctional linkers as are well known (see, 1994 Pierce Chemical Company catalog, technical section on cross-linkers, pages 155-200, incorporated entirely by reference). A number of strategies may be used to covalently link molecules together. These include, but are not limited to polypeptide linkages between N- and C-termini of proteins or protein domains, linkage via disulfide bonds, and linkage via chemical cross-linking reagents. In one aspect of this embodiment, the linker is a peptide bond, generated by recombinant

techniques or peptide synthesis. The linker may contain amino acid residues that provide flexibility. Thus, the linker peptide may predominantly include the following amino acid residues: Gly, Ser, Ala, or Thr. The linker peptide should have a length that is adequate to link two molecules in such a way that they assume the correct conformation relative to one another so that they retain the desired activity. Suitable lengths for this purpose include at least one and not more than 50 amino acid residues. Preferably, the linker is from about 1 to 30 amino acids in length, with linkers of 1 to 20 amino acids in length being most preferred. Useful linkers include glycine-serine polymers (including, for example, (GS)n, (GSGGS)n (GGGGS)n and (GGGS)n, where n is an integer of at least one), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers, as will be appreciated by those in the art.-Alternatively, a variety of nonproteinaceous polymers, including but not limited to polyethylene glycol (PEG), polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol, may find use as linkers, that is may find use to link the antibodies of the present invention to a fusion or conjugate partner, or to link the antibodies of the present invention to a conjugate.

Production of antibodies

The present invention provides methods for producing and experimentally testing antibodies. The described methods are not meant to constrain the present invention to any particular application or theory of operation. Rather, the provided methods are meant to illustrate generally that one or more antibodies may be produced and experimentally tested to obtain variant antibodies. General methods for antibody molecular biology, expression, purification, and screening are described in Antibody Engineering, edited by Duebel & Kontermann, Springer-Verlag, Heidelberg, 2001; and Hayhurst & Georgiou, 2001, *Curr Opin Chem Biol* 5:683-689; Maynard & Georgiou, 2000, *Annu Rev Biomed Eng* 2:339-76; Antibodies: A Laboratory Manual by Harlow & Lane, New York: Cold Spring Harbor Laboratory Press, 1988, all incorporated entirely by reference.

In one embodiment of the present invention, nucleic acids are created that encode the antibodies, and that may then be cloned into host cells, expressed and assayed, if desired. Thus, nucleic acids, and particularly DNA, may be made that encode each protein sequence. These practices are carried out using well-known procedures. For example, a variety of methods that may find use in the present invention are described in Molecular Cloning - A Laboratory Manual, 3rd Ed. (Maniatis, Cold Spring Harbor Laboratory Press, New York, 2001), and Current Protocols in Molecular Biology (John Wiley & Sons), both incorporated entirely by reference. As will be appreciated by those skilled in the art, the generation of exact sequences for a library comprising

a large number of sequences is potentially expensive and time consuming. By "library" herein is meant a set of variants in any form, including but not limited to a list of nucleic acid or amino acid sequences, a list of nucleic acid or amino acid substitutions at variable positions, a physical library comprising nucleic acids that encode the library sequences, or a physical library comprising the variant proteins, either in purified or unpurified form. Accordingly, there are a variety of techniques that may be used to efficiently generate libraries of the present invention. Such methods that may find use in the present invention are described or referenced in US 6,403,312; USSN 09/782,004; USSN 09/927,790; USSN 10/218,102; PCT WO 01/40091; and PCT WO 02/25588, all incorporated entirely by reference. Such methods include but are not limited to gene assembly methods, PCR-based method and methods which use variations of PCR, ligase chain reaction-based methods, pooled oligo methods such as those used in synthetic shuffling, error-prone amplification methods and methods which use oligos with random mutations, classical site-directed mutagenesis methods, cassette mutagenesis, and other amplification and gene synthesis methods. As is known in the art, there are a variety of commercially available kits and methods for gene assembly, mutagenesis, vector subcloning, and the like, and such commercial products find use in the present invention for generating nucleic acids that encode antibodies.

The antibodies of the present invention may be produced by culturing a host cell transformed with nucleic acid, preferably an expression vector, containing nucleic acid encoding the antibodies, under the appropriate conditions to induce or cause expression of the protein. The conditions appropriate for expression will vary with the choice of the expression vector and the host cell, and will be easily ascertained by one skilled in the art through routine experimentation. A wide variety of appropriate host cells may be used, including but not limited to mammalian cells, bacteria, insect cells, and yeast. For example, a variety of cell lines that may find use in the present invention are described in the ATCC® cell line catalog, available from the American Type Culture Collection.

In a preferred embodiment, the antibodies are expressed in mammalian expression systems, including systems in which the expression constructs are introduced into the mammalian cells using virus such as retrovirus or adenovirus. Any mammalian cells may be used, with human, mouse, rat, hamster, and primate cells being particularly preferred. Suitable cells also include known research cells, including but not limited to Jurkat T cells, NIH3T3, CHO, BHK, COS, HEK293, PER C.6, HeLa, Sp2/0, NS0 cells and variants thereof. In an alternately preferred embodiment, library proteins are expressed in bacterial cells. Bacterial expression systems are well known in the art, and include *Escherichia coli* (*E. coli*), *Bacillus*

subtilis, *Streptococcus cremoris*, and *Streptococcus lividans*. In alternate embodiments, antibodies are produced in insect cells (e.g. Sf21/Sf9, Trichoplusia ni Bti-Tn5b1-4) or yeast cells (e.g. *S. cerevisiae*, *Pichia*, etc). In an alternate embodiment, antibodies are expressed *in vitro* using cell free translation systems. *In vitro* translation systems derived from both prokaryotic (e.g. *E. coli*) and eukaryotic (e.g. wheat germ, rabbit reticulocytes) cells are available and may be chosen based on the expression levels and functional properties of the protein of interest. For example, as appreciated by those skilled in the art, *in vitro* translation is required for some display technologies, for example ribosome display. In addition, the antibodies may be produced by chemical synthesis methods. Also transgenic expression systems both animal (e.g. cow, sheep or goat milk, embryonated hen's eggs, whole insect larvae, etc.) and plant (e.g. corn, tobacco, duckweed, etc.)

The nucleic acids that encode the antibodies of the present invention may be incorporated into an expression vector in order to express the protein. A variety of expression vectors may be utilized for protein expression. Expression vectors may comprise self-replicating extra-chromosomal vectors or vectors which integrate into a host genome. Expression vectors are constructed to be compatible with the host cell type. Thus expression vectors which find use in the present invention include but are not limited to those which enable protein expression in mammalian cells, bacteria, insect cells, yeast, and in *in vitro* systems. As is known in the art, a variety of expression vectors are available, commercially or otherwise, that may find use in the present invention for expressing antibodies.

Expression vectors typically comprise a protein operably linked with control or regulatory sequences, selectable markers, any fusion partners, and/or additional elements. By "operably linked" herein is meant that the nucleic acid is placed into a functional relationship with another nucleic acid sequence. Generally, these expression vectors include transcriptional and translational regulatory nucleic acid operably linked to the nucleic acid encoding the antibody, and are typically appropriate to the host cell used to express the protein. In general, the transcriptional and translational regulatory sequences may include promoter sequences, ribosomal binding sites, transcriptional start and stop sequences, translational start and stop sequences, and enhancer or activator sequences. As is also known in the art, expression vectors typically contain a selection gene or marker to allow the selection of transformed host cells containing the expression vector. Selection genes are well known in the art and will vary with the host cell used.

Antibodies may be operably linked to a fusion partner to enable targeting of the expressed protein, purification, screening, display, and the like. Fusion partners may be linked to the

antibody sequence via a linker sequences. The linker sequence will generally comprise a small number of amino acids, typically less than ten, although longer linkers may also be used. Typically, linker sequences are selected to be flexible and resistant to degradation. As will be appreciated by those skilled in the art, any of a wide variety of sequences may be used as linkers. For example, a common linker sequence comprises the amino acid sequence GGGGS. A fusion partner may be a targeting or signal sequence that directs antibody and any associated fusion partners to a desired cellular location or to the extracellular media. As is known in the art, certain signaling sequences may target a protein to be either secreted into the growth media, or into the periplasmic space, located between the inner and outer membrane of the cell. A fusion partner may also be a sequence that encodes a peptide or protein that enables purification and/or screening. Such fusion partners include but are not limited to polyhistidine tags (His-tags) (for example H_6 and H_{10} or other tags for use with Immobilized Metal Affinity Chromatography (IMAC) systems (e.g. Ni^{+2} affinity columns)), GST fusions, MBP fusions, Strep-tag, the BSP biotinylation target sequence of the bacterial enzyme BirA, and epitope tags which are targeted by antibodies (for example c-myc tags, flag-tags, and the like). As will be appreciated by those skilled in the art, such tags may be useful for purification, for screening, or both. For example, an antibody may be purified using a His-tag by immobilizing it to a Ni^{+2} affinity column, and then after purification the same His-tag may be used to immobilize the antibody to a Ni^{+2} coated plate to perform an ELISA or other binding assay (as described below). A fusion partner may enable the use of a selection method to screen antibodies (see below). Fusion partners that enable a variety of selection methods are well-known in the art, and all of these find use in the present invention. For example, by fusing the members of an antibody library to the gene III protein, phage display can be employed (Kay *et al.*, Phage display of peptides and proteins: a laboratory manual, Academic Press, San Diego, CA, 1996; Lowman *et al.*, 1991, *Biochemistry* 30:10832-10838; Smith, 1985, *Science* 228:1315-1317, incorporated entirely by reference). Fusion partners may enable antibodies to be labeled. Alternatively, a fusion partner may bind to a specific sequence on the expression vector, enabling the fusion partner and associated antibody to be linked covalently or noncovalently with the nucleic acid that encodes them.

The methods of introducing exogenous nucleic acid into host cells are well known in the art, and will vary with the host cell used. Techniques include but are not limited to dextran-mediated transfection, calcium phosphate precipitation, calcium chloride treatment, polybrene mediated transfection, protoplast fusion, electroporation, viral or phage infection, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei. In the case of mammalian cells, transfection may be either transient or stable.

In a preferred embodiment, antibodies are purified or isolated after expression. Proteins may be isolated or purified in a variety of ways known to those skilled in the art. Standard purification methods include chromatographic techniques, including ion exchange, hydrophobic interaction, affinity, sizing or gel filtration, and reversed-phase, carried out at atmospheric pressure or at high pressure using systems such as FPLC and HPLC. Purification methods also include electrophoretic, immunological, precipitation, dialysis, and chromatofocusing techniques. Ultrafiltration and diafiltration techniques, in conjunction with protein concentration, are also useful. As is well known in the art, a variety of natural proteins bind Fc and antibodies, and these proteins can find use in the present invention for purification of antibodies. For example, the bacterial proteins A and G bind to the Fc region. Likewise, the bacterial protein L binds to the Fab region of some antibodies, as of course does the antibody's target antigen. Purification can often be enabled by a particular fusion partner. For example, antibodies may be purified using glutathione resin if a GST fusion is employed, Ni^{+2} affinity chromatography if a His-tag is employed, or immobilized anti-flag antibody if a flag-tag is used. For general guidance in suitable purification techniques, see, e.g. incorporated entirely by reference Protein Purification: Principles and Practice, 3rd Ed., Scopes, Springer-Verlag, NY, 1994, incorporated entirely by reference. The degree of purification necessary will vary depending on the screen or use of the antibodies. In some instances no purification is necessary. For example in one embodiment, if the antibodies are secreted, screening may take place directly from the media. As is well known in the art, some methods of selection do not involve purification of proteins. Thus, for example, if a library of antibodies is made into a phage display library, protein purification may not be performed.

In vitro experimentation

Antibodies may be screened using a variety of methods, including but not limited to those that use *in vitro* assays, *in vivo* and cell-based assays, and selection technologies. Automation and high-throughput screening technologies may be utilized in the screening procedures. Screening may employ the use of a fusion partner or label. The use of fusion partners has been discussed above. By "labeled" herein is meant that the antibodies of the invention have one or more elements, isotopes, or chemical compounds attached to enable the detection in a screen. In general, labels fall into three classes: a) immune labels, which may be an epitope incorporated as a fusion partner that is recognized by an antibody, b) isotopic labels, which may be radioactive or heavy isotopes, and c) small molecule labels, which may include fluorescent and colorimetric dyes, or molecules such as biotin that enable other labeling methods. Labels may be incorporated into the compound at any position and may be incorporated *in vitro* or *in vivo* during protein

expression.

In a preferred embodiment, the functional and/or biophysical properties of antibodies are screened in an *in vitro* assay. *In vitro* assays may allow a broad dynamic range for screening properties of interest. Properties of antibodies that may be screened include but are not limited to stability, solubility, and affinity for Fc ligands, for example FcγRs. Multiple properties may be screened simultaneously or individually. Proteins may be purified or unpurified, depending on the requirements of the assay. In one embodiment, the screen is a qualitative or quantitative binding assay for binding of antibodies to a protein or nonprotein molecule that is known or thought to bind the antibody. In a preferred embodiment, the screen is a binding assay for measuring binding to the target antigen. In an alternately preferred embodiment, the screen is an assay for binding of antibodies to an Fc ligand, including but are not limited to the family of FcγRs, the neonatal receptor FcRn, the complement protein C1q, and the bacterial proteins A and G. Said Fc ligands may be from any organism, with humans, mice, rats, rabbits, and monkeys preferred. Binding assays can be carried out using a variety of methods known in the art, including but not limited to FRET (Fluorescence Resonance Energy Transfer) and BRET (Bioluminescence Resonance Energy Transfer) -based assays, AlphaScreen™ (Amplified Luminescent Proximity Homogeneous Assay), Scintillation Proximity Assay, ELISA (Enzyme-Linked Immunosorbent Assay), SPR (Surface Plasmon Resonance, also known as Biacore™), isothermal titration calorimetry, differential scanning calorimetry, gel electrophoresis, and chromatography including gel filtration. These and other methods may take advantage of some fusion partner or label of the antibody. Assays may employ a variety of detection methods including but not limited to chromogenic, fluorescent, luminescent, or isotopic labels.

The biophysical properties of antibodies, for example stability and solubility, may be screened using a variety of methods known in the art. Protein stability may be determined by measuring the thermodynamic equilibrium between folded and unfolded states. For example, antibodies of the present invention may be unfolded using chemical denaturant, heat, or pH, and this transition may be monitored using methods including but not limited to circular dichroism spectroscopy, fluorescence spectroscopy, absorbance spectroscopy, NMR spectroscopy, calorimetry, and proteolysis. As will be appreciated by those skilled in the art, the kinetic parameters of the folding and unfolding transitions may also be monitored using these and other techniques. The solubility and overall structural integrity of an antibody may be quantitatively or qualitatively determined using a wide range of methods that are known in the art. Methods which may find use in the present invention for characterizing the biophysical properties of antibodies include gel electrophoresis, isoelectric focusing, capillary electrophoresis, chromatography such

as size exclusion chromatography, ion-exchange chromatography, and reversed-phase high performance liquid chromatography, peptide mapping, oligosaccharide mapping, mass spectrometry, ultraviolet absorbance spectroscopy, fluorescence spectroscopy, circular dichroism spectroscopy, isothermal titration calorimetry, differential scanning calorimetry, analytical ultra-centrifugation, dynamic light scattering, proteolysis, and cross-linking, turbidity measurement, filter retardation assays, immunological assays, fluorescent dye binding assays, protein-staining assays, microscopy, and detection of aggregates via ELISA or other binding assay. Structural analysis employing X-ray crystallographic techniques and NMR spectroscopy may also find use. In one embodiment, stability and/or solubility may be measured by determining the amount of protein solution after some defined period of time. In this assay, the protein may or may not be exposed to some extreme condition, for example elevated temperature, low pH, or the presence of denaturant. Because function typically requires a stable, soluble, and/or well-folded/structured protein, the aforementioned functional and binding assays also provide ways to perform such a measurement. For example, a solution comprising an antibody could be assayed for its ability to bind target antigen, then exposed to elevated temperature for one or more defined periods of time, then assayed for antigen binding again. Because unfolded and aggregated protein is not expected to be capable of binding antigen, the amount of activity remaining provides a measure of the antibody's stability and solubility.

In a preferred embodiment, the library is screened using one or more cell-based or *in vitro* assays. For such assays, antibodies, purified or unpurified, are typically added exogenously such that cells are exposed to individual variants or groups of variants belonging to a library. These assays are typically, but not always, based on the biology of the ability of the antibody to bind to antigen and mediate some biochemical event, for example effector functions like cellular lysis, phagocytosis, ligand/receptor binding inhibition, inhibition of growth and/or proliferation, apoptosisand the like. Such assays often involve monitoring the response of cells to antibody, for example cell survival, cell death, cellular phagocytosis, cell lysis, change in cellular morphology, or transcriptional activation such as cellular expression of a natural gene or reporter gene. For example, such assays may measure the ability of antibodies to elicit ADCC, ADCP, or CDC. For some assays additional cells or components, that is in addition to the target cells, may need to be added, for example serum complement, or effector cells such as peripheral blood monocytes (PBMCs), NK cells, macrophages, and the like. Such additional cells may be from any organism, preferably humans, mice, rat, rabbit, and monkey. Crosslinked or monomeric antibodies may cause apoptosis of certain cell lines expressing the antibody's target antigen, or they may mediate attack on target cells by immune cells which have been added to the assay. Methods for

monitoring cell death or viability are known in the art, and include the use of dyes, fluorophores, immunochemical, cytochemical, and radioactive reagents. For example, caspase assays or annexin-flourconjugates may enable apoptosis to be measured, and uptake or release of radioactive substrates (e.g. Chromium-51 release assays) or the metabolic reduction of fluorescent dyes such as alamar blue may enable cell growth, proliferationor activation to be monitored. In a preferred embodiment, the DELFIA® EuTDA-based cytotoxicity assay (Perkin Elmer, MA) is used. Alternatively, dead or damaged target cells may be monitored by measuring the release of one or more natural intracellular proteins, for example lactate dehydrogenase. Transcriptional activation may also serve as a method for assaying function in cell-based assays. In this case, response may be monitored by assaying for natural genes or proteins which may be upregulated or down-regulated, for example the release of certain interleukins may be measured, or alternatively readout may be via a luciferase or GFP-reporter construct. Cell-based assays may also involve the measure of morphological changes of cells as a response to the presence of an antibody. Cell types for such assays may be prokaryotic or eukaryotic, and a variety of cell lines that are known in the art may be employed. Alternatively, cell-based screens are performed using cells that have been transformed or transfected with nucleic acids encoding the antibodies.

In vitro assays include but are not limited to binding assays, ADCC, CDC, phagocytosis, cytotoxicity, proliferation, apoptosis, necrosis, cell cycle arrest, peroxide/ozone release, chemotaxis of effector cells, inhibition of such assays by reduced effector function antibodies; ranges of activities such as >100x improvement or >100x reduction, blends of receptor activation and the assay outcomes that are expected from such receptor profiles.

In vivo experimentation

The biological properties of the antibodies of the present invention may be characterized in cell, tissue, and whole organism experiments. As is know in the art, drugs are often tested in animals, including but not limited to mice, rats, rabbits, dogs, cats, pigs, and monkeys, in order to measure a drug's efficacy for treatment against a disease or disease model, or to measure a drug's pharmacokinetics, toxicity, and other properties. Said animals may be referred to as disease models. With respect to the antibodies of the present invention, a particular challenge arises when using animal models to evaluate the potential for in-human efficacy of candidate polypeptides – this is due, at least in part, to the fact that antibodies that have a specific effect on the affinity for a human Fc receptor may not have a similar affinity effect with the orthologous animal receptor. These problems can be further exacerbated by the inevitable ambiguities associated with correct assignment of true orthologues (Mechetina *et al.*, *Immunogenetics*, 2002 54:463–468, incorporated entirely by reference), and the fact that some orthologues simply do not exist in the

animal (e.g. humans possess an FcγRIIa whereas mice do not). Therapeutics are often tested in mice, including but not limited to nude mice, SCID mice, xenograft mice, and transgenic mice (including knockins and knockouts). For example, an antibody of the present invention that is intended as an anti-cancer therapeutic may be tested in a mouse cancer model, for example a xenograft mouse. In this method, a tumor or tumor cell line is grafted onto or injected into a mouse, and subsequently the mouse is treated with the therapeutic to determine the ability of the antibody to reduce or inhibit cancer growth and metastasis. An alternative approach is the use of a SCID murine model in which immune-deficient mice are injected with human Periferal Blood Lymphocytes (PBLs), conferring a semi-functional and human immune system – with an appropriate array of human FcRs - to the mice that have subsequently been injected with antibodies or Fc-polypeptides that target injected human tumor cells. In such a model, the Fc-polypeptides that target the desired antigen (such as her2/neu on SkOV3 ovarian cancer cells) interact with human PBLs within the mice to engage tumoricidal effector functions. Such experimentation may provide meaningful data for determination of the potential of said antibody to be used as a therapeutic. Any organism, preferably mammals, may be used for testing. For example because of their genetic similarity to humans, monkeys can be suitable therapeutic models, and thus may be used to test the efficacy, toxicity, pharmacokinetics, or other property of the antibodies of the present invention. Tests of the antibodies of the present invention in humans are ultimately required for approval as drugs, and thus of course these experiments are contemplated. Thus the antibodies of the present invention may be tested in humans to determine their therapeutic efficacy, toxicity, pharmacokinetics, and/or other clinical properties.

The antibodies of the present invention may confer superior performance on Fc-containing therapeutics in animal models or in humans. The receptor binding profiles of such antibodies, as described in this specification, may, for example, be selected to increase the potency of cytotoxic drugs or to target specific effector functions or effector cells to improve the selectivity of the drug's action. Further, receptor binding profiles can be selected that may reduce some or all effector functions thereby reducing the side-effects or toxicity of such Fc-containing drug. For example, an antibody with reduced binding to FcγRIIIa, FcγRI and FcγRIIa can be selected to eliminate most cell-mediated effector function, or an antibody with reduced binding to C1q may be selected to limit complement-mediated effector functions. In some contexts, such effector functions are known to have potential toxic effects, therefore eliminating them may increase the safety of the Fc-bearing drug and such improved safety may be characterized in animal models. In some contexts, such effector functions are known to mediate the desirable therapeutic activity, therefore enhancing them may increase the activity or potency of the Fc-

bearing drug and such improved activity or potency may be characterized in animal models.

Optimized antibodies can be tested in a variety of orthotopic tumor models. These clinically relevant animal models are important in the study of pathophysiology and therapy of aggressive cancers like pancreatic, prostate and breast cancer. Immune deprived mice including, but not limited to athymic nude or SCID mice are frequently used in scoring of local and systemic tumor spread from the site of intraorgan (e.g. pancreas, prostate or mammary gland) injection of human tumor cells or fragments of donor patients.

In preferred embodiments, antibodies of the present invention may be assessed for efficacy in clinically relevant animal models of various human diseases. In many cases, relevant models include various transgenic animals for specific tumor antigens.

Relevant transgenic models such as those that express human Fc receptors (e.g., CD16 including the gamma chain, FcγR1, RIIa/b, and others) could be used to evaluate and test antibodies and Fc-fusions in their efficacy. The evaluation of antibodies by the introduction of human genes that directly or indirectly mediate effector function in mice or other rodents that may enable physiological studies of efficacy in tumor toxicity or other diseases such as autoimmune disorders and RA. Human Fc receptors such as FcγRIIIa may possess polymorphisms such as that in position 158 V or F which would further enable the introduction of specific and combinations of human polymorphisms into rodents. The various studies involving polymorphism-specific FcRs are not limited to this section, however, and encompasses all discussions and applications of FcRs in general as specified in throughout this application. antibodies of the present invention may confer superior activity on Fc-containing drugs in such transgenic models, in particular variants with binding profiles optimized for human FcγRIIIa mediated activity may show superior activity in transgenic CD16 mice. Similar improvements in efficacy in mice transgenic for the other human Fc receptors, e.g. FcγRIIa, FcγRI, etc., may be observed for antibodies with binding profiles optimized for the respective receptors. Mice transgenic for multiple human receptors would show improved activity for antibodies with binding profiles optimized for the corresponding multiple receptors, for example as outlined in Figure 5.

Because of the difficulties and ambiguities associated with using animal models to characterize the potential efficacy of candidate therapeutic antibodies in a human patient, some variant polypeptides of the present invention may find utility as proxies for assessing potential in-human efficacy. Such proxy molecules would preferably mimic – in the animal system - the FcR and/or complement biology of a corresponding candidate human antibody. This mimicry is most

likely to be manifested by relative association affinities between specific antibodies and animal vs. human receptors. For example, if one were using a mouse model to assess the potential in-human efficacy of an antibody that has enhanced affinity for human FcγRIIIa, an appropriate proxy variant would have enhanced affinity for mouse FcγRIII-2 (mouse CD16-2). Alternatively if one were using a mouse model to assess the potential in-human efficacy of an antibody that has reduced affinity for the inhibitory human FcγRIIb, an appropriate proxy variant would have reduced affinity for mouse FcγRII. It should also be noted that the proxy antibodies could be created in the context of a human antibody, an animal antibody, or both.

In a preferred embodiment, the testing of antibodies may include study of efficacy in primates (e.g. cynomolgus monkey model) to facilitate the evaluation of depletion of specific target cells harboring target antigen. Additional primate models include but not limited to that of the rhesus monkey and Fc polypetides in therapeutic studies of autoimmune, transplantation and cancer.

Toxicity studies are performed to determine the antibody or Fc-fusion related-effects that cannot be evaluated in standard pharmacology profile or occur only after repeated administration of the agent. Most toxicity tests are performed in two species – a rodent and a non-rodent – to ensure that any unexpected adverse effects are not overlooked before new therapeutic entities are introduced into man. In general, these models may measure a variety of toxicities including genotoxicity, chronic toxicity, immunogenicity, reproductive/developmental toxicity and carcinogenicity. Included within the aforementioned parameters are standard measurement of food consumption, bodyweight, antibody formation, clinical chemistry, and macro- and microscopic examination of standard organs/tissues (e.g. cardiotoxicity). Additional parameters of measurement are injection site trauma and the measurement of neutralizing antibodies, if any. Traditionally, monoclonal antibody therepeutics, naked or conjugated are evaluated for cross-reactivity with normal tissues, immunogenicity/antibody production, conjugate or linker toxicity and "bystander" toxicity of radiolabeled species. Nonetheless, such studies may have to be individualized to address specific concerns and following the guidance set by ICH S6 (Safety studies for biotechnological products also noted above). As such, the general principles are that the products are sufficiently well characterized and for which impurities/contaminants have been removed, that the test material is comparable throughout development, and GLP compliance.

The pharmacokinetics (PK) of the antibodies of the invention can be studied in a variety of animal systems, with the most relevant being non-human primates such as the cynomolgus, rhesus monkeys. Single or repeated i.v./s.c. administrations over a dose range of 6000-fold (0.05-

300 mg/kg) can be evaluated for the half-life (days to weeks) using plasma concentration and clearance as well as volume of distribution at a steady state and level of systemic absorbance can be measured. Examples of such parameters of measurement generally include maximum observed plasma concentration (Cmax), the time to reach Cmax (Tmax), the area under the plasma concentration-time curve from time 0 to infinity [AUC(0-inf] and apparent elimination half-life (T1/2). Additional measured parameters could include compartmental analysis of concentration-time data obtained following i.v. administration and bioavailability. Examples of pharmacological/toxicological studies using cynomolgus have been established for Rituxan and Zevalin in which monoclonal antibodies to CD20 are cross-reactive. Biodistribution, dosimetry (for radiolabled antibodies), and PK studies can also be done in rodent models. Such studies would evaluate tolerance at all doses administered, toxicity to local tissues, preferential localization to rodent xenograft animal models, depletion of target cells (e.g. CD20 positive cells).

The antibodies of the present invention may confer superior pharmacokinetics on Fc-containing therapeutics in animal systems or in humans. For example, increased binding to FcRn may increase the half-life and exposure of the Fc-containing drug. Alternatively, decreased binding to FcRn may decrease the half-life and exposure of the Fc-containing drug in cases where reduced exposure is favorable such as when such drug has side-effects.

It is known in the art that the array of Fc receptors is differentially expressed on various immune cell types, as well as in different tissues. Differential tissue distribution of Fc receptors may ultimately have an impact on the pharmacodynamic (PD) and pharmacokinetic (PK) properties of antibodies of the present invention. Because antibodies of the presentation have varying affinities for the array of Fc receptors, further screening of the polypeptides for PD and/or PK properties may be extremely useful for defining the optimal balance of PD, PK, and therapeutic efficacy conferred by each candidate polypeptide.

Pharmacodynamic studies may include, but are not limited to, targeting specific tumor cells or blocking signaling mechanisms, measuring depletion of target antigen expressing cells or signals, etc. The antibodies of the present invention may target particular effector cell populations and thereby direct Fc-containing drugs to recruit certain activities to improve potency or to increase penetration into a particularly favorable physiological compartment. For example, neutrophil activity and localization can be targeted by an antibody that preferentially targets FcγRIIIb. Such pharmacodynamic effects may be demonstrated in animal models or in humans.

Clinical use

The antibodies of the present invention may be used for various therapeutic purposes. As will be appreciated by those in the art, the antibodies of the present invention may be used for any therapeutic purpose that uses antibodies and the like. In a preferred embodiment, the antibodies are administered to a patient to treat disorders including but not limited to cancer, autoimmune and inflammatory diseases, and infectious diseases.

A "patient" for the purposes of the present invention includes both humans and other animals, preferably mammals and most preferably humans. Thus the antibodies of the present invention have both human therapy and veterinary applications. The term "treatment" or "treating" in the present invention is meant to include therapeutic treatment, as well as prophylactic, or suppressive measures for a disease or disorder. Thus, for example, successful administration of an antibody prior to onset of the disease results in treatment of the disease. As another example, successful administration of an optimized antibody after clinical manifestation of the disease to combat the symptoms of the disease comprises treatment of the disease. "Treatment" and "treating" also encompasses administration of an optimized antibody after the appearance of the disease in order to eradicate the disease. Successful administration of an agent after onset and after clinical symptoms have developed, with possible abatement of clinical symptoms and perhaps amelioration of the disease, comprises treatment of the disease. Those "in need of treatment" include mammals already having the disease or disorder, as well as those prone to having the disease or disorder, including those in which the disease or disorder is to be prevented.

In one embodiment, an antibody of the present invention is administered to a patient having a disease involving inappropriate expression of a protein or other molecule. Within the scope of the present invention this is meant to include diseases and disorders characterized by aberrant proteins, due for example to alterations in the amount of a protein present, protein localization, posttranslational modification, conformational state, the presence of a mutant or pathogen protein, etc. Similarly, the disease or disorder may be characterized by alterations molecules including but not limited to polysaccharides and gangliosides. An overabundance may be due to any cause, including but not limited to overexpression at the molecular level, prolonged or accumulated appearance at the site of action, or increased activity of a protein relative to normal. Included within this definition are diseases and disorders characterized by a reduction of a protein. This reduction may be due to any cause, including but not limited to reduced expression at the molecular level, shortened or reduced appearance at the site of action, mutant forms of a protein, or decreased activity of a protein relative to normal. Such an overabundance

or reduction of a protein can be measured relative to normal expression, appearance, or activity of a protein, and said measurement may play an important role in the development and/or clinical testing of the antibodies of the present invention.

By "cancer" and "cancerous" herein refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to carcinoma, lymphoma, blastoma, sarcoma (including liposarcoma), neuroendocrine tumors, mesothelioma, schwanoma, meningioma, adenocarcinoma, melanoma, and leukemia or lymphoid malignancies.

More particular examples of such cancers include hematologic malignancies, such as non-Hodgkin's lymphomas (NHL). NHL cancers include but are not limited to Burkitt's lymphoma (BL), small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL), mantle cell lymphoma (MCL), follicular lymphoma (FL), diffuse large B-cell lymphoma (DLCL), marginal zone lymphoma (MZL), hairy cell leukemia (HCL) and lymphoplasmacytic leukemia (LPL), extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT), nodal marginal zone B cell lymphoma, mediastinal large cell lymphoma, intravascular large cell lymphoma, primary effusion lymphoma, precursor B-lymphoblastic leukemia/lymphoma, precursor T- and NK-cells lymphoma (precursor T lymphoblastic lymphoma, blastic NK cell lymphoma), tumors of the mature T and NK cells, including peripheral T-cell lymphoma and leukemia (PTL), adult T-cell leukemia/T-cell lymphomas and large granular lymphocytic leukemia, T-cell chronic lymphocytic leukemia/prolymphocytic leukemia, T-cell large granular lymphocytic leukemia, aggressive NK-cell leukemia, extranodal T-/NK cell lymphoma, enteropathy-type T-cell lymphoma, hepatosplenic T-cell lymphoma, anaplastic large cell lymphoma (ALCL), angiocetric and angioimmunoblastic T-cell lymphoma, mycosis fungoides/Sézary syndrome, and cutaneous T-cell lymphoma (CTCL). Other cancers that may be treatable by the antibodies of the invention include but are not limited to Hodgkin's lymphoma, tumors of lymphocyte precursor cells, including B-cell acute lymphoblastic leukemia/lymphoma (B-ALL), and T-cell acute lymphoblastic leukemia/lymphoma (T-ALL), thymoma, Langerhans cell histocytosis, multiple myeloma, myeloid neoplasias such as acute myelogenous leukemias (AML), including AML with maturation, AML without differentiation, acute promyelocytic leukemia, acute myelomonocytic leukemia, and acute monocytic leukemias, myelodysplastic syndromes, and chronic myeloproliferative disorders (MDS), including chronic myelogenous leukemia (CML). Other cancers that may be treatable by the antibodies of the invenntion include but are not limited to tumors of the central nervous system such as glioma, glioblastoma, neuroblastoma, astrocytoma, medulloblastoma, ependymoma, and retinoblastoma; solid tumors

of the head and neck (eg. nasopharyngeal cancer, salivary gland carcinoma, and esophagael cancer), lung (eg. small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung), digestive system (eg. gastric or stomach cancer including gastrointestinal cancer, cancer of the bile duct or biliary tract, colon cancer, rectal cancer, colorectal cancer, and anal carcinoma), reproductive system (eg. testicular, penile, or prostate cancer, uterine, vaginal, vulval, cervical, ovarian, and endometrial cancer), skin (eg. melanoma, basal cell carcinoma, squamous cell cancer, actinic keratosis), liver (eg. liver cancer, hepatic carcinoma, hepatocellular cancer, and hepatoma), bone (eg. osteoclastoma, and osteolytic bone cancers) additional tissues and organs (eg. pancreatic cancer, bladder cancer, kidney or renal cancer, thyroid cancer, breast cancer, cancer of the peritoneum, and Kaposi's sarcoma), and tumors of the vascular system (eg. angiosarcoma and hemagiopericytoma).

Preferred oncology indications that may be treated by anti-CD19 antibodies of the invention include but are not limited to all non-Hodgkin's lymphomas (NHL), especially refractory/resistant NHL, chronic lymphocytic leukemia (CLL), B-cell acute lymphoblastic leukemia/lymphoma (B-ALL), and mantle cell lymphoma (MCL).

Autoimmunity results from a breakdown of self-tolerance involving humoral and/or cell-mediated immune mechanisms in. Among of the consequences of failure in central and/or peripheral tolerance, are survival and activation of self-reactive B cells and T cells. Several autoimmune diseases are defined by excessive activation of both B and/or T lymphocytes. Activation of these cells requires in cooperation, antigen engagement and co-stimulatory signals from interacting lymphocytes. Antibody-mediated depletion, inhibition, anti-proliferation, and/or blockade of B cells are therapeutic approaches for the treatment of autoimmune disease.

By "autoimmune diseases" herein include allogenic islet graft rejection, alopecia areata, ankylosing spondylitis, antiphospholipid syndrome, autoimmune Addison's disease, antineutrophil cytoplasmic autoantibodies (ANCA), autoimmune diseases of the adrenal gland, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune myocarditis, autoimmune neutropenia, autoimmune oophoritis and orchitis, autoimmune thrombocytopenia, autoimmune urticaria, Behcet's disease, bullous pemphigoid, cardiomyopathy, Castleman's syndrome, celiac spruce-dermatitis, chronic fatigue immune disfunction syndrome, chronic inflammatory demyelinating polyneuropathy, Churg-Strauss syndrome, cicatrical pemphigoid, CREST syndrome, cold agglutinin disease, Crohn's disease, dermatomyositis, discoid lupus, essential mixed cryoglobulinemia, factor VIII deficiency, fibromyalgia-fibromyositis, glomerulonephritis, Grave's disease, Guillain-Barre, Goodpasture's syndrome, graft-versus-host disease (GVHD), Hashimoto's thyroiditis, hemophilia A, idiopathic pulmonary fibrosis, idiopathic

thrombocytopenia purpura (ITP), IgA neuropathy, IgM polyneuropathies, immune mediated thrombocytopenia, juvenile arthritis, Kawasaki's disease, lichen plantus, lupus erthematosis, Meniere's disease, mixed connective tissue disease, multiple sclerosis (MS), type 1 diabetes mellitus, myasthenia gravis, pemphigus vulgaris, pernicious anemia, polyarteritis nodosa, polychrondritis, polyglandular syndromes, polymyalgia rheumatica, polymyositis and dermatomyositis, primary agammaglobinulinemia, primary biliary cirrhosis, psoriasis, psoriatic arthritis, Reynauld's phenomenon, Reiter's syndrome, rheumatoid arthritis (RA), sarcoidosis, scleroderma, Sjogren's syndrome, solid organ transplant rejection, stiff-man syndrome, systemic lupus erythematosus (SLE), takayasu arteritis, temporal arteristis / giant cell arteritis, thrombotic thrombocytopenia purpura, ulcerative colitis, uveitis, vasculitides such as dermatitis herpetiformis vasculitis, vitiligo, and Wegner's granulomatosis.

Preferred autoimmune indications that may be treated by anti-CD19 antibodies of the invention include but are not limited to rheumatoid arthritis (RA), systemic lupus erythematosus (SLE or lupus), multiple sclerosis, Sjogren's syndrome, and idiopathic thrombocytopenia purpura (ITP).

By "inflammatory disorders" herein include acute respiratory distress syndrome (ARDS), acute septic arthritis, adjuvant arthritis, juvenile idiopathic arthritis, allergic encephalomyelitis, allergic rhinitis, allergic vasculitis, allergy, asthma, atherosclerosis, chronic inflammation due to chronic bacterial or viral infectionis, chronic obstructive pulmonary disease (COPD), coronary artery disease, encephalitis, inflammatory bowel disease, inflammatory osteolysis, inflammation associated with acute and delayed hypersensitivity reactions, inflammation associated with tumors, peripheral nerve injury or demyelinating diseases, inflammation associated with tissue trauma such as burns and ischemia, inflammation due to meningitis, multiple organ injury syndrome, pulmonary fibrosis, sepsis and septic shock, Stevens-Johnson syndrome, undifferentiated arthropy, and undifferentiated spondyloarthropathy.

By "infectious diseases" herein include diseases caused by pathogens such as viruses, bacteria, fungi, protozoa, and parasites. Infectious diseases may be caused by viruses including adenovirus, cytomegalovirus, dengue, Epstein-Barr, hanta, hepatitis A, hepatitis B, hepatitis C, herpes simplex type I, herpes simplex type II, human immunodeficiency virus, (HIV), human papilloma virus (HPV), influenza, measles, mumps, papova virus, polio, respiratory syncytial virus, rinderpest, rhinovirus, rotavirus, rubella, SARS virus, smallpox, viral meningitis, and the like. Infections diseases may also be caused by bacteria including Bacillus antracis, Borrelia burgdorferi, Campylobacter jejuni, Chlamydia trachomatis, Clostridium botulinum, Clostridium tetani, Diptheria, E. coli, Legionella, Helicobacter pylori, Mycobacterium rickettsia, Mycoplasma

nesisseria, Pertussis, Pseudomonas aeruginosa, S. pneumonia, Streptococcus, Staphylococcus, Vibria cholerae, Yersinia pestis, and the like. Infectious diseases may also be caused by fungi such as Aspergillus fumigatus, Blastomyces dermatitidis, Candida albicans, Coccidioides immitis, Cryptococcus neoformans, Histoplasma capsulatum, Penicillium marneffei, and the like. Infectious diseases may also be caused by protozoa and parasites such as chlamydia, kokzidioa, leishmania, malaria, rickettsia, trypanosoma, and the like.

Furthermore, antibodies of the present invention may be used to prevent or treat additional conditions including but not limited to heart conditions such as congestive heart failure (CHF), myocarditis and other conditions of the myocardium; skin conditions such as rosecea, acne, and eczema; bone and tooth conditions such as bone loss, osteoporosis, Paget's disease, Langerhans' cell histiocytosis, periodontal disease, disuse osteopenia, osteomalacia, monostotic fibrous dysplasia, polyostotic fibrous dysplasia, bone metastasis, bone pain management, humoral malignant hypercalcemia, periodontal reconstruction, spinal cord injury, and bone fractures; metabolic conditions such as Gaucher's disease; endocrine conditions such as Cushing's syndrome; and neurological conditions.

A number of the receptors that may interact with the antibodies of the present invention are polymorphic in the human population. For a given patient or population of patients, the efficacy of the antibodies of the present invention may be affected by the presence or absence of specific polymorphisms in proteins. For example, FcγRIIIA is polymorphic at position 158, which is commonly either V (high affinity) or F (low affinity). Patients with the V/V homozygous genotype are observed to have a better clinical response to treatment with the anti-CD20 antibody Rituxan® (rituximab), likely because these patients mount a stronger NK response (Dall'Ozzo *et al.* (2004) Cancer Res. 64:4664-9, incorporated entirely by reference). Additional polymorphisms include but are not limited to FcγRIIA R131 or H131, and such polymorphisms are known to either increase or decrease Fc binding and subsequent biological activity, depending on the polymorphism. antibodies of the present invention may bind preferentially to a particular polymorphic form of a receptor, for example FcγRIIIA 158 V, or to bind with equivalent affinity to all of the polymorphisms at a particular position in the receptor, for example both the 158V and 158F polymorphisms of FcγRIIIA. In a preferred embodiment, antibodies of the present invention may have equivalent binding to polymorphisms may be used in an antibody to eliminate the differential efficacy seen in patients with different polymorphisms. Such a property may give greater consistency in therapeutic response and reduce non-responding patient populations. Such variant Fc with indentical binding to receptor polymorphisms may have increased biological activity, such as ADCC, CDC or circulating half-

life, or alternatively decreased activity, via modulation of the binding to the relevant Fc receptors. In a preferred embodiment, antibodies of the present invention may bind with higher or lower affinity to one of the polymorphisms of a receptor, either accentuating the existing difference in binding or reversing the difference. Such a property may allow creation of therapeutics particularly tailored for efficacy with a patient population possessing such polymorphism. For example, a patient population possessing a polymorphism with a higher affinity for an inhibitory receptor such as FcγRIIB could receive a drug containing an antibody with reduced binding to such polymorphic form of the receptor, creating a more efficacious drug.

In a preferred embodiment, patients are screened for one or more polymorphisms in order to predict the efficacy of the antibodies of the present invention. This information may be used, for example, to select patients to include or exclude from clinical trials or, post-approval, to provide guidance to physicians and patients regarding appropriate dosages and treatment options. For example, in patients that are homozygous or heterozygous for FcγRIIIA 158F antibody drugs such as the anti-CD20 mAb, Rituximab are minimially effective (Carton 2002 Blood 99: 754-758; Weng 2003 J. Clin. Oncol. 21:3940-3947, both incorporated entirely by reference); such patients may show a much better clinical response to the antibodies of the present invention. In one embodiment, patients are selected for inclusion in clinical trials for an antibody of the present invention if their genotype indicates that they are likely to respond significantly better to an antibody of the present invention as compared to one or more currently used antibody therapeutics. In another embodiment, appropriate dosages and treatment regimens are determined using such genotype information. In another embodiment, patients are selected for inclusion in a clinical trial or for receipt of therapy post-approval based on their polymorphism genotype, where such therapy contains an antibody engineered to be specifically efficacious for such population, or alternatively where such therapy contains an antibody that does not show differential activity to the different forms of the polymorphism.

Included in the present invention are diagnostic tests to identify patients who are likely to show a favorable clinical response to an antibody of the present invention, or who are likely to exhibit a significantly better response when treated with an antibody of the present invention versus one or more currently used antibody therapeutics. Any of a number of methods for determining FcγR polymorphisms in humans known in the art may be used.

Furthermore, the present invention comprises prognostic tests performed on clinical samples such as blood and tissue samples. Such tests may assay for effector function activity, including but not limited to ADCC, CDC, phagocytosis, and opsonization, or for killing, regardless of mechanism, of cancerous or otherwise pathogenic cells. In a preferred embodiment,

ADCC assays, such as those described previously, are used to predict, for a specific patient, the efficacy of a given antibody of the present invention. Such information may be used to identify patients for inclusion or exclusion in clinical trials, or to inform decisions regarding appropriate dosages and treatment regemins. Such information may also be used to select a drug that contains a particular antibody that shows superior activity in such assay.

Formulation

Pharmaceutical compositions are contemplated wherein an antibody of the present invention and and one or more therapeutically active agents are formulated. Formulations of the antibodies of the present invention are prepared for storage by mixing said antibody having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed., 1980, incorporated entirely by reference), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, acetate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl orbenzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; sweeteners and other flavoring agents; fillers such as microcrystalline cellulose, lactose, corn and other starches; binding agents; additives; coloring agents; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG). In a preferred embodiment, the pharmaceutical composition that comprises the antibody of the present invention may be in a water-soluble form, such as being present as pharmaceutically acceptable salts, which is meant to include both acid and base addition salts. "Pharmaceutically acceptable acid addition salt" refers to those salts that retain the biological effectiveness of the free bases and that are not biologically or otherwise undesirable, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and organic acids such as acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric

acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like. "Pharmaceutically acceptable base addition salts" include those derived from inorganic bases such as sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like. Particularly preferred are the ammonium, potassium, sodium, calcium, and magnesium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, and ethanolamine. The formulations to be used for *in vivo* administration are preferably sterile. This is readily accomplished by filtration through sterile filtration membranes or other methods.

The antibodies disclosed herein may also be formulated as immunoliposomes. A liposome is a small vesicle comprising various types of lipids, phospholipids and/or surfactant that is useful for delivery of a therapeutic agent to a mammal. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein *et al.*, 1985, *Proc Natl Acad Sci USA*, 82:3688; Hwang *et al.*, 1980, *Proc Natl Acad Sci USA*, 77:4030; US 4,485,045; US 4,544,545; and PCT WO 97/38731, all incorporated entirely by reference. Liposomes with enhanced circulation time are disclosed in US 5,013,556, incorporated entirely by reference. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes. Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. A chemotherapeutic agent or other therapeutically active agent is optionally contained within the liposome (Gabizon *et al.*, 1989, *J National Cancer Inst* 81:1484, incorporated entirely by reference).

The antibody and other therapeutically active agents may also be entrapped in microcapsules prepared by methods including but not limited to coacervation techniques, interfacial polymerization (for example using hydroxymethylcellulose or gelatin-microcapsules, or poly-(methylmethacylate) microcapsules), colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), and macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed., 1980, incorporated entirely by reference. Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable

matrices of solid hydrophobic polymer, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (US 3,773,919, incorporated entirely by reference), copolymers of L-glutamic acid and gamma ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the Lupron Depot® (which are injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), poly-D-(-)-3-hydroxybutyric acid, and ProLease® (commercially available from Alkermes), which is a microsphere-based delivery system composed of the desired bioactive molecule incorporated into a matrix of poly-DL-lactide-co-glycolide (PLG).

Administration

Administration of the pharmaceutical composition comprising an antibody of the present invention, preferably in the form of a sterile aqueous solution, may be done in a variety of ways, including, but not limited to orally, subcutaneously, intravenously, intranasally, intraotically, transdermally, topically (e.g., gels, salves, lotions, creams, etc.), intraperitoneally, intramuscularly, intrapulmonary, vaginally, parenterally, rectally, or intraocularly. In some instances, for example for the treatment of wounds, inflammation, etc., the antibody may be directly applied as a solution or spray. As is known in the art, the pharmaceutical composition may be formulated accordingly depending upon the manner of introduction.

Subcutaneous administration may be preferable in some circumstances because the patient may self-administer the pharmaceutical composition. Many protein therapeutics are not sufficiently potent to allow for formulation of a therapeutically effective dose in the maximum acceptable volume for subcutaneous administration. This problem may be addressed in part by the use of protein formulations comprising arginine-HCl, histidine, and polysorbate (see WO 04091658, incorporated entirely by reference). Antibodies of the present invention may be more amenable to subcutaneous administration due to, for example, increased potency, improved serum half-life, or enhanced solubility.

As is known in the art, protein therapeutics are often delivered by IV infusion or bolus. The antibodies of the present invention may also be delivered using such methods. For example, administration may venious be by intravenous infusion with 0.9% sodium chloride as an infusion vehicle.

Pulmonary delivery may be accomplished using an inhaler or nebulizer and a formulation comprising an aerosolizing agent. For example, AERx® inhalable technology commercially

available from Aradigm, or Inhance™ pulmonary delivery system commercially available from Nektar Therapeutics may be used. Antibodies of the present invention may be more amenable to intrapulmonary delivery. FcRn is present in the lung, and may promote transport from the lung to the bloodstream (e.g. Syntonix WO 04004798, Bitonti *et al.* (2004) Proc. Nat. Acad. Sci. 101:9763-8, both incorporated entirely by reference). Accordingly, antibodes that bind FcRn more effectively in the lung or that are released more efficiently in the bloodstream may have improved bioavailability following intrapulmonary administration. Antibodies of the present invention may also be more amenable to intrapulmonary administration due to, for example, improved solubility or altered isoelectric point.

Furthermore, antibodies of the present invention may be more amenable to oral delivery due to, for example, improved stability at gastric pH and increased resistance to proteolysis. Furthermore, FcRn appears to be expressed in the intestinal epithelia of adults (Dickinson *et al.* (1999) J. Clin. Invest. 104:903-11, incorporated entirely by reference), so antibodies of the present invention with improved FcRn interaction profiles may show enhanced bioavailability following oral administration. FcRn mediated transport of antibodies may also occur at other mucus membranes such as those in the gastrointestinal, respiratory, and genital tracts (Yoshida *et al.* (2004) Immunity 20:769-83, incorporated entirely by reference).

In addition, any of a number of delivery systems are known in the art and may be used to administer the antibodies of the present invention. Examples include, but are not limited to, encapsulation in liposomes, microparticles, microspheres (eg. PLA/PGA microspheres), and the like. Alternatively, an implant of a porous, non-porous, or gelatinous material, including membranes or fibers, may be used. Sustained release systems may comprise a polymeric material or matrix such as polyesters, hydrogels, poly(vinylalcohol),polylactides, copolymers of L-glutamic acid and ethyl-L-gutamate, ethylene-vinyl acetate, lactic acid-glycolic acid copolymers such as the Lupron Depot®, and poly-D-(-)-3-hydroxyburyric acid. It is also possible to administer a nucleic acid encoding the antibody of the current invention, for example by retroviral infection, direct injection, or coating with lipids, cell surface receptors, or other transfection agents. In all cases, controlled release systems may be used to release the antibody at or close to the desired location of action.

Dosing

The dosing amounts and frequencies of administration are, in a preferred embodiment, selected to be therapeutically or prophylactically effective. As is known in the art, adjustments for protein degradation, systemic versus localized delivery, and rate of new protease synthesis, as

well as the age, body weight, general health, sex, diet, time of administration, drug interaction and the severity of the condition may be necessary, and will be ascertainable with routine experimentation by those skilled in the art.

The concentration of the therapeutically active antibody in the formulation may vary from about 0.1 to 100 weight %. In a preferred embodiment, the concentration of the antibody is in the range of 0.003 to 1.0 molar. In order to treat a patient, a therapeutically effective dose of the antibody of the present invention may be administered. By "therapeutically effective dose" herein is meant a dose that produces the effects for which it is administered. The exact dose will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques. Dosages may range from 0.0001 to 100 mg/kg of body weight or greater, for example 0.1, 1, 10, or 50 mg/kg of body weight, with 1 to 10mg/kg being preferred.

In some embodiments, only a single dose of the antibody is used. In other embodiments, multiple doses of the antibody are administered. The elapsed time between administrations may be less than 1 hour, about 1 hour, about 1-2 hours, about 2-3 hours, about 3-4 hours, about 6 hours, about 12 hours, about 24 hours, about 48 hours, about 2-4 days, about 4-6 days, about 1 week, about 2 weeks, or more than 2 weeks.

In other embodiments the antibodies of the present invention are administered in metronomic dosing regimes, either by continuous infusion or frequent administration without extended rest periods. Such metronomic administration may involve dosing at constant intervals without rest periods. Typically such regimens encompass chronic low-dose or continuous infusion for an extended period of time, for example 1-2 days, 1-2 weeks, 1-2 months, or up to 6 months or more. The use of lower doses may minimize side effects and the need for rest periods.

In certain embodiments the antibody of the present invention and one or more other prophylactic or therapeutic agents are cyclically administered to the patient. Cycling therapy involves administration of a first agent at one time, a second agent at a second time, optionally additional agents at additional times, optionally a rest period, and then repeating this sequence of administration one or more times. The number of cycles is typically from 2 – 10. Cycling therapy may reduce the development of resistance to one or more agents, may minimize side effects, or may improve treatment efficacy.

Combination therapies

The antibodies of the present invention may be administered concomitantly with one or more other therapeutic regimens or agents. The additional therapeutic regimes or agents may be used to improve the efficacy or safety of the antibody. Also, the additional therapeutic regimes or

agents may be used to treat the same disease or a comorbidity rather than to alter the action of the antibody. For example, an antibody of the present invention may be administered to the patient along with chemotherapy, radiation therapy, or both chemotherapy and radiation therapy. The antibody of the present invention may be administered in combination with one or more other prophylactic or therapeutic agents, including but not limited to cytotoxic agents, chemotherapeutic agents, cytokines, growth inhibitory agents, anti-hormonal agents, kinase inhibitors, anti-angiogenic agents, cardioprotectants, immunostimulatory agents, immunosuppressive agents, agents that promote proliferation of hematological cells, angiogenesis inhibitors, protein tyrosine kinase (PTK) inhibitors, additional antibodies, FcγRIIb or other Fc receptor inhibitors, or other therapeutic agents.

The terms "in combination with" and "co-administration" are not limited to the administration of said prophylactic or therapeutic agents at exactly the same time. Instead, it is meant that the antibody of the present invention and the other agent or agents are administered in a sequence and within a time interval such that they may act together to provide a benefit that is increased versus treatment with only either the antibody of the present invention or the other agent or agents. It is preferred that the antibody and the other agent or agents act additively, and especially preferred that they act synergistically. Such molecules are suitably present in combination in amounts that are effective for the purpose intended. The skilled medical practitioner can determine empirically, or by considering the pharmacokinetics and modes of action of the agents, the appropriate dose or doses of each therapeutic agent, as well as the appropriate timings and methods of administration.

In one embodiment, the antibodies of the present invention are administered with one or more additional molecules comprising antibodies or Fc. The antibodies of the present invention may be co-administered with one or more other antibodies that have efficacy in treating the same disease or an additional comorbidity; for example two antibodies may be administered that recognize two antigens that are overexpressed in a given type of cancer, or two antigens that mediate pathogenesis of an autoimmune or infectious disease.

Examples of anti-cancer antibodies that may be co-administered include, but are not limited to, anti-17-1A cell surface antigen antibodies such as Panorex™ (edrecolomab); anti-4-1BB antibodies; anti-4Dc antibodies; anti-A33 antibodies such as A33 and CDP-833; anti-α4β1 integrin antibodies such as natalizumab; anti-α4β7 integrin antibodies such as LDP-02; anti-αVβ1 integrin antibodies such as F-200, M-200, and SJ-749; anti-αVβ3 integrin antibodies such as abciximab, CNTO-95, Mab-17E6, and Vitaxin™; anti-complement factor 5 (C5) antibodies such as 5G1.1; anti-CA125 antibodies such as OvaRex® (oregovomab); anti-CD3 antibodies

such as Nuvion® (visilizumab) and Rexomab; anti-CD4 antibodies such as IDEC-151, MDX-CD4, OKT4A; anti-CD6 antibodies such as Oncolysin B and Oncolysin CD6; anti-CD7 antibodies such as HB2; anti-CD19 antibodies such as B43, MT-103, and Oncolysin B; anti-CD20 antibodies such as 2H7, 2H7.v16, 2H7.v114, 2H7.v115, Bexxar® (tositumomab, I-131 labeled anti-CD20), Rituxan® (rituximab), and Zevalin® (Ibritumomab tiuxetan, Y-90 labeled anti-CD20); anti-CD22 antibodies such as Lymphocide™ (epratuzumab, Y-90 labeled anti-CD22); anti-CD23 antibodies such as IDEC-152; anti-CD25 antibodies such as basiliximab and Zenapax® (daclizumab); anti-CD30 antibodies such as AC10, MDX-060, and SGN-30; anti-CD33 antibodies such as Mylotarg® (gemtuzumab ozogamicin), Oncolysin M, and Smart M195; anti-CD38 antibodies; anti-CD40 antibodies such as SGN-40 and toralizumab; anti-CD40L antibodies such as 5c8, Antova™, and IDEC-131; anti-CD44 antibodies such as bivatuzumab; anti-CD46 antibodies; anti-CD52 antibodies such as Campath® (alemtuzumab); anti-CD55 antibodies such as SC-1; anti-CD56 antibodies such as huN901-DM1; anti-CD64 antibodies such as MDX-33; anti-CD66e antibodies such as XR-303; anti-CD74 antibodies such as IMMU-110; anti-CD80 antibodies such as galiximab and IDEC-114; anti-CD89 antibodies such as MDX-214; anti-CD123 antibodies; anti-CD138 antibodies such as B-B4-DM1; anti-CD146 antibodies such as AA-98; anti-CD148 antibodies; anti-CEA antibodies such as cT84.66, labetuzumab, and Pentacea™; anti-CTLA-4 antibodies such as MDX-101; anti-CXCR4 antibodies; anti-EGFR antibodies such as ABX-EGF, Erbitux® (cetuximab), IMC-C225, and Merck Mab 425; anti-EpCAM antibodies such as Crucell's anti-EpCAM, ING-1, and IS-IL-2; anti-ephrin B2/EphB4 antibodies; anti-Her2 antibodies such as Herceptin®, MDX-210; anti-FAP (fibroblast activation protein) antibodies such as sibrotuzumab; anti-ferritin antibodies such as NXT-211; anti-FGF-1 antibodies; anti-FGF-3 antibodies; anti-FGF-8 antibodies; anti-FGFR antibodies, anti-fibrin antibodies; anti-G250 antibodies such as WX-G250 and Rencarex®; anti-GD2 ganglioside antibodies such as EMD-273063 and TriGem; anti-GD3 ganglioside antibodies such as BEC2, KW-2871, and mitumomab; anti-gpIIb/IIIa antibodies such as ReoPro; anti-heparinase antibodies; anti-Her2/ErbB2 antibodies such as Herceptin® (trastuzumab), MDX-210, and pertuzumab; anti-HLA antibodies such as Oncolym®, Smart 1D10; anti-HM1.24 antibodies; anti-ICAM antibodies such as ICM3; anti-IgA receptor antibodies; anti-IGF-1 antibodies such as CP-751871 and EM-164; anti-IGF-1R antibodies such as IMC-A12; anti-IL-6 antibodies such as CNTO-328 and elsilimomab; anti-IL-15 antibodies such as HuMax™-IL15; anti-KDR antibodies; anti-laminin 5 antibodies; anti-Lewis Y antigen antibodies such as Hu3S193 and IGN-311; anti-MCAM antibodies; anti-Muc1 antibodies such as BravaRex and TriAb; anti-NCAM antibodies such as ERIC-1 and ICRT; anti-PEM antigen antibodies such as Theragyn and Therex; anti-PSA antibodies; anti-PSCA antibodies such as IG8; anti-Ptk antbodies; anti-PTN

antibodies; anti-RANKL antibodies such as AMG-162; anti-RLIP76 antibodies; anti-SK-1 antigen antibodies such as Monopharm C; anti-STEAP antibodies; anti-TAG72 antibodies such as CC49-SCA and MDX-220; anti-TGF-β antibodies such as CAT-152; anti-TNF-α antibodies such as CDP571, CDP870, D2E7, Humira® (adalimumab), and Remicade® (infliximab); anti-TRAIL-R1 and TRAIL-R2 antibodies; anti-VE-cadherin-2 antibodies; and anti-VLA-4 antibodies such as Antegren™. Furthermore, anti-idiotype antibodies including but not limited to the GD3 epitope antibody BEC2 and the gp72 epitope antibody 105AD7, may be used. In addition, bispecific antibodies including but not limited to the anti-CD3/CD20 antibody Bi20 may be used.

Examples of antibodies that may be co-administered to treat autoimmune or inflammatory disease, transplant rejection, GVHD, and the like include, but are not limited to, anti-α4β7 integrin antibodies such as LDP-02, anti-beta2 integrin antibodies such as LDP-01, anti-complement (C5) antibodies such as 5G1.1, anti-CD2 antibodies such as BTI-322, MEDI-507, anti-CD3 antibodies such as OKT3, SMART anti-CD3, anti-CD4 antibodies such as IDEC-151, MDX-CD4, OKT4A, anti-CD11a antibodies, anti-CD14 antibodies such as IC14, anti-CD18 antibodies, anti-CD23 antibodies such as IDEC 152, anti-CD25 antibodies such as Zenapax, anti-CD40L antibodies such as 5c8, Antova, IDEC-131, anti-CD64 antibodies such as MDX-33, anti-CD80 antibodies such as IDEC-114, anti-CD147 antibodies such as ABX-CBL, anti-E-selectin antibodies such as CDP850, anti-gpIIb/IIIa antibodies such as ReoPro/Abcixima, anti-ICAM-3 antibodies such as ICM3, anti-ICE antibodies such as VX-740, anti-FcγR1 antibodies such as MDX-33, anti-IgE antibodies such as rhuMab-E25, anti-IL-4 antibodies such as SB-240683, anti-IL-5 antibodies such as SB-240563, SCH55700, anti-IL-8 antibodies such as ABX-IL8, anti-interferon gamma antibodies, and anti-TNFa antibodies such as CDP571, CDP870, D2E7, Infliximab, MAK-195F, anti-VLA-4 antibodies such as Antegren. Examples of other Fc-containing molecules that may be co-administered to treat autoimmune or inflammatory disease, transplant rejection, GVHD, and the like include, but are not limited to, the p75 TNF receptor/Fc fusion Enbrel® (etanercept) and Regeneron's IL-1 trap.

Examples of antibodies that may be co-administered to treat infectious diseases include, but are not limited to, anti-anthrax antibodies such as ABthrax, anti-CMV antibodies such as CytoGam and sevirumab, anti-cryptosporidium antibodies such as CryptoGAM, Sporidin-G, anti-helicobacter antibodies such as Pyloran, anti-hepatitis B antibodies such as HepeX-B, Nabi-HB, anti-HIV antibodies such as HRG-214, anti-RSV antibodies such as felvizumab, HNK-20, palivizumab, RespiGam, and anti-staphylococcus antibodies such as Aurexis, Aurograb, BSYX-A110, and SE-Mab.

Alternatively, the antibodies of the present invention may be co-administered or with one or more other molecules that compete for binding to one or more Fc receptors. For example, co-administering inhibitors of the inhibitory receptor FcγRIIb may result in increased effector function. Similarly, co-administering inhibitors of the activating receptors such as FcγRIIIa may minimize unwanted effector function. Fc receptor inhibitors include, but are not limited to, Fc molecules that are engineered to act as competitive inhibitors for binding to FcγRIIb FcγRIIIa, or other Fc receptors, as well as other immunoglobulins and specificially the treatment called IVIg (intravenous immunoglobulin). In one embodiment, the inhibitor is administered and allowed to act before the antibody is administered. An alternative way of achieving the effect of sequential dosing would be to provide an immediate release dosage form of the Fc receptor inhibitor and then a sustained release formulation of the antibody of the invention. The immediate release and controlled release formulations could be administered separately or be combined into one unit dosage form. Administration of an FcγRIIb inhibitor may also be used to limit unwanted immune responses, for example anti-Factor VIII antibody response following Factor VIII administration to hemophiliacs.

In one embodiment, the antibodies of the present invention are administered with a chemotherapeutic agent. By "chemotherapeutic agent" as used herein is meant a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include but are not limited to alkylating agents such as thiotepa and cyclosphosphamide (CYTOXAN™); alkyl sulfonates such as busulfan, improsulfan and piposulfan; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabicin, caminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY 117018, onapristone, and toremifene (Fareston); anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphaoramide and

trimethylolomelamine; folic acid replenisher such as frolinic acid; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; proteins such as arginine deiminase and asparaginase; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; taxanes, e.g. paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, N.J.) and docetaxel (TAXOTERE®, Rhne-Poulenc Rorer, Antony, France); topoisomerase inhibitor RFS 2000; thymidylate synthase inhibitor (such as Tomudex); additional chemotherapeutics including aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; difluoromethylornithine (DMFO); elformithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK®; razoxane; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2, 2',2"-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-11;retinoic acid; esperamicins; capecitabine. Pharmaceutically acceptable salts, acids or derivatives of any of the above may also be used.

A chemotherapeutic or other cytotoxic agent may be administered as a prodrug. By "prodrug" as used herein is meant a precursor or derivative form of a pharmaceutically active substance that is less cytotoxic to tumor cells compared to the parent drug and is capable of being enzymatically activated or converted into the more active parent form. See, for example Wilman, 1986, Biochemical Society Transactions, 615th Meeting Belfast, 14:375-382; Stella *et al.*, "Prodrugs: A Chemical Approach to Targeted Drug Delivery," Directed Drug Delivery; and Borchardt *et al.*, (ed.): 247-267, Humana Press, 1985, all incorporated entirely by reference. The prodrugs that may find use with the present invention include but are not limited to phosphate-containing prodrugs, thiophosphate-containing prodrugs, sulfate-containing prodrugs, peptide-containing prodrugs, D-amino acid-modified prodrugs, glycosylated prodrugs, beta-lactam-

containing prodrugs, optionally substituted phenoxyacetamide-containing prodrugs or optionally substituted phenylacetamide-containing prodrugs, 5-fluorocytosine and other 5-fluorouridine prodrugs which can be converted into the more active cytotoxic free drug. Examples of cytotoxic drugs that can be derivatized into a prodrug form for use with the antibodies of the present invention include but are not limited to any of the aforementioned chemotherapeutic agents.

A variety of other therapeutic agents may find use for administration with the antibodies of the present invention. In one embodiment, the antibody is administered with an anti-angiogenic agent. By "anti-angiogenic agent" as used herein is meant a compound that blocks, or interferes to some degree, the development of blood vessels. The anti-angiogenic factor may, for instance, be a small molecule or a protein, for example an antibody, Fc fusion, or cytokine, that binds to a growth factor or growth factor receptor involved in promoting angiogenesis. The preferred anti-angiogenic factor herein is an antibody that binds to Vascular Endothelial Growth Factor (VEGF). Other agents that inhibit signaling through VEGF may also be used, for example RNA-based therapeutics that reduce levels of VEGF or VEGF-R expression, VEGF-toxin fusions, Regeneron's VEGF-trap, and antibodies that bind VEGF-R. In an alternate embodiment, the antibody is administered with a therapeutic agent that induces or enhances adaptive immune response, for example an antibody that targets CTLA-4. Additional anti-angiogenesis agents include, but are not limited to, angiostatin (plasminogen fragment), antithrombin III, angiozyme, ABT-627, Bay 12-9566, benefin, bevacizumab, bisphosphonates, BMS-275291, cartilage-derived inhibitor (CDI), CAI, CD59 complement fragment, CEP-7055, Col 3, combretastatin A-4, endostatin (collagen XVIII fragment), farnesyl transferase inhibitors, fibronectin fragment, gro-beta, halofuginone, heparinases, heparin hexasaccharide fragment, HMV833, human chorionic gonadotropin (hCG), IM-862, interferon alpha, interferon beta, interferon gamma, interferon inducible protein 10 (IP-10), interleukin-12, kringle 5 (plasminogen fragment), marimastat, metalloproteinase inhibitors (eg. TIMPs), 2-methodyestradiol, MMI 270 (CGS 27023A), plasminogen activiator inhibitor (PAI), platelet factor-4 (PF4), prinomastat, prolactin 16kDa fragment, proliferin-related protein (PRP), PTK 787/ZK 222594, retinoids, solimastat, squalamine, SS3304, SU5416, SU6668, SU11248, tetrahydrocortisol-S, tetrathiomolybdate, thalidomide, thrombospondin-1 (TSP-1), TNP-470, transforming growth factor beta (TGF-β), vasculostatin, vasostatin (calreticulin fragment), ZS6126,and ZD6474.

In a preferred embodiment, the antibody is administered with a tyrosine kinase inhibitor. By "tyrosine kinase inhibitor" as used herein is meant a molecule that inhibits to some extent tyrosine kinase activity of a tyrosine kinase. Examples of such inhibitors include but are not limited to quinazolines, such as PD 153035, 4-(3-chloroanilino) quinazoline; pyridopyrimidines;

pyrimidopyrimidines; pyrrolopyrimidines, such as CGP 59326, CGP 60261 and CGP 62706; pyrazolopyrimidines, 4-(phenylamino)-7H-pyrrolo(2,3-d) pyrimidines; curcumin (diferuloyl methane, 4,5-bis (4-fluoroanilino)phthalimide); tyrphostines containing nitrothiophene moieties; PD-0183805 (Warner-Lambert); antisense molecules (e.g. those that bind to ErbB-encoding nucleic acid); quinoxalines (US 5,804,396); tryphostins (US 5,804,396); ZD6474 (Astra Zeneca); PTK-787 (Novartis/Schering A G); pan-ErbB inhibitors such as C1-1033 (Pfizer); Affinitac (ISIS 3521; Isis/Lilly); Imatinib mesylate (STI571,Gleevec®; Novartis); PKI 166 (Novartis); GW2016 (Glaxo SmithKline); C1-1033 (Pfizer); EKB-569 (Wyeth); Semaxinib (Sugen); ZD6474 (AstraZeneca); PTK-787 (Novartis/Schering AG); INC-1C11 (Imclone); or as described in any of the following patent publications: US 5,804,396; PCT WO 99/09016 (American Cyanimid); PCT WO 98/43960 (American Cyanamid); PCT WO 97/38983 (Warner-Lambert); PCT WO 99/06378 (Warner-Lambert); PCT WO 99/06396 (Warner-Lambert); PCT WO 96/30347 (Pfizer, Inc); PCT WO 96/33978 (AstraZeneca); PCT WO96/3397 (AstraZeneca); PCT WO 96/33980 (AstraZeneca), gefitinib (IRESSA™, ZD1839, AstraZeneca), and OSI-774 (Tarceva™, OSI Pharmaceuticals/Genentech), all patent publications incorporated entirely by reference.

In another embodiment, the antibody is administered with one or more immunomodulatory agents. Such agents may increase or decrease production of one or more cytokines, up- or down-regulate self-antigen presentation, mask MHC antigens, or promote the proliferation, differentiation, migration, or activation state of one or more types of immune cells. Immunomodulatory agents include but not limited to: non-steroidal anti-inflammatory drugs (NSAIDs) such as asprin, ibuprofed, celecoxib, diclofenac, etodolac, fenoprofen, indomethacin, ketoralac, oxaprozin, nabumentone, sulindac, tolmentin, rofecoxib, naproxen, ketoprofen, and nabumetone; steroids (eg. glucocorticoids, dexamethasone, cortisone, hydroxycortisone, methylprednisolone, prednisone, prednisolone, trimcinolone, azulfidineicosanoids such as prostaglandins, thromboxanes, and leukotrienes; as well as topical steroids such as anthralin, calcipotriene, clobetasol, and tazarotene); cytokines such as TGFb, IFNa, IFNb, IFNg, IL-2, IL-4, IL-10; cytokine, chemokine, or receptor antagonists including antibodies, soluble receptors, and receptor-Fc fusions against BAFF, B7, CCR2, CCR5, CD2, CD3, CD4, CD6, CD7, CD8, CD11, CD14, CD15, CD17, CD18, CD20, CD23, CD28, CD40, CD40L, CD44, CD45, CD52, CD64, CD80, CD86, CD147, CD152, complement factors (C5, D) CTLA4, eotaxin, Fas, ICAM, ICOS, IFNα, IFNβ, IFNγ, IFNAR, IgE, IL-1, IL-2, IL-2R, IL-4, IL-5R, IL-6, IL-8, IL-9 IL-12, IL-13, IL-13R1, IL-15, IL-18R, IL-23, integrins, LFA-1, LFA-3, MHC, selectins, TGFβ, TNFα, TNFβ, TNF-R1, T-cell receptor, including Enbrel® (etanercept), Humira® (adalimumab), and Remicade® (infliximab); heterologous anti-lymphocyte globulin; other immunomodulatory

molecules such as 2-amino-6-aryl-5 substituted pyrimidines, anti-idiotypic antibodies for MHC binding peptides and MHC fragments, azathioprine, brequinar, bromocryptine, cyclophosphamide, cyclosporine A, D-penicillamine, deoxyspergualin, FK506, glutaraldehyde, gold, hydroxychloroquine, leflunomide, malononitriloamides (eg. leflunomide), methotrexate, minocycline, mizoribine, mycophenolate mofetil, rapamycin, and sulfasasazine.

In an alternate embodiment, antibody of the present invention are administered with a cytokine. By "cytokine" as used herein is meant a generic term for proteins released by one cell population that act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor-alpha and -beta; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF-beta; platelet-growth factor; transforming growth factors (TGFs) such as TGF-alpha and TGF-beta; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-alpha, beta, and -gamma; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1alpha, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12; IL-15, a tumor necrosis factor such as TNF-alpha or TNF-beta; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture, and biologically active equivalents of the native sequence cytokines.

In a preferred embodiment, cytokines or other agents that stimulate cells of the immune system are co-administered with the antibody of the present invention. Such a mode of treatment may enhance desired effector function. For examle, agents that stimulate NK cells, including but not limited to IL-2 may be co-administered. In another embodiment, agents that stimulate macrophages, including but not limited to C5a, formyl peptides such as N-formyl-methionyl-leucyl-phenylalanine (Beigier-Bompadre *et al.* (2003) Scand. J. Immunol. 57: 221-8, incorporated entirely by reference), may be co-administered. Also, agents that stimulate neutrophils, including but not limited to G-CSF, GM-CSF, and the like may be administered. Furthermore, agents that promote migration of such immunostimulatory cytokines may be used.

Also additional agents including but not limited to interferon gamma, IL-3 and IL-7 may promote one or more effector functions.

In an alternate embodiment, cytokines or other agents that inhibit effector cell function are co-administered with the antibody of the present invention. Such a mode of treatment may limit unwanted effector function.

In an additional embodiment, the antibody is administered with one or more antibiotics, including but not limited to: aminoglycoside antibiotics (eg. apramycin, arbekacin, bambermycins, butirosin, dibekacin, gentamicin, kanamycin, neomycin, netilmicin, paromomycin, ribostamycin, sisomycin, spectrinomycin), aminocyclitols (eg. sprctinomycin), amphenicol antibiotics (eg. azidamfenicol, chloramphenicol, florfrnicol, and thiamphemicol), ansamycin antibiotics (eg. rifamide and rifampin), carbapenems (eg. imipenem, meropenem, panipenem); cephalosporins (eg. cefaclor, cefadroxil, cefamandole, cefatrizine, cefazedone, cefozopran, cefpimizole, cefpiramide, cefpirome, cefprozil, cefuroxine, cefixime, cephalexin, cephradine), cephamycins (cefbuperazone, cefoxitin, cefminox, cefmetazole, and cefotetan); lincosamides (eg. clindamycin, lincomycin); macrolide (eg. azithromycin, brefeldin A, clarithromycin, erythromycin, roxithromycin, tobramycin), monobactams (eg. aztreonam, carumonam, and tigernonam); mupirocin; oxacephems (eg. flomoxef, latamoxef, and moxalactam); penicillins (eg. amdinocillin, amdinocillin pivoxil, amoxicillin, bacampicillin, bexzylpenicillinic acid, benzylpenicillin sodium, epicillin, fenbenicillin, floxacillin, penamecillin, penethamate hydriodide, penicillin o-benethamine, penicillin O, penicillin V, penicillin V benzoate, penicillin V hydrabamine, penimepicycline, and phencihicillin potassium); polypeptides (eg. bacitracin, colistin, polymixin B, teicoplanin, vancomycin); quinolones (amifloxacin, cinoxacin, ciprofloxacin, enoxacin, enrofloxacin, feroxacin, flumequine, gatifloxacin, gemifloxacin, grepafloxacin, lomefloxacin, moxifloxacin, nalidixic acid, norfloxacin, ofloxacin, oxolinic acid, pefloxacin, pipemidic acid, rosoxacin, rufloxacin, sparfloxacin, temafloxacin, tosufloxacin, trovafloxacin); rifampin; streptogramins (eg. quinupristin, dalfopristin); sulfonamides (sulfanilamide, sulfamethoxazole); tetracyclenes (chlortetracycline, demeclocycline hydrochloride, demethylchlortetracycline, doxycycline, duramycin, minocycline, neomycin, oxytetracycline, streptomycin, tetracycline, vancomycin).

Anti-fungal agents such as amphotericin B, ciclopirox, clotrimazole, econazole, fluconazole, flucytosine, itraconazole, ketoconazole, niconazole, nystatin, terbinafine, terconazole, and tioconazole may also be used.

Antiviral agents including protease inhibitors, reverse transcriptase inhibitors, and others,

including type I interferons, viral fusion inhibitors, and neuramidase inhibitors, may also be used. Examples of antiviral agents include, but are not limited to, acyclovir, adefovir, amantadine, amprenavir, clevadine, enfuvirtide, entecavir, foscarnet, gangcyclovir, idoxuridine, indinavir, lopinavir, pleconaril, ribavirin, rimantadine, ritonavir, saquinavir, trifluridine, vidarabine, and zidovudine, may be used.

The antibodies of the present invention may be combined with other therapeutic regimens. For example, in one embodiment, the patient to be treated with an antibody of the present invention may also receive radiation therapy. Radiation therapy can be administered according to protocols commonly employed in the art and known to the skilled artisan. Such therapy includes but is not limited to cesium, iridium, iodine, or cobalt radiation. The radiation therapy may be whole body irradiation, or may be directed locally to a specific site or tissue in or on the body, such as the lung, bladder, or prostate. Typically, radiation therapy is administered in pulses over a period of time from about 1 to 2 weeks. The radiation therapy may, however, be administered over longer periods of time. For instance, radiation therapy may be administered to patients having head and neck cancer for about 6 to about 7 weeks. Optionally, the radiation therapy may be administered as a single dose or as multiple, sequential doses. The skilled medical practitioner can determine empirically the appropriate dose or doses of radiation therapy useful herein. In accordance with another embodiment of the invention, the antibody of the present invention and one or more other anti-cancer therapies are employed to treat cancer cells *ex vivo*. It is contemplated that such *ex vivo* treatment may be useful in bone marrow transplantation and particularly, autologous bone marrow transplantation. For instance, treatment of cells or tissue(s) containing cancer cells with antibody and one or more other anti-cancer therapies, such as described above, can be employed to deplete or substantially deplete the cancer cells prior to transplantation in a recipient patient.

It is of course contemplated that the antibodies of the invention may employ in combination with still other therapeutic techniques such as surgery or phototherapy.

EXAMPLES

Examples are provided below to illustrate the present invention. These examples are not meant to constrain the present invention to any particular application or theory of operation.

For reference to immunoglobulin variable regions, positions are numbered according to the Kabat numbering scheme. For reference to immunoglobulin constant regions, positions are numbered according to the EU index as in Kabat (Kabat *et al.*, 1991, *Sequences of Proteins of*

Immunological Interest, 5th Ed., United States Public Health Service, National Institutes of Health, Bethesda).

Example 1. Anti-CD19 antibodies with amino acid modifications that enhance effector function

The anti-CD19 antibodies of the invention are intended as clinical candidates for anti-cancer therapeutics. To investigate the possibility of improving the effector function of an antibody that targets CD19, variant versions of anti-CD19 antibodies were engineered.

Figure 6 provides some heavy and light chain variable region sequences of the anti-CD19 antibodies 4G7 (Meeker, T.C. et al. 1984. Hybridoma. 3: 305-320) and HD37 (Pezzuto, A. et al. 1987. J. Immunol. 138: 2793-2799) used in the present study. The mouse, parent chimeric heavy and light chains are labeled H0 4G7, H0 HD37, L0 4G7, and L0 HD37 respectively. Variants of the present invention could also be made in the context of the anti-CD19 antibody B43 (Uckun, F.M. et al. 1998. Blood. 71: 13-29) which has similar properties to HD37 and shares identical CDRs and an overall 97% sequence identity relative to the HD37 H0 and L0 sequences shown in Figure 6. The genes for murine WT 4G7 and HD37 VH and VL, designated H0 and L0 respectively, were constructed using gene synthesis techniques and subcloned into the mammalian expression vector pcDNA3.1Zeo (Invitrogen) comprising the full length light kappa (Cκ) and heavy chain IgG1 constant regions. Variant S239D/I332E (effector function enhanced anti-CD19) was constructed in the Fc region of a hybrid IgG1/IgG2 (referred to as "Hybrid", Figure 2) antibody in the pcDNA3.1Zeo vector using QuikChange mutagenesis techniques (Stratagene). All sequences were sequenced to confirm the fidelity of the sequence. Plasmids containing heavy chain gene (VH-CH1-CH2-CH3) (wild-type or variants) were co-transfected with plasmid containing light chain gene (VL-CLκ) into 293T cells. Media were harvested 5 days after transfection, and antibodies were purified from the supernatant using protein A affinity chromatography (Pierce, Catalog # 20334)

The relative binding affinities of 4G7 Hybrid S239D/I332E and 4G7 IgG1 antibody were calculated by determining binding parameters on Biacore™ using a panel of Fc receptors (Figure 7). Briefly, protein A/G was coupled to a flow cell of a CM5 chip. IgG was first diluted to 25 nM and immobilized to protein A/G channel to ~ 1000 RUs. FcγR-His was serially diluted and injected at 30 mL/min for 2 min followed by dissociation for 3 min. To determine KD the resulting sensorgrams are "group-fitted" using the 1:1 interaction model available in BIAevaluation software. Values of K_D that were higher than 5×10^{-6} M are labeled as ND (not determined) in Figure 7. The data indicate that WT IgG1 antibody binds V158 FcγRIIIa with an

affnity of approximately 240 nM, consistent with the literature (Okazaki et al, 2004, J Mol Bio 336:1239-49; Lazar et al, Proc Natl Acad Sci USA 103(11):4005-4010). The Fc variant version binds with an affinity to V158 FcγRIIIa of about 4.7 nM, indicating an affinity enhancement of about 50-fold relative to WT. Binding of variant anti-CD19 to F158 FcγRIIIa is about 16.7 nM.

To assess the capacity of the antibody variants to mediate effector function against CD19 expressing cells, effector function enhanced anti-CD19 was tested in a cell-based ADCC assay. Human peripheral blood monocytes (PBMCs) were isolated from leukopaks and used as effector cells, and CD19 positive cancer cells were used as target cells. Target cells were seeded at 10,000 (Raji and MEC-1) and 20,000 (SUP-B15) cells/well in 96-well plates and treated with designated antibodies in triplicates. PBMCs isolated using a Ficoll gradient were added in excess to target cells and co-cultured for 4 hrs before processing for LDH activity using the Cytotoxicity Detection Kit according to the manufacturer's instructions. Figure 8a shows the results of the ADCC assay comparing 4G7 IgG1 and 4G7 Hybrid S239D/I332E antibodies, and HD37 IgG1 and HD37 Hybrid S239D/I332E on the cell line Daudi (BL). Figure 8b shows the results of the ADCC assay comparing 4G7 IgG1 and 4G7 Hybrid S239D/I332E antibodies, and anti-CD20 rituximab on the cell lines SUP-B15 (ALL) and Raji (Burkitt's Lymphoma). The graphs show that the antibodies differ not only in their EC50, reflecting their relative potency, but also in the maximal level of ADCC attainable by the antibodies at saturating concentrations, reflecting their relative efficacy. Considerable enhancements in potency and efficacy are observed for the Fc variant antibodies as compared to the antibody with WT Fc region. The chimeric IgG1 antibody has very little efficacy or potency.

EC50 of a dose response curve such as that in Figure 8 represents the concentration of a compound where 50% of its maximal effect is observed. In a clinical setting, potency reflects the concentration of antibody needed to carry out its therapeutic effect. Thus the data in Figure 8 show that the Fc optimized anti-CD19 antibodies act in vivo at a concentration or dose lower than that of a WT anti-CD19 or anti-CD20 antibody. In Figure 8b, whereas WT IgG1 anti-CD19 at saturating concentration mediates approximately 10% maximal ADCC, Fc variant anti-CD19 lyses approximately 60% of the target cells. In a clinical setting, efficacy reflects the maximal therapeutic benefit from the administered drug.

Example 2. Binding of an effector function enhanced anti-CD19 antibody to a B-cell derived tumor cell line

The relative binding of 4G7 Hybrid S239D/I332E to the Raji cell line was measured. Affinities of enhanced effector function anti-CD19 variants were determined by using the

DELFIA® system (PerkinElmer Life Sciences) which is based on Time-Resolved Fluorometry (TRF). Anti-CD19 (H0L0) is labeled with Europium using the Eu-Labeling kit available from PerkinElmer Biosciences. Unlabeled wild-type (WT) or variants (cold) are serially diluted (typically starting from 1 uM) in ½ log steps and mixed with a fixed concentration of labeled (or hot) anti-CD19. The mix of "hot" and "cold" antibodies are then added to 100,000 Raji Cells (that have a high density of surface expressed CD-19 antigen) and incubated on ice for 30 min. The assay is essentially applied as a competition assay for screening anti-CD19 antibodies of different affinities. In the absence of competing affinity variants, Eu-anti-CD19 and surface CD19 interact and produce a signal at 613 nm when the Europium is excited at 340 nm. Addition of wild type or variant competes with Eu-anti-CD19 - CD19 interaction, reducing fluorescence quantitatively to enable determination of relative binding affinities. Figure 9 shows results of a cell-surface binding assay of enhanced effector function anti-CD19 to Raji cells. As can be seen, the calculated EC50 value is 1.2 nM.

Example 3. ADCC of an anti-CD19 antibody with enhanced cytotoxicity against multiple lymphoma cell lines.

In order to evaluate cytotoxic properties of effector function enhanced anti-CD19, ADCC assays were performed on a panel of 14 cell lines representing various lymphomas and leukemias (Figure 10a). Cell lines tested were the Follicular Lymphoma (FL) cell lines DoHH-2 and SC1; Mantle Cell Lymphoma (MCL) cell line Jeko-1; Burkitt's Lymphoma (BL) cell lines Daudi and Raji; Chronic Lymphocytic Leukemia (CLL) cell lines MEC1 and WaC3CD5; Hairy Cell Leukemia (HCL) cell line Bonna-12; Chronic Myelogenous Leukemia (CML) cell line BV-173; and Acute Lymphoblastic Leukemia (ALL) cell lines VAL, SUP-B15, NALM-6, RS4;11, and 697.Human peripheral blood monocytes (PBMCs) were isolated from leukopaks and used as effector cells, and CD19 positive cancer cells were used as target cells. Target cells were seeded in 96-well plates and treated with designated antibodies in triplicate. PBMCs isolated using a Ficoll gradient were added in excess to target cells and co-cultured for 4 hrs before processing for LDH activity using the Cytotoxicity Detection Kit according to the manufacturer's instructions. Both parameters, potency (EC50) and efficacy (% ADCC) were normalized to that of rituximab (anti-CD20). This screen has demonstrated the cytotoxic superiority *in vitro* of effector function enhanced anti-CD19 over a broad range of cell lines, especially representing the lympho-proliferative disease that originates in early stages of B cell development. Figure 10b lists cell lines used and their corresponding cancer type.

Example 4. Anti-CD19 antibodies with reduced potential for immunogenicity.

Due to the wide use of hybridoma technology, a substantial number of antibodies are derived from nonhuman sources. However, nonhuman proteins are often immunogenic when administered to humans, thereby greatly reducing their therapeutic utility. Immunogenicity is the result of a complex series of responses to a substance that is perceived as foreign, and may include production of neutralizing and non-neutralizing antibodies, formation of immune complexes, complement activation, mast cell activation, inflammation, hypersensitivity responses, and anaphylaxis. Several factors can contribute to protein immunogenicity, including but not limited to protein sequence, route and frequency of administration, and patient population. Immunogenicity may limit the efficacy and safety of a protein therapeutic in multiple ways. Efficacy can be reduced directly by the formation of neutralizing antibodies. Efficacy may also be reduced indirectly, as binding to either neutralizing or non-neutralizing antibodies typically leads to rapid clearance from serum. Severe side effects and even death may occur when an immune reaction is raised. Thus in a preferred embodiment, protein engineering is used to reduce the immunogenicity of the CD19 targeting proteins of the present invention.

In order to reduce the potential for immunogenicity of the anti-CD19 proteins of the present invention, the immunogenicity of the anti-CD19 antibodies 4G7 and HD37 were reduced using a method described in USSN 60/619,483, filed October 14, 2004 and USSN 11/004,590, entitled "Methods of Generating Variant Proteins with Increased Host String Content and Compositions Thereof", filed on December 6, 2004. The methods reduce the potential for immunogenicity by increasing the human string content of the antibody through mutations. The heavy and light chains with reduced potential for immunogenicity are named H1, H2, H3, H4, etc and L1, L2, L3, etc. and are shown in Figures 11 thru 14. The heavy and light chains of the original antibodies, 4G7 and HD37, are referred to as H0 and L0, respectively. Combinations of the different heavy and light chains were expressed and the resulting antibodies, with names such as H3L3, H3/L3 or H3_L3, were purified and examined. Anti-CD19 antibodies were expressed by transient transfection of vectors encoding the heavy and light chains into 293T cells grown in 10% ultra low IgG fetal bovine serum with 1mM sodium pyruvate and 1X non-essential amino acids (Gibco®, Invitrogen Hayward CA). Five days after transfection, the culture media was removed and passed through a protein A column (Pierce Biotechnology Inc, Rockford MD.) The heavy chains may be made with any type of constant domain including, in humans, IgG1, IgG2 and hybrids comprising IgG1 and IgG2 as well as mouse constant domains such as IgG1 and IgG2a, which may be reffered to as mIgG1 and mIgG2a. The sequences of human heavy chains may be found in Figure 2. The relative binding of anti-CD19 variants with reduced immunogenicity to the Raji cell line was measured. Affinities of reduced immunogenicity anti-

CD19 variants were determined by using the DELFIA® system (PerkinElmer Life Sciences) which is based on Time-Resolved Fluorometry (TRF). Anti-CD19 is labeled with Europium using the Eu-Labeling kit available from PerkinElmer Biosciences. Unlabeled wild-type (WT) or variants (cold) are serially diluted (typically starting from 1 uM) in ½ log steps and mixed with a fixed concentration of labeled (or hot) anti-CD19. The mix of "hot" and "cold" antibodies are then added to 100,000 Raji Cells (that have a high density of surface expressed CD-19 antigen) and incubated on ice for 30 min. The assay is essentially applied as a competition assay for screening anti-CD19 antibodies of different affinities. In the absence of competing affinity variants, Eu-anti-CD19 and surface CD19 interact and produce a signal at 613 nm when the Europium is excited at 340 nm. Addition of wild type or variant competes with Eu-anti-CD19 - CD19 interaction, reducing fluorescence quantitatively to enable determination of relative binding affinities. Figure 15a shows results of a cell-surface binding assay of reduced immunogenicity 4G7 variants to Raji cells. Based on binding affinity and stability, the variable region 4G7 H1L1 was chosen for further development. Figure 15b shows results of an ADCC assay on reduced immunogenicity templates HD37_H2L1 Hybrid S239D/I332E and 4G7_H1L3 Hybrid S239D/I332E on the cell line MEC-1 (CLL). This ADCC assay was performed as in the previous assays. Both antibodies are active on this cell line and therefore may be potential treatments for CLL.

Example 5. Affinity and stability enhancement of effector function enhanced anti-CD19.

Affinity maturation of 4G7 mAb H1L1 was carried out in order to further increase CD19 binding affinity as well as ADCC potency. The affinity maturation was performed in three stages using a computational/protein engineering approach. First, operating under the hypothesis that the specificity determining residues (SDRs) (Padlan, E.A. et al. 1995. FASEB J. 9: 133-139) in the CDRs of an antibody have already been optimized by B-cells in the process of *in vivo* somatic hypermutation, a library of 94 variants was designed to determine those residues in the CDRs that were critical for antigen binding, and thus should not be changed during the engineering process. This library consisted of one or two "probing" mutations made at positions in the CDRs with sites chosen using structural modeling as well as the likelihood that a position is often an SDR, which was compiled from analysis of available antigen-antibody complex structures in the Protein Data Bank (PDB) (MacCallum, R.M. et al. 1996. JMB 262: 732-745; Almagro, J.C. 2004. J. Mol. Recognit. 17:132-143).

Variant mutations were introduced using the QuikChange mutagenesis kit in the Fab format of the H1L1 template and contained a 6X-His tag. Variant Fabs were expressed in 293T cells using 24-well plates and were analyzed by AlphaScreen or flow cytometry using Raji or

RS4;11 cells, and with the concentration of each variant determined using a His-binding chip by Biacore™. Out of 50 positions, 17 positions were identified that were critical to antigen binding, enabling us to reduce the library size in the next round of affinity maturation and giving us valuable structural information as to which positions lie close to the antigen interface and would make good targets for finding increased affinity variants. The 17 SDRs identified in our analysis are in excellent agreement with the average number of SDRs present in antibodies whose antigen-antibody complexes have been solved (Almagro, J.C. 2004. J. Mol. Recognit. 17:132-143). In addition to the valuable structural information gained from this library, some variants were obtained that had an increased affinity.

The remaining 33 CDR positions were ranked in order of importance based on analysis of the first library results and by mapping the SDRs onto a structural model of the H1L1 template. Through this analysis it was determined that nearly the entire antigen-antibody binding interface could be explored with a total frequency of 12.2 amino acids per position (~9.3 new variants per position) with a second round library size of 279 variants. Library Design Automation (LDA™) (USSN 11/367,184, filed March 3, 2006) was used to design an optimized library of variants that was tuned for both fitness and coverage based on the number of variants desired. The final second round library when adjusted for high-throughput format contained 265 variants at 30 positions. This library yielded several variants displaying increased binding affinity. Anti-CD19 Fab variants were screened by flow cytometry to determine the affinity. The cell line RS4;11, known to express CD19, were suspended in PBS and plated at 200,000 cells/ well in a 96-well round bottom plate. A serial dilution of CD19 antibodies were added to the RS4;11 cells at an unknown concentration. The cells were incubated on ice for 30 minutes and then washed 4 times in PBS. An anti-Fab PE-labeled $F(ab')_2$ was diluted 1/50 in PBS, which was then used to resuspend the anti-CD19 Fab coated RS4;11. Cells were incubated for 30 minutes and washed two times. The cells were then fixed and the binding assay was evaluated on a FACS Canto II flow cytometer. The MFI was used to measure the tightness of binding. From both libraries one and two, a total of 30 increased affinity single variants were obtained at 11 positions.

Analysis of the binding data from the first two libraries as well as further structural analysis enabled us to design a third and final library containing combinations of 2-8 single variants. This library consisted of 149 variants at 8 positions. From these, 20 variants showed a significant increase in affinity and were selected for conversion to full length format for simultaneous measurement of binding affinity and ADCC. To assess solution properties, stability assays on these variants were performed. The final set of mutations included in the final 20 were heavy chain variants T57P, K58E, S100cT, R100dS, and light chain variants L27cQ, S27eV,

A55N, F96I, and F96N. Accelerated stability studies revealed that at least one of the affinity enhancing mutations created instability in the protein and caused these variants to lose all potency after only 8 hrs at 37 °C. Taking the binding and stability data into account, a final affinity matured candidate mAb was able to be selected which displayed an ~10-fold increase in binding affinity on RS4;11 cells relative to the H1L1 mAb (Figure 16). Variants designed to increase the long-term stability of the anti-CD19 molecule were also designed and screened. Figure 17 shows binding data for variants incubated for 5 days at 37°C, pH 9.0 in 200 mM Tris-HCl, demonstrating the improvement in stability obtained from an anti-CD19 variant.

All single subsitutions made for enhanced stability and/or affinity are shown in Figure 27. Figure 28 lists all anti-CD19 variable region variants constructed to optimize affinity and stability. Figure 29 lists preferred variants and relative increase in binding affinity versus the parent H1L1 mAb. Sequences for the preferred affinity and/or stability enhanced heavy chain variants are shown in Figure 18. Sequences for the preferred affinity and/or stability enhanced light chain variants are shown in Figure 19. Amino acid sequences of full length hybrid S239D/I332E variants containing the affinity and stability improved variable regions are provided as SEQ ID NOs: 86-110. Affinity and stability improved CDR's are provided as SEQ ID NOs: 111-131.

Example 6. Anti-proliferative properties of 4G7 Hybrid S239D/I332 on Raji cells.

To observe an anti-proliferative effect *in vitro*, many antibodies require cross-linking, usually accomplished by a secondary antibody. It has been proposed that corresponding *in vivo* effects for these antibodies may be dependent on cross-linking mediated by Fc receptors expressed on the surface of effector cells. In this experiment Raji cells were grown for 3 days in the presence of 100 ng/mL 4G7 Hybrid S239D/I332E, 4G7 IgG1, or anti-CD20 (rituximab) or control antibodies (non-CD19 binding variable region with Hybrid S239D/I332E variants Fc) at varying concentrations with 10x molar excess of cross-linking antibody. Cell growth was measured using an ATP-dependent luminescence assay. Results for the anti-proliferation assay are shown in Figure 20. Both 4G7 Hybrid S239D/I332E and 4G7 IgG1 show stronger anti-proliferation effects than rituximab.

Example 7. Anti-proliferative properties of 4G7 stability and affinity improved Hybrid S239D/I332E on SU-DHL-6 cells.

In this experiment SU-DHL-6 cells were either grown for 3 days in the presence of humanized 4G7 stability and affinity improved Hybrid S239D/I332E and control antibodies at varying concentrations with 10x molar excess of cross-linking antibody and 6000 cells/well or

were grown in the presence of a fixed concentration of antibody at 3000 cells/well and viability at specific time points measured for a total of 72 hours. Results for the anti-proliferation assay are shown in Figure 21. 4G7 stability and affinity improved Hybrid S239D/I332E shows stronger anti-proliferation effects than rituximab. 4G7 stability and affinity improved Hybrid S239D/I332E also shows anti-proliferative effects even in the absence of cross-linking antibody.

Example 8. Phagocytosis of Raji and RS4;11 cells with 4G7 stability and affinity improved Hybrid S239D/I332E.

Unlike NK cells which only express FcγRIIIa and sometimes FcγRIIc, monocytes and monocyte-derived effector cells express the range of FcγRs, including FcγRI, FcγRIIa, FcγRIIb, and FcγRIIIa. Thus the activation and function of monocyte-derived effector cells, including for example macrophages, may be dependent on engagement of antibody immune complexes with receptors other than only FcγRIIIa. Indeed as described in PCT/US2006/038842, Desjarlais J.R. et al., filed October 3rd, 2006, phagocytosis by macrophages is mediated in part by engagement of antibody with FcγRIIa.

To assess the ability of 4G7 stability and affinity improved Hybrid S239D/I332E to mediate phagocytosis a flow cytometry based phagocytosis assay was performed. Purified $CD14^+$ monocytes were cultured in macrophage colony stimulating factor (50ng/ml) for 5 days in a humidified incubator to differentiate macrophages. RS4;11 or Raji cells were used as targets. The target cells were labeled with PKH67 (Sigma) according to the manufacture's instructions. Cells were added to a 96 well plate after which a serial dilution of WT and Fc modified anti-CD19 antibodies were added. Monocyte-derived macrophages were then added to the wells at an effector to target ratio of 4:1. These assays were performed in the presence of human serum. The co-culture of cells were briefly spun down and then incubated in a humidified incubator for 4 hours. The cells were harvested, and macrophages were stained with a second fluorescent color to distinguish them from the target. The cells were fixed in 1% PFA and phagocytosis was evaluated on a FACS Canto II flow cytometer. The read out of phagocytosis was determined by the number of double positive cells divided by the total number of tumor cells. Results of the phagocytosis assay are shown in Figure 22. 4G7 stability and affinity improved Hybrid S239D/I332E shows an increased level of phagocytosis on both cell lines compared to the IgG1 anti-CD19 antibody.

Macrophages are phagocytes that act as scavengers to engulf dead cells, foreign substances, and other debris. Importantly, macrophages are professional antigen presenting cells (APCs), taking up pathogens and foreign structures in peripheral tissues, then migrating to

secondary lymphoid organs to initiate adaptive immune responses by activating naive T-cells. Thus the results of the previous experiment suggest that modification of anti-CD19 antibodies may enable mechanisms of action that include both innate cytotoxic effector functions, as well as effector functions that can potentially lead to long-term adaptive immune response.

Example 9. ADCC of 4G7 stability and affinity improved Hybrid S239D/I332E against multiple lymphoma cell lines using purified natural killer (NK) cells

In order to evaluate cytotoxic properties of 4G7 stability and affinity improved Hybrid S239D/I332E, ADCC assays were performed with purified NK cells on a panel of 6 cell lines representing various lymphomas and leukemias (Figure 23). ADCC with purified NK cells is done in 96-well microtiter plates. The NK cells were purified from human PBMC using the kit from Miltenyi Biotec (Cat #130-091-152) and incubated in 10%FBS/RPMI1640 overnight with 10 ng/ml IL-2. The following day, 10,000 (WaC3CD5, Namalwa, Bonna-12, Ramos) or 20,000 (RS4;11, BV-173) cancer target cells are opsonized with varying concentrations of antibody and 50k NK cells are used for each antibody concentration in triplicates. The target cells are washed three times while NK cells are washed twice with RPMI1640 and both resuspended in 1%FBS/RPMI1640 and added to the antibody solutions. After 4 hours of incubation at 37°C in a humidified incubator with 5% CO_2, the assay was quantified using LDH dependent CytoTox-One fluorescence dependent detection system from Promega (#PAG7891). Total LDH signal is determined from the Triton-X100 lysed target cells (Total Target LDH) and used to normalize against the spontaneous LDH background (Spontaneous Background) adjusted experimental values. Thus %ADCC = ((Experimental Value – Spontaneous Background)/(Total Target LDH – Target LDH))*100. Spontaneous background is the value obtained from the Target and NK cells co-incubated in the absence of antibody. Target LDH is the value from the target cancer cells alone spontaneously releasing LDH during the incubation. Figure 23 shows results of the ADCC assay for 6 cell lines using 4G7 stability and affinity improved Hybrid S239D/I332E, 4G7 IgG1 (with affinity/stability optimized variable region), rituximab (anti-CD20), and an isotype control antibody. For all cell lines tested, 4G7 stability and affinity improved Hybrid S239D/I332E performs better in both potency and efficacy when compared to 4G7 IgG1 and rituximab.

Example 10. 4G7 stability and affinity improved Hybrid S239D/I332E binding to CD19 transfected 293T cells

A human CD19 clone was ordered from Origene (catalog No. SC127938) and transfected into 293T cells. Cells were suspended in PBS and plated at 100 000 cells/ well. A serial dilution of 4G7 stability and affinity improved Hybrid S239D/I332E was added to the cells and then the

cells were incubated on ice for 30 minutes and then washed 4 times in PBS. An anti-Fab PE-labeled $F(ab')_2$ was diluted 1/50 in PBS, which was then used to resuspend the 4G7 stability and affinity improved Hybrid S239D/I332E anti-CD19 coated 293T cells. Cells were incubated for 30 minutes and washed two times. The cells were then fixed and the binding was evaluated on a FACS Canto II flow cytometer. Figure 24 displays results for this assay. The results show that 4G7 stability and affinity improved Hybrid S239D/I332E binds to 293T cells transfected with CD19 and does not bind to the control cells (normal 293T cells).

Example 11. 4G7 stability and affinity improved Hybrid S239D/I332E is cross-reactive with CD19 from cynomolgus and rhesus monkeys.

Pre-clinical testing of drugs in monkeys is typically an important step in drug discovery in order to assess potential toxicity. Blood samples from five cynomolgus (*Macaca fascicularis*; genus = *Macaca* (Latin) or Macaque (English); species = *fascicularis*) and five rhesus (*Macaca mulatta*) monkeys were obtained. 4G7 stability +affinity improved Hybrid S239D/I332E anti-CD19, anti-CD19 IgG1 (reduced immunogenicity, but without affinity/stability optimized variable region), rituximab (anti-CD20), and negative control (enhanced Fc, non-binding variable region) were directly labeled with FITC. Rituximab was also labeled with APC to identify the B-cell fraction of cells. Human PBMCs were used as positive controls throughout. Blood samples and PBMCs were pre-incubated with 2 mg/mL of an isotype control antibody with enhanced Fc to block any potential FcγR binding. In each experiment, rituximab-APC and one of the test variants were included in the assay. Detection is made using a FACS Canto II flow cytometer with gate lymphocyte fractions based on the forward and side scattering. Results are shown in Figure 25. Non-affinity/stability matured anti-CD19 (as well as its parental murine antibody) does not cross-react with cynomolgus or rhesus CD19. Variants that increased binding and stability of the anti-CD19 molecule enabled cross-reactivity of 4G7 stability and affinity improved Hybrid S239D/I332E to both cynomolgus and rhesus CD19.

Example 12. ADCC of an enhanced effector function anti-CD19 antibody with reduced fucose content

Anti-CD19 antibodies with enhanced effector function (4G7 H1L1 Hybrid S239D/I332E) were evaluated with reduced fucose content. The Lec13 cell line (Ripka et al. Arch. Biochem. Biophys. 49:533-545 (1986)) was utilized to express anti-CD19 antibodies with reduced fucose content. Lec13 refers to the lectin-resistant Chinese Hamster Ovary (CHO) mutant cell line which displays a defective fucose metabolism and therefore has a diminished ability to add fucose to complex carbohydrates. That cell line is described in Ripka & Stanley, 1986, Somatic

Cell & Molec. Gen. 12(1):51-62; and Ripka et al., 1986, Arch. Biochem. Biophys. 249(2):533-545. Lec13 cells are believed to lack the transcript for GDP-D-mannose-4,6-dehydratase, a key enzyme for fucose metabolism. Ohyama et al., 1988, J. Biol. Chem. 273(23):14582-14587. GDP-D-mannose-4,6-dehydratase generates GDP-mannose-4-keto-6-D-deoxymannose from GDP-mannose, which is then converted by the FX protein to GDP-L-fucose. Expression of fucosylated oligosaccharides is dependent on the GDP-L-fucose donor substrates and fucosyltransferase(s). The Lec13 CHO cell line is deficient in its ability to add fucose, but provides IgG with oligosaccharide which is otherwise similar to that found in normal CHO cell lines and from human serum (Jefferis, R. et al., 1990, Biochem. J. 268, 529–537; Raju, S. et al., 2000, Glycobiology 10, 477–486; Routier, F. H., et al., 1997, Glycoconj. J. 14, 201–207). Normal CHO and HEK293 cells add fucose to IgG oligosaccharide to a high degree, typically from 80–98%, and IgGs from sera are also highly fucosylated (Jefferis, R. et al., 1990, Biochem. J. 268, 529–537; Raju, S. et al., 2000, Glycobiology 10, 477–486; Routier, F. H., et al., 1997, Glycoconj. J. 14, 201–207; Shields et al., 2002, J Biol Chem 277(90):26733-26740). It is well established that antibodies expressed in transfected Lec13 cells consistently produce about 10% fucosylated carbohydrate (Shields et al., 2002, J Biol Chem 277(90):26733-26740).

ADCC assays were performed on RS4;11 and MEC-1 cells using anti-CD19 antibodies with and without enhanced effector function variants and with and without reduced fucosylation. Figure 26 shows the results of these ADCC assays. Both ADCC potency and efficacy are similar for anti-CD19 antibody with amino acid modifications (4G7_H1L1_Hybrid_239D/I332E + fucose) and anti-CD19 IgG1 with reduced fucose content (4G7_H1L1_IgG1_WT –fucose). ADCC potency is further increased by combining amino acid modification with reduced fucose content (4G7_H1L1_Hybrid_239D/332E –fucose). (Figure 26). This experiment thus illustrates that combinations of amino acid modifications and modified glycoforms may be used to optimize anti-CD19 antibodies for effector function properties.

The use of the Lec13 cell line is not meant to limit the present invention to that particular mode of reducing fucose content. A variety of other methods are known in the art for controlling the level of fucosylated and/or bisecting oligosaccharides that are covalently attached to the Fc region, including but not limited to expression in various organisms or cell lines, engineered or otherwise (for example Lec13 CHO cells or rat hybridoma YB2/0 cells), regulation of enzymes involved in the glycosylation pathway (for example FUT8 [α1,6-fucosyltranserase] and/or β1-4-N-acetylglucosaminyltransferase III [GnTIII]), and modification of modifying carbohydrate(s) after the IgG has been expressed (Umaña et al., 1999, Nat Biotechnol 17:176-180; Davies et al., 2001, Biotechnol Bioeng 74:288-294; Shields et al., 2002, J Biol Chem 277:26733-26740;

Shinkawa et al., 2003, J Biol Chem 278:3466-3473; Yamane-Ohnuki et al., 2004, Biotechnology and Bioengineering 87(5):614-621); (US 6,602,684; USSN 10/277,370; USSN 10/113,929; PCT WO 00/61739A1; PCT WO 01/29246A1; PCT WO 02/31140A1; PCT WO 02/30954A1).

The use of particular modifications to enhance effector function, for example the substitutions 239D and 332E and the reduced level of fucose, are not meant to constrain the anti-CD19 antibodies to these particular modifications. As described above in the section entitled "Modifications for optimizing effector function", a large number of modifications, including amino acid modifications and modified glycoforms, are contemplated for anti-CD19 antibodies to improve their effector function properties.

Example 13. Anti-CD19 antibodies inhibit proliferation of primary B cells – applications of anti-CD19 antibodies to treat autoimmune diseases

The ability of the anti-CD19 antibodies of this invention to deplete B cells through ADCC effector function is exemplified by their ability to lyse a variety of cell lines representative of a range of B cell lineages, as shown in the preceding examples. This function is mediated by effector cells such as NK cells and macrophages that express FcγRs, triggering of which induces lysis of the CD19-coated target cells. An additional mechanism of action may also be mediated against antigen-activated B cells. Antigen activation of B cells can be mimicked by the use of antibodies to the B-cell receptor (BCR). This leads to their proliferation in culture, a generic measure of activation.

Antigen binding can be mimicked in vitro by cross-linking BCR (mu or IgM) with anti-mu (anti-μ, anti-IgM) antibody. In order to demonstrate this activity, Peripheral Blood Mononuclear Cells (PBMCs) were prepared from Leukophoresis Pack by Ficoll density gradient, and primary human B cells were purified from PBMCs using magnetic negative selection kit purchased from Miltenyi Biotec. The proliferation assay was performed in 10%FBS/RPMI1640 medium in total of 100 ul volume in 96 well micro-titer plates in triplicates. B cell activation was induced using F(ab')2 fragment of goat anti-mu antibody (Jackson Immunoresearch, Inc.). In 50 ul of medium, serial dilutions of the anti-mu antibody was aliquoted in 96 well micro-titer plate, to which 83,000 purified B cells were added in 50 ul volume. Then the micro-titer plate was incubated at 37 °C for 3 days after which, ATP luminescence assay format (Cell TiterGlo Kit from Promega) was used to detect the live cells using luminometer. Figure 30a shows that there is a dose-dependence of B cell proliferation on anti-mu antibody concentration.

In order to evaluate the capacity of the WT (4G7_H3_L1 IgG1_WT) and variant (4G7_H3_L1_Hybrid_239D/332E) anti-CD19 antibodies to modulate B-cell proliferation, an

assay was carried out to monitor viability of primary human B cells in the presence of anti-CD19 and co-stimular anti-mu antibody. As described above, PBMCs were prepared from Leukophoresis Pack by Ficoll density gradient, and primary human B cells were purified from PBMCs using magnetic negative selection. The proliferation assay was performed in 10%FBS/RPMI1640 medium in total of 100 ul volume in 96 well micro-titer plates in triplicates. To induce activation of B cells, the F(ab')2 fragment of goat anti-mu antibody was used. In 50 ul of medium, a fixed concentration (2 mg/ml) of anti-mu with five fold serial dilutions of the antibodies were performed in 96 well micro-titer plate, to which 100,000 purified B cells were added in 50 ul volume. Then the micro-titer plate was incubated at 37 °C for 3 days after which, ATP luminescence assay format was used to detect the live cells using luminometer.

The results, provided in Figure 30b, show that WT anti-CD19 antibody has no effect on primary B-cell proliferation, similar to negative control with anti-CD30 antibody (CD30 is not expressed on B cells). In contrast, the anti-CD19 antibody comprising Fc modifications has significant inhibitory activity against B-cell viability. Notably, in vitro signaling as a result of anti-mu antibody cross-linking mimicks antigen engagement of BCR, and is a proxy for BCR engagement by autoantigen in a clinical autoimmune setting.

The pathogenesis of most autoimmune diseases is coupled to the production of autoantibodies against self antigens, leading to a variety of associated pathologies. For example, SLE is characterized by production of auto- or self- antibodies to double stranded DNA. Accordingly, in the aforedescribed experiment BCR engagement in vitro by anti-mu antibody mimicks stimulation of B cells in lupus patients in vivo by anti- double-stranded DNA antibodies. Autoantibodies are produced by terminally differentiated plasma cells that are derived from naïve or memory B cells. Furthermore, B cells can have other effects on autoimmune pathology, as antigen-presenting cells (APCs) that can interact with and stimulate helper T cells, further stimulating the cycle of anti-self immune response. Given the expression of CD19 on most of the B-cell lineage, ranging from pre-B to plasma cells, the antibodies of this invention may have broad utility for the treatment of autoimmune diseases. Examples of such autoimmune diseases include, but are not limited to, rheumatoid arthritis (RA), systemic lupus erythematosus (SLE or lupus), multiple sclerosis, Sjogren's syndrome, and idiopathic thrombocytopenia purpura (ITP).

The current example demonstrates that anti-CD19 antibodies of the invention can substantially inhibit B cell proliferation in a dose-dependent manner, indicating that they can inhibit antigen-stimulated activation of B cells. B-cell activation by antigen can also initiate the process of class-switching and ultimately terminal differentiation into antibody-secreting plasma

cells. The antibodies of this invention are thus capable of inhibiting these processes via an additional mechanism of action that does not require effector cells. This inhibition is expected to have beneficial impact on autoimmune disease by preventing the terminal differentiation of naïve and memory B cell populations, thus preventing the differentiation of autoantibody-secreting plasma cells. It is also possible that additional aspects of B-cell biology such as antigen presentation will be affected by the anti-CD19 antibodies.

> IgG1 G1m(a,z) allotype (SEQ ID NO:80)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP
ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT
LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL
TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

> IgG1 G1m(a,x,z) allotype (SEQ ID NO:81)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP
ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT
LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL
TVDKSRWQQGNVFSCSVMHEGLHNHYTQKSLSLSPGK

> IgG1 G1m(f) allotype (SEQ ID NO:82)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAP
ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT
LPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL
TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

> IgG1 G1m(a,f) allotype (SEQ ID NO:83)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAP
ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT
LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL
TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

> IgG2 G2m(n+) allotype (SEQ ID NO:84)

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQF
NSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSRE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

> IgG2 G2m(n-) allotype (SEQ ID NO:85)

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGMEVHNAKTKPREEQF
NSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSRE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK.

> 4G7 H1 Hybrid S239D/I332E (SEQ ID NO:86)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPY
NDGTKYNEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGSRVFDYWG
QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP
CPAPELLGGPDVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNA
KTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKALPAPEEKTISKTKGQPREP
QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

> 4G7 H1.52 Hybrid S239D/I332E (SEQ ID NO:87)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPY
NDGTKYNEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWG
QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP
CPAPELLGGPDVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNA
KTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKALPAPEEKTISKTKGQPREP
QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

> 4G7 H1.78 Hybrid S239D/I332E (SEQ ID NO:88)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPY
NAGTKYNEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGSRVFDYWG
QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP
CPAPELLGGPDVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNA
KTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKALPAPEEKTISKTKGQPREP
QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

> 4G7 H1.191 Hybrid S239D/I332E (SEQ ID NO:89)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPY
NDGTEYNEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWG
QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP
CPAPELLGGPDVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNA
KTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKALPAPEEKTISKTKGQPREP
QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

> 4G7 H1.192 Hybrid S239D/I332E (SEQ ID NO:90)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPY
NDGPKYNEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWG
QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP
CPAPELLGGPDVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNA
KTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKALPAPEEKTISKTKGQPREP
QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

> 4G7 H1.196 Hybrid S239D/I332E (SEQ ID NO:91)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPY
NDGPKYNEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTSVFDYWG
QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP
CPAPELLGGPDVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNA
KTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKALPAPEEKTISKTKGQPREP
QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

> 4G7 H1.201 Hybrid S239D/I332E (SEQ ID NO:92)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPY
NSGTKYNEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWG
QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP
CPAPELLGGPDVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNA
KTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKALPAPEEKTISKTKGQPREP
QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

> 4G7 H1.202 Hybrid S239D/I332E (SEQ ID NO:93)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPY
NEGTKYNEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWG
QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP
CPAPELLGGPDVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNA
KTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKALPAPEEKTISKTKGQPREP
QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

> 4G7 H1.203 Hybrid S239D/I332E (SEQ ID NO:94)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPY
NSGTEYNEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWGQ
GTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC
PAPELLGGPDVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAK
TKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKALPAPEEKTISKTKGQPREPQ
VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFL
YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

> 4G7 H1.204 Hybrid S239D/I332E (SEQ ID NO:95)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPY
NEGTEYNEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWG
QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP
CPAPELLGGPDVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNA
KTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKALPAPEEKTISKTKGQPREP
QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

> 4G7 L1 (SEQ ID NO:96)

DIVMTQSPATLSLSPGERATLSCRSSKSLLNSNGNTYLYWFQQKPGQSPQLLIYRM
SNLASGVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPFTFGAGTKLEIKRTVAA
PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS
TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

> 4G7 L1.26 (SEQ ID NO:97)

DIVMTQSPATLSLSPGERATLSCRSSKSLQNSNGNTYLYWFQQKPGQSPQLLIYR
MSNLASGVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPFTFGAGTKLEIKRTV
AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK
DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

> 4G7 L1.32 (SEQ ID NO:98)

DIVMTQSPATLSLSPGERATLSCRSSKSLLNVNGNTYLYWFQQKPGQSPQLLIYR
MSNLASGVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPFTFGAGTKLEIKRTV
AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK
DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

> 4G7 L1.64 (SEQ ID NO:99)

DIVMTQSPATLSLSPGERATLSCRSSKSLLNSNGNTYLYWFQQKPGQSPQLLIYRM
SNLASGVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPITFGAGTKLEIKRTVAA
PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS
TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

> 4G7 L1.68 (SEQ ID NO:100)

DIVMTQSPATLSLSPGERATLSCRSSKSLLNSNGNTYLYWFQQKPGQSPQLLIYRM
SNLASGVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPNTFGAGTKLEIKRTVA
APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

> 4G7 L1.96 (SEQ ID NO:101)

DIVMTQSPATLSLSPGERATLSCRSSKSLLNSNGNTYLYWFQQKPGQSPQLLIYRM
SNLNSGVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPFTFGAGTKLEIKRTVAA
PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS
TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

> 4G7 L1.145 (SEQ ID NO:102)

DIVMTQSPATLSLSPGERATLSCRSSKSLQNSNGNTYLYWFQQKPGQSPQLLIYR
MSNLASGVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPITFGAGTKLEIKRTVA
APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

> 4G7 L1.148 (SEQ ID NO:103)

DIVMTQSPATLSLSPGERATLSCRSSKSLQNSNGNTYLYWFQQKPGQSPQLLIYR
MSNLNSGVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPNTFGAGTKLEIKRTV
AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK
DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

> 4G7 L1.149 (SEQ ID NO:104)

DIVMTQSPATLSLSPGERATLSCRSSKSLQNSNGNTYLYWFQQKPGQSPQLLIYR
MSNLNSGVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPITFGAGTKLEIKRTVA
APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

> 4G7 L1.154 (SEQ ID NO:105)

DIVMTQSPATLSLSPGERATLSCRSSKSLQNVNGNTYLYWFQQKPGQSPQLLIYR
MSNLNSGVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPNTFGAGTKLEIKRTV
AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK
DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

> 4G7 L1.155 (SEQ ID NO:106)

DIVMTQSPATLSLSPGERATLSCRSSKSLQNVNGNTYLYWFQQKPGQSPQLLIYR
MSNLNSGVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPITFGAGTKLEIKRTVA
APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

> 4G7 L1.160 (SEQ ID NO:107)

DIVMTQSPATLSLSPGERATLSCRSSKSLQNVNANTYLYWFQQKPGQSPQLLIYR
MSNLNSGVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPITFGAGTKLEIKRTVA
APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

> 4G7 L1.162 (SEQ ID NO:108)

DIVMTQSPATLSLSPGERATLSCRSSKSLQNANANTYLYWFQQKPGQSPQLLIYR
MSNLNSGVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPITFGAGTKLEIKRTVA
APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

> 4G7 L1.163 (SEQ ID NO:109)

DIVMTQSPATLSLSPGERATLSCRSSKSLQNANSNTYLYWFQQKPGQSPQLLIYR
MSNLNSGVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPITFGAGTKLEIKRTVA
APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

> 4G7 L1.164 (SEQ ID NO:110)

DIVMTQSPATLSLSPGERATLSCRSSKSLQNANGNTYLYWFQQKPGQSPQLLIYR
MSNLNSGVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPITFGAGTKLEIKRTVA
APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

> 4G7 VH CDR2 D55A (SEQ ID NO:111)

YINPYNAGTKYNEKFKG

> 4G7 VH CDR2 T57P (SEQ ID NO:112)

YINPYNDGPKYNEKFKG

> 4G7 VH CDR2 K58E (SEQ ID NO:113)

YINPYNDGTEYNEKFKG

> 4G7 VH CDR2 D55S (SEQ ID NO:114)

YINPYNSGTKYNEKFKG

> 4G7 VH CDR2 D55E (SEQ ID NO:115)

YINPYNEGTKYNEKFKG

> 4G7 VH CDR3 S100T (SEQ ID NO:116)

GTYYYGTRVFDY

> 4G7 VH CDR3 R100dS (SEQ ID NO:117)

GTYYYGSSVFDY

> 4G7 VH CDR3 S100cT/R100dS (SEQ ID NO:118)

GTYYYGTSVFDY

> 4G7 VL CDR1 L27cQ (SEQ ID NO:119)

RSSKSLQNSNGNTYLY

> 4G7 VL CDR1 L27cQ/S27eV (SEQ ID NO:120)

RSSKSLQNVNGNTYLY

> 4G7 VL CDR1 S27eV (SEQ ID NO:121)

RSSKSLLNVNGNTYLY

> 4G7 VL CDR1 G29A (SEQ ID NO:122)

RSSKSLLNSNANTYLY

> 4G7 VL CDR1 L27cQ/S27eV/G29A (SEQ ID NO:123)

RSSKSLQNVNANTYLY

> 4G7 VL CDR1 S27eA (SEQ ID NO:124)

RSSKSLLNANGNTYLY

> 4G7 VL CDR1 L27cQ/S27eA/G29A (SEQ ID NO:125)

RSSKSLQNANANTYLY

> 4G7 VL CDR1 G29S (SEQ ID NO:126)

RSSKSLLNSNSNTYLY

> 4G7 VL CDR1 L27cQ/S27eA/G29S (SEQ ID NO:127)

RSSKSLQNANSNTYLY

> 4G7 VL CDR1 L27cQ/S27eA (SEQ ID NO:128)

RSSKSLQNANGNTYLY

> 4G7 VL CDR2 A55N (SEQ ID NO:129)

RMSNLNS

> 4G7 VL CDR3 F96I (SEQ ID NO:130)

MQHLEYPIT

> 4G7 VL CDR3 F96N (SEQ ID NO:131)

MQHLEYPNT

> 4G7 VH CDR1 (SEQ ID NO:132): SYVMH

> 4G7 VH CDR2 (SEQ ID NO:133): YINPYNDGTKYNEKFKG

> 4G7 VH CDR3 (SEQ ID NO:134): GTYYYGSRVFDY

> 4G7 VL CDR1 (SEQ ID NO:135): RSSKSLLNSNGNTYLY

> 4G7 VL CDR2 (SEQ ID NO:136): RMSNLAS

> 4G7 VL CDR3 (SEQ ID NO:137): MQHLEYPFT

> HD37 VH CDR1 (SEQ ID NO:138): SYWMN

> HD37 VH CDR2 (SEQ ID NO:139): QIWPGDGDTNYNGKFKG

> HD37 VH CDR3 (SEQ ID NO:140): RETTTVGRYYYAMDY

> HD37 VL CDR1 (SEQ ID NO:141): KASQSVDYDGDSYLN

> HD37 VL CDR2 (SEQ ID NO:142): DASNLVS

> HD37 VL CDR3 (SEQ ID NO:143): QQSTEDPWT

All cited references are herein expressly incorporated by reference in their entirety.

Whereas particular embodiments of the invention have been described above for purposes of illustration, it will be appreciated by those skilled in the art that numerous variations of the details may be made without departing from the invention as described in the appended claims.

CD-19 CLAIMS

1. An antibody that binds CD19, said antibody comprising a heavy chain and/or a light chain, said heavy chain having a CDR1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 132 and 138, a CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:111-115 and a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:116-118; and said light chain having a CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 119-128, a CDR2 comprising the amino acid sequence of SEQ ID NOs:129, and a CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:130-131.

2. An antibody according to claim 1, wherein said antibody comprises a variable heavy chain sequence selected from the group consisting of SEQ ID NOS: 13-16, 20-23, and 27-44, and/or a variable light chain sequence selected from the group consisting of SEQ ID NOS: 17-19, 24-26, and 45-79.

3. An antibody according to claim 2, wherein said antibody comprises a variable heavy chain sequence selected from the group consisting of SEQ ID NOS: 86-95, and/or a variable light chain sequence selected from the group consisting of SEQ ID NOS: 96-110.

4. An antibody according to claim 1, wherein said antibody binds with increased affinity to the FcγRIIIa receptor or enhances ADCC effector function as compared to the parent antibody.

5. An antibody according to claim 4, wherein said modification is an amino acid.

6. The antibody according to claim 5, wherein said amino acid modification is at a position selected from the group consisting of 221, 222, 223, 224, 225, 227, 228, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 243, 244, 245, 246, 247, 249, 255, 258, 260, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 278, 280, 281, 282, 283, 284, 285, 286, 288, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 313, 317, 318, 320, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, and 337, wherein numbering is according to the EU index.

7. An antibody according to claim 5, wherein said amino acid modification is a substitution selected from the group consisting of 221K, 221Y, 222E, 222Y, 223E, 223K, 224E, 224Y, 225E, 225K, 225W, 227E, 227G, 227K, 227Y, 228E, 228G, 228K, 228Y, 230A, 230E, 230G, 230Y, 231E, 231G, 231K, 231P, 231Y, 232E, 232G, 232K, 232Y, 233A,

233D, 233F, 233G, 233H, 233I, 233K, 233L, 233M, 233N, 233Q, 233R, 233S, 233T, 233V, 233W, 233Y, 234A, 234D, 234E, 234F, 234G, 234H, 234I, 234K, 234M, 234N, 234P, 234Q, 234R, 234S, 234T, 234V, 234W, 234Y, 235A, 235D, 235E, 235F, 235G, 235H, 235I, 235K, 235M, 235N, 235P, 235Q, 235R, 235S, 235T, 235V, 235W, 235Y, 236A, 236D, 236E, 236F, 236H, 236I, 236K, 236L, 236M, 236N, 236P, 236Q, 236R, 236S, 236T, 236V, 236W, 236Y, 237D, 237E, 237F, 237H, 237I, 237K, 237L, 237M, 237N, 237P, 237Q, 237R, 237S, 237T, 237V, 237W, 237Y, 238D, 238E, 238F, 238G, 238H, 238I, 238K, 238L, 238M, 238N, 238Q, 238R, 238S, 238T, 238V, 238W, 238Y, 239D, 239E, 239F, 239G, 239H, 239I, 239K, 239L, 239M, 239N, 239P, 239Q, 239R, 239T, 239V, 239W, 239Y, 240A, 240I, 240M, 240T, 241D, 241E, 241L, 241R, 241S, 241W, 241Y, 243E, 243H, 243L, 243Q, 243R, 243W, 243Y, 244H, 245A, 246D, 246E, 246H, 246Y, 247G, 247V, 249H, 249Q, 249Y, 255E, 255Y, 258H, 258S, 258Y, 260D, 260E, 260H, 260Y, 262A, 262E, 262F, 262I, 262T, 263A, 263I, 263M, 263T, 264A, 264D, 264E, 264F, 264G, 264H, 264I, 264K, 264L, 264M, 264N, 264P, 264Q, 264R, 264S, 264T, 264W, 264Y, 265F, 265G, 265H, 265I, 265K, 265L, 265M, 265N, 265P, 265Q, 265R, 265S, 265T, 265V, 265W, 265Y, 266A, 266I, 266M, 266T, 267D, 267E, 267F, 267H, 267I, 267K, 267L, 267M, 267N, 267P, 267Q, 267R, 267T, 267V, 267W, 267Y, 268D, 268E, 268F, 268G, 268I, 268K, 268L, 268M, 268P, 268Q, 268R, 268T, 268V, 268W, 269F, 269G, 269H, 269I, 269K, 269L, 269M, 269N, 269P, 269R, 269S, 269T, 269V, 269W, 269Y, 270F, 270G, 270H, 270I, 270L, 270M, 270P, 270Q, 270R, 270S, 270T, 270W, 270Y, 271A, 271D, 271E, 271F, 271G, 271H, 271I, 271K, 271L, 271M, 271N, 271Q, 271R, 271S, 271T, 271V, 271W, 271Y, 272D, 272F, 272G, 272H, 272I, 272K, 272L, 272M, 272P, 272R, 272S, 272T, 272V, 272W, 272Y, 273I, 274D, 274E, 274F, 274G, 274H, 274I, 274L, 274M, 274N, 274P, 274R, 274T, 274V, 274W, 274Y, 275L, 275W, 276D, 276E, 276F, 276G, 276H, 276I, 276L, 276M, 276P, 276R, 276S, 276T, 276V, 276W, 276Y, 278D, 278E, 278G, 278H, 278I, 278K, 278L, 278M, 278N, 278P, 278Q, 278R, 278S, 278T, 278V, 278W, 280G, 280K, 280L, 280P, 280W, 281D, 281E, 281K, 281N, 281P, 281Q, 281Y, 282E, 282G, 282K, 282P, 282Y, 283G, 283H, 283K, 283L, 283P, 283R, 283Y, 284D, 284E, 284L, 284N, 284Q, 284T, 284Y, 285D, 285E, 285K, 285Q, 285W, 285Y, 286E, 286G, 286P, 286Y, 288D, 288E, 288Y, 290D, 290H, 290L, 290N, 290W, 291D, 291E, 291G, 291H, 291I, 291Q, 291T, 292D, 292E, 292T, 292Y, 293F, 293G, 293H, 293I, 293L, 293M, 293N, 293P, 293R, 293S, 293T, 293V, 293W, 293Y, 294F, 294G, 294H, 294I, 294K, 294L, 294M, 294P, 294R, 294S, 294T, 294V, 294W, 294Y, 295D, 295E, 295F, 295G, 295H, 295I, 295M, 295N, 295P, 295R, 295S, 295T, 295V, 295W, 295Y, 296A, 296D, 296E, 296G, 296H, 296I,

296K, 296L, 296M, 296N, 296Q, 296R, 296S, 296T, 296V, 297D, 297E, 297F, 297G, 297H, 297I, 297K, 297L, 297M, 297P, 297Q, 297R, 297S, 297T, 297V, 297W, 297Y, 298A, 298D, 298E, 298F, 298H, 298I, 298K, 298M, 298N, 298Q, 298R, 298T, 298W, 298Y, 299A, 299D, 299E, 299F, 299G, 299H, 299I, 299K, 299L, 299M, 299N, 299P, 299Q, 299R, 299S, 299V, 299W, 299Y, 300A, 300D, 300E, 300G, 300H, 300K, 300M, 300N, 300P, 300Q, 300R, 300S, 300T, 300V, 300W, 301D, 301E, 301H, 301Y, 302I, 303D, 303E, 303Y, 304D, 304H, 304L, 304N, 304T, 305E, 305T, 305Y, 313F, 317E, 317Q, 318H, 318L, 318Q, 318R, 318Y, 320D, 320F, 320G, 320H, 320I, 320L, 320N, 320P, 320S, 320T, 320V, 320W, 320Y, 322D, 322F, 322G, 322H, 322I, 322P, 322S, 322T, 322V, 322W, 322Y, 323I, 324D, 324F, 324G, 324H, 324I, 324L, 324M, 324P, 324R, 324T, 324V, 324W, 324Y, 325A, 325D, 325E, 325F, 325G, 325H, 325I, 325K, 325L, 325M, 325P, 325Q, 325R, 325S, 325T, 325V, 325W, 325Y, 326E, 326I, 326L, 326P, 326T, 327D, 327E, 327F, 327H, 327I, 327K, 327L, 327M, 327N, 327P, 327R, 327S, 327T, 327V, 327W, 327Y, 328A, 328D, 328E, 328F, 328G, 328H, 328I, 328K, 328M, 328N, 328P, 328Q, 328R, 328S, 328T, 328V, 328W, 328Y, 329D, 329E, 329F, 329G, 329H, 329I, 329K, 329L, 329M, 329N, 329Q, 329R, 329S, 329T, 329V, 329W, 329Y, 330E, 330F, 330G, 330H, 330I, 330L, 330M, 330N, 330P, 330R, 330S, 330T, 330V, 330W, 330Y, 331D, 331F, 331H, 331I, 331L, 331M, 331Q, 331R, 331T, 331V, 331W, 331Y, 332A, 332D, 332E, 332F, 332H, 332K, 332L, 332M, 332N, 332P, 332Q, 332R, 332S, 332T, 332V, 332W, 332Y, 333A, 333F, 333H, 333I, 333L, 333M, 333P, 333T, 333Y, 334A, 334F, 334I, 334L, 334P, 334T, 335D, 335F, 335G, 335H, 335I, 335L, 335M, 335N, 335P, 335R, 335S, 335V, 335W, 335Y, 336E, 336K, 336Y, 337E, 337H, and 337N, wherein numbering is according to the EU index.

8. An antibody according to claim 5, wherein said amino acid modification is at a position selected from the group consisting of 221, 222, 223, 224, 225, 227, 228, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 243, 245, 246, 247, 249, 255, 258, 260, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 274, 275, 276, 278, 280, 281, 282, 283, 284, 285, 286, 288, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 313, 317, 318, 320, 322, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, and 337.

9. An antibody according to claim 5, wherein said amino acid modification is a substitution selected from the group consisting of 221K, 222Y, 223E, 223K, 224E, 224Y, 225E, 225W, 227E, 227G, 227K, 227Y, 228E, 228G, 228K, 228Y, 230A, 230E, 230G, 230Y, 231E, 231G, 231K, 231P, 231Y, 232E, 232G, 232K, 232Y, 233A, 233F, 233H, 233I,

233K, 233L, 233M, 233N, 233Q, 233R, 233S, 233T, 233V, 233W, 233Y, 234D, 234E, 234F, 234G, 234H, 234I, 234K, 234M, 234N, 234P, 234Q, 234R, 234S, 234T, 234W, 234Y, 235D, 235F, 235G, 235H, 235I, 235K, 235M, 235N, 235Q, 235R, 235S, 235T, 235V, 235W, 235Y, 236D, 236E, 236F, 236H, 236I, 236K, 236L, 236M, 236N, 236P, 236Q, 236R, 236S, 236T, 236V, 236W, 236Y, 237D, 237E, 237F, 237H, 237I, 237K, 237L, 237M, 237N, 237P, 237Q, 237R, 237S, 237T, 237V, 237W, 237Y, 238D, 238E, 238F, 238G, 238H, 238I, 238K, 238L, 238M, 238N, 238Q, 238R, 238S, 238T, 238V, 238W, 238Y, 239D, 239E, 239F, 239G, 239H, 239I, 239K, 239L, 239M, 239N, 239P, 239Q, 239R, 239T, 239V, 239W, 239Y, 240M, 240T, 241D, 241E, 241R, 241S, 241W, 241Y, 243E, 243H, 243Q, 243R, 243W, 243Y, 245A, 246D, 246H, 246Y, 247G, 247V, 249H, 249Q, 249Y, 255E, 255Y, 258H, 258S, 258Y, 260D, 260E, 260H, 260Y, 262A, 262E, 262F, 262I, 262T, 263A, 263I, 263M, 263T, 264D, 264E, 264F, 264G, 264H, 264I, 264K, 264L, 264M, 264N, 264P, 264Q, 264R, 264S, 264T, 264W, 264Y, 265F, 265G, 265H, 265I, 265K, 265L, 265M, 265P, 265Q, 265R, 265S, 265T, 265V, 265W, 265Y, 266A, 266I, 266M, 266T, 267D, 267E, 267F, 267H, 267I, 267K, 267L, 267M, 267N, 267P, 267Q, 267R, 267V, 267W, 267Y, 268F, 268G, 268I, 268M, 268P, 268T, 268V, 268W, 269F, 269G, 269H, 269I, 269L, 269M, 269N, 269P, 269R, 269S, 269T, 269V, 269W, 269Y, 270F, 270G, 270H, 270I, 270L, 270M, 270P, 270Q, 270R, 270S, 270T, 270W, 270Y, 271A, 271D, 271E, 271F, 271G, 271H, 271I, 271K, 271L, 271M, 271N, 271Q, 271R, 271S, 271T, 271V, 271W, 271Y, 272F, 272G, 272H, 272I, 272K, 272L, 272M, 272P, 272R, 272S, 272T, 272V, 272W, 272Y, 274D, 274E, 274F, 274G, 274H, 274I, 274L, 274M, 274P, 274R, 274T, 274V, 274W, 274Y, 275W, 276D, 276E, 276F, 276G, 276H, 276I, 276L, 276M, 276P, 276R, 276S, 276T, 276V, 276W, 278D, 278E, 278G, 278H, 278I, 278K, 278L, 278M, 278N, 278P, 278Q, 278R, 278S, 278T, 278V, 278W, 280G, 280P, 280W, 281E, 281K, 281N, 281P, 281Y, 282G, 282P, 282Y, 283G, 283H, 283K, 283L, 283P, 283R, 283Y, 284L, 284N, 284Q, 284T, 284Y, 285K, 285Q, 285W, 285Y, 286G, 286P, 286Y, 288Y, 290H, 290L, 290W, 291D, 291E, 291G, 291H, 291I, 291Q, 291T, 292D, 292E, 292T, 292Y, 293F, 293G, 293H, 293I, 293L, 293M, 293N, 293P, 293R, 293S, 293T, 293W, 293Y, 294F, 294G, 294H, 294I, 294K, 294L, 294M, 294P, 294R, 294S, 294T, 294V, 294W, 294Y, 295D, 295F, 295G, 295H, 295I, 295M, 295N, 295P, 295R, 295S, 295T, 295V, 295W, 295Y, 296A, 296D, 296E, 296G, 296I, 296K, 296L, 296M, 296N, 296Q, 296R, 296S, 296T, 296V, 297D, 297E, 297F, 297G, 297H, 297I, 297K, 297L, 297M, 297P, 297R, 297S, 297T, 297V, 297W, 297Y, 298E, 298F, 298H, 298I, 298K, 298M, 298Q, 298R, 298W, 298Y, 299A, 299D, 299E, 299F, 299G, 299H, 299I, 299K, 299L, 299M, 299N, 299P, 299Q, 299R, 299S, 299V,

299W, 299Y, 300A, 300D, 300E, 300G, 300H, 300K, 300M, 300N, 300P, 300Q, 300R, 300S, 300T, 300V, 300W, 301D, 301E, 301Y, 302I, 303D, 303E, 303Y, 304H, 304L, 304N, 304T, 305E, 305T, 305Y, 313F, 317E, 317Q, 318H, 318L, 318Q, 318R, 318Y, 320D, 320F, 320G, 320H, 320I, 320L, 320N, 320P, 320S, 320T, 320V, 320W, 320Y, 322D, 322F, 322G, 322H, 322I, 322P, 322S, 322T, 322V, 322W, 322Y, 324D, 324F, 324G, 324H, 324I, 324L, 324M, 324P, 324R, 324T, 324V, 324W, 324Y, 325A, 325D, 325E, 325F, 325G, 325H, 325I, 325K, 325L, 325M, 325P, 325Q, 325R, 325S, 325T, 325V, 325W, 325Y, 326L, 326P, 326T, 327D, 327E, 327F, 327H, 327I, 327K, 327L, 327M, 327P, 327R, 327V, 327W, 327Y, 328A, 328D, 328E, 328F, 328G, 328H, 328K, 328M, 328N, 328P, 328Q, 328R, 328S, 328T, 328V, 328W, 328Y, 329D, 329E, 329F, 329G, 329H, 329I, 329K, 329L, 329M, 329N, 329Q, 329R, 329S, 329T, 329V, 329W, 329Y, 330E, 330F, 330H, 330I, 330L, 330M, 330N, 330P, 330W, 330Y, 331D, 331F, 331H, 331I, 331L, 331M, 331Q, 331R, 331T, 331V, 331W, 331Y, 332A, 332F, 332H, 332L, 332M, 332N, 332P, 332Q, 332S, 332T, 332V, 332W, 332Y, 333F, 333H, 333I, 333L, 333M, 333P, 333T, 333Y, 334F, 334P, 334T, 335D, 335F, 335G, 335H, 335I, 335L, 335M, 335P, 335R, 335S, 335V, 335W, 335Y, 336E, 336K, 336Y, 337H, and 337N.

10. The antibody of claim 5, wherein said modification is at a position selected from the group consisting of 221, 222, 223, 224, 225, 228, 230, 231, 232, 240, 244, 245, 247, 262, 263, 266, 271, 273, 275, 281, 284, 291, 299, 302, 304, 313, 323, 325, 328, 332, 336, wherein the positional numbering is according to the EU index.

11. The antibody of claim 5, wherein said modification is selected from the group consisting of 221K, 221Y, 222E, 222Y, 223E, 223K, 224E, 224Y, 225E, 225K, 225W, 228E, 228G, 228K, 228Y, 230A, 230E, 230G, 230Y, 231E, 231G, 231K, 231P, 231Y, 232E, 232G, 232K, 232Y, 240A, 240I, 240M, 240T, 244H, 245A, 247G, 247V, 262A, 262E, 262F, 262I, 262T, 263A, 263I, 263M, 263T, 266A, 266I, 266M, 266T, 271A, 271D, 271E, 271F, 271G, 271H, 271I, 271K, 271L, 271M, 271N, 271Q, 271R, 271S, 271T, 271V, 271W, 271Y, 273I, 275L, 275W, 281D, 281E, 281K, 281N, 281P, 281Q, 281Y, 284D, 284E, 284L, 284N, 284Q, 284T, 284Y, 291D, 291E, 291G, 291H, 291I, 291Q, 291T, 299A, 299D, 299E, 299F, 299G, 299H, 299I, 299K, 299L, 299M, 299N, 299P, 299Q, 299R, 299S, 299V, 299W, 299Y, 304D, 304H, 304L, 304N, 304T, 313F, 323I, 325A, 325D, 325E, 325F, 325G, 325H, 325I, 325K, 325L, 325M, 325P, 325Q, 325R, 325S, 325T, 325V, 325W, 325Y, 328A, 328D, 328E, 328F, 328G, 328H, 328I, 328K, 328M, 328N, 328P, 328Q, 328R, 328S, 328T, 328V, 328W, 328Y, 332A, 332D, 332E, 332F,

332H, 332K, 332L, 332M, 332N, 332P, 332Q, 332R, 332S, 332T, 332V, 332W, 332Y, 336E, 336K, and 336Y.

12. An antibody according to claim 10, further comprising a second amino acid modification is at a position selected from the group consisting of 221, 222, 223, 224, 225, 227, 228, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 243, 244, 245, 246, 247, 249, 255, 258, 260, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 278, 280, 281, 282, 283, 284, 285, 286, 288, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 313, 317, 318, 320, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, and 337, wherein numbering is according to the EU index.

13. An antibody according to claim 10, wherein said second amino acid modification is a substitution selected from the group consisting of 221K, 221Y, 222E, 222Y, 223E, 223K, 224E, 224Y, 225E, 225K, 225W, 227E, 227G, 227K, 227Y, 228E, 228G, 228K, 228Y, 230A, 230E, 230G, 230Y, 231E, 231G, 231K, 231P, 231Y, 232E, 232G, 232K, 232Y, 233A, 233D, 233F, 233G, 233H, 233I, 233K, 233L, 233M, 233N, 233Q, 233R, 233S, 233T, 233V, 233W, 233Y, 234A, 234D, 234E, 234F, 234G, 234H, 234I, 234K, 234M, 234N, 234P, 234Q, 234R, 234S, 234T, 234V, 234W, 234Y, 235A, 235D, 235E, 235F, 235G, 235H, 235I, 235K, 235M, 235N, 235P, 235Q, 235R, 235S, 235T, 235V, 235W, 235Y, 236A, 236D, 236E, 236F, 236H, 236I, 236K, 236L, 236M, 236N, 236P, 236Q, 236R, 236S, 236T, 236V, 236W, 236Y, 237D, 237E, 237F, 237H, 237I, 237K, 237L, 237M, 237N, 237P, 237Q, 237R, 237S, 237T, 237V, 237W, 237Y, 238D, 238E, 238F, 238G, 238H, 238I, 238K, 238L, 238M, 238N, 238Q, 238R, 238S, 238T, 238V, 238W, 238Y, 239D, 239E, 239F, 239G, 239H, 239I, 239K, 239L, 239M, 239N, 239P, 239Q, 239R, 239T, 239V, 239W, 239Y, 240A, 240I, 240M, 240T, 241D, 241E, 241L, 241R, 241S, 241W, 241Y, 243E, 243H, 243L, 243Q, 243R, 243W, 243Y, 244H, 245A, 246D, 246E, 246H, 246Y, 247G, 247V, 249H, 249Q, 249Y, 255E, 255Y, 258H, 258S, 258Y, 260D, 260E, 260H, 260Y, 262A, 262E, 262F, 262I, 262T, 263A, 263I, 263M, 263T, 264A, 264D, 264E, 264F, 264G, 264H, 264I, 264K, 264L, 264M, 264N, 264P, 264Q, 264R, 264S, 264T, 264W, 264Y, 265F, 265G, 265H, 265I, 265K, 265L, 265M, 265N, 265P, 265Q, 265R, 265S, 265T, 265V, 265W, 265Y, 266A, 266I, 266M, 266T, 267D, 267E, 267F, 267H, 267I, 267K, 267L, 267M, 267N, 267P, 267Q, 267R, 267T, 267V, 267W, 267Y, 268D, 268E, 268F, 268G, 268I, 268K, 268L, 268M, 268P, 268Q, 268R, 268T, 268V, 268W, 269F, 269G, 269H, 269I, 269K, 269L, 269M, 269N, 269P, 269R, 269S, 269T, 269V, 269W, 269Y, 270F, 270G, 270H, 270I, 270L, 270M, 270P, 270Q,

270R, 270S, 270T, 270W, 270Y, 271A, 271D, 271E, 271F, 271G, 271H, 271I, 271K, 271L, 271M, 271N, 271Q, 271R, 271S, 271T, 271V, 271W, 271Y, 272D, 272F, 272G, 272H, 272I, 272K, 272L, 272M, 272P, 272R, 272S, 272T, 272V, 272W, 272Y, 273I, 274D, 274E, 274F, 274G, 274H, 274I, 274L, 274M, 274N, 274P, 274R, 274T, 274V, 274W, 274Y, 275L, 275W, 276D, 276E, 276F, 276G, 276H, 276I, 276L, 276M, 276P, 276R, 276S, 276T, 276V, 276W, 276Y, 278D, 278E, 278G, 278H, 278I, 278K, 278L, 278M, 278N, 278P, 278Q, 278R, 278S, 278T, 278V, 278W, 280G, 280K, 280L, 280P, 280W, 281D, 281E, 281K, 281N, 281P, 281Q, 281Y, 282E, 282G, 282K, 282P, 282Y, 283G, 283H, 283K, 283L, 283P, 283R, 283Y, 284D, 284E, 284L, 284N, 284Q, 284T, 284Y, 285D, 285E, 285K, 285Q, 285W, 285Y, 286E, 286G, 286P, 286Y, 288D, 288E, 288Y, 290D, 290H, 290L, 290N, 290W, 291D, 291E, 291G, 291H, 291I, 291Q, 291T, 292D, 292E, 292T, 292Y, 293F, 293G, 293H, 293I, 293L, 293M, 293N, 293P, 293R, 293S, 293T, 293V, 293W, 293Y, 294F, 294G, 294H, 294I, 294K, 294L, 294M, 294P, 294R, 294S, 294T, 294V, 294W, 294Y, 295D, 295E, 295F, 295G, 295H, 295I, 295M, 295N, 295P, 295R, 295S, 295T, 295V, 295W, 295Y, 296A, 296D, 296E, 296G, 296H, 296I, 296K, 296L, 296M, 296N, 296Q, 296R, 296S, 296T, 296V, 297D, 297E, 297F, 297G, 297H, 297I, 297K, 297L, 297M, 297P, 297Q, 297R, 297S, 297T, 297V, 297W, 297Y, 298A, 298D, 298E, 298F, 298H, 298I, 298K, 298M, 298N, 298Q, 298R, 298T, 298W, 298Y, 299A, 299D, 299E, 299F, 299G, 299H, 299I, 299K, 299L, 299M, 299N, 299P, 299Q, 299R, 299S, 299V, 299W, 299Y, 300A, 300D, 300E, 300G, 300H, 300K, 300M, 300N, 300P, 300Q, 300R, 300S, 300T, 300V, 300W, 301D, 301E, 301H, 301Y, 302I, 303D, 303E, 303Y, 304D, 304H, 304L, 304N, 304T, 305E, 305T, 305Y, 313F, 317E, 317Q, 318H, 318L, 318Q, 318R, 318Y, 320D, 320F, 320G, 320H, 320I, 320L, 320N, 320P, 320S, 320T, 320V, 320W, 320Y, 322D, 322F, 322G, 322H, 322I, 322P, 322S, 322T, 322V, 322W, 322Y, 323I, 324D, 324F, 324G, 324H, 324I, 324L, 324M, 324P, 324R, 324T, 324V, 324W, 324Y, 325A, 325D, 325E, 325F, 325G, 325H, 325I, 325K, 325L, 325M, 325P, 325Q, 325R, 325S, 325T, 325V, 325W, 325Y, 326E, 326I, 326L, 326P, 326T, 327D, 327E, 327F, 327H, 327I, 327K, 327L, 327M, 327N, 327P, 327R, 327S, 327T, 327V, 327W, 327Y, 328A, 328D, 328E, 328F, 328G, 328H, 328I, 328K, 328M, 328N, 328P, 328Q, 328R, 328S, 328T, 328V, 328W, 328Y, 329D, 329E, 329F, 329G, 329H, 329I, 329K, 329L, 329M, 329N, 329Q, 329R, 329S, 329T, 329V, 329W, 329Y, 330E, 330F, 330G, 330H, 330I, 330L, 330M, 330N, 330P, 330R, 330S, 330T, 330V, 330W, 330Y, 331D, 331F, 331H, 331I, 331L, 331M, 331Q, 331R, 331T, 331V, 331W, 331Y, 332A, 332D, 332E, 332F, 332H, 332K, 332L, 332M, 332N, 332P, 332Q, 332R, 332S, 332T, 332V, 332W, 332Y, 333A, 333F, 333H, 333I, 333L, 333M,

333P, 333T, 333Y, 334A, 334F, 334I, 334L, 334P, 334T, 335D, 335F, 335G, 335H, 335I, 335L, 335M, 335N, 335P, 335R, 335S, 335V, 335W, 335Y, 336E, 336K, 336Y, 337E, 337H, and 337N, wherein numbering is according to the EU index.

14. An antibody according to claim 5, wherein the amino acid modification is 332E.

15. An antibody according to claim 14, further comprising a second amino acid modification selected from the group consisting of: 236A, 239D, 332E, 268D, 268E, 330Y, and 330L.

16. An antibody according to claim 15, wherein the second amino acid modification is 239D.

17. An antibody according to claim 4, wherein said modification is a glycoform modification that reduces the level of fucose relative to the parent antibody.

18. A composition comprising plurality of glycosylated antibodies, wherein about 80-100% of the glycosylated antibody in the composition comprises a mature core carbohydrate structure which lacks fucose.

19. An antibody according to claim 1, wherein said antibody further reduces binding to FcγRIIb as compared to said parent anti-CD19 antibody.

20. A nucleic acid sequence encoding an antibody of one of claims 1-17.

21. A method of treating a B-cell related disease, wherein said method comprises administering an antibody according to claim 1.

22. A method of claim 21, wherein said disease is selected from the group consisting of: non-Hodgkin's lymphomas (NHL), chronic lymphocytic leukemia (CLL), B-cell acute lymphoblastic leukemia/lymphoma (B-ALL), and mantle cell lymphoma (MCL).

23. A method of claim 21, wherein said disease is an autoimmune disease.

24. The method of claim 23, wherein said autoimmune disease is selected from the group consisting of: rheumatoid arthritis (RA), systemic lupus erythematosus (SLE or lupus), multiple sclerosis, Sjogren's syndrome, and idiopathic thrombocytopenia purpura (ITP).

25. A pharmaceutical composition comprising an antibody according to claim 1 and a pharmaceutically acceptable carrier.

Figure 1

> Human CD19 (SEQ ID NO:1).

MPPPRLLFFLLFLTPMEVRPEEPLVVKVEEGDNAVLQCLKGTSDGPTQQLTWSRESPLKPF
LKLSLGLPGLGIHMRPLAIWLFIFNVSQQMGGFYLCQPGPPSEKAWQPGWTVNVEGSGEL
FRWNVSDLGGLGCGLKNRSSEGPSSPSGKLMSPKLYVWAKDRPEIWEGEPPCLPPRDSL
NQSLSQDLTMAPGSTLWLSCGVPPDSVSRGPLSWTHVHPKGPKSLLSLELKDDRPARDM
WVMETGLLLPRATAQDAGKYYCHRGNLTMSFHLEITARPVLWHWLLRTGGWKVSAVTLAY
LIFCLCSLVGILHLQRALVLRRKRKRMTDPTRRFFKVTPPPGSGPQNQYGNVLSLPTPTSGL
GRAQRWAAGLGGTAPSYGNPSSDVQADGALGSRSPPGVGPEEEEGEGYEEPDSEEDSE
FYENDSNLGQDQLSQDGSGYENPEDEPLGPEDEDSFSNAESYENEDEELTQPVARTMDFL
SPHGSAWDPSREATSLGSQSYEDMRGILYAAPQLRSIRGQPGPNHEEDADSYENMDNPD
GPDPAWGGGGRMGTWSTR

Figure 2

> Kappa constant light chain (Cκ) (SEQ ID NO:2)

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

> IgG1 constant heavy chain (CH) (SEQ ID NO:3)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPGK

> IgG2 constant heavy chain (CH) (SEQ ID NO:4)

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLF
PPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVV
SVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGK

> IgG3 constant heavy chain (CH) (SEQ ID NO:5)

ASTKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYTCNVNHKPSNTKVDKRVELKTPLGDTTHTCPRCPEPKSCD
TPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPAPELLGGPSVFLFPPKPKDTL
MISRTPEVTCVVVDVSHEDPEVQFKWYVDGVEVHNAKTKPREEQYNSTFRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF
YPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEAL
HNRFTQKSLSLSPGK

> IgG4 constant heavy chain (CH) (SEQ ID NO:6)

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSVFL
FPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVF
SCSVMHEALHNHYTQKSLSLSLGK

> Hybrid constant heavy chain (CH) (SEQ ID NO:7)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTF
RVVSVLTVVHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPGK

> Hybrid constant heavy chain (CH) with 239D and 332E substitutions (SEQ ID NO:8)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPD
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTF
RVVSVLTVVHQDWLNGKEYKCKVSNKALPAPEEKTISKTKGQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK

Figure 3a

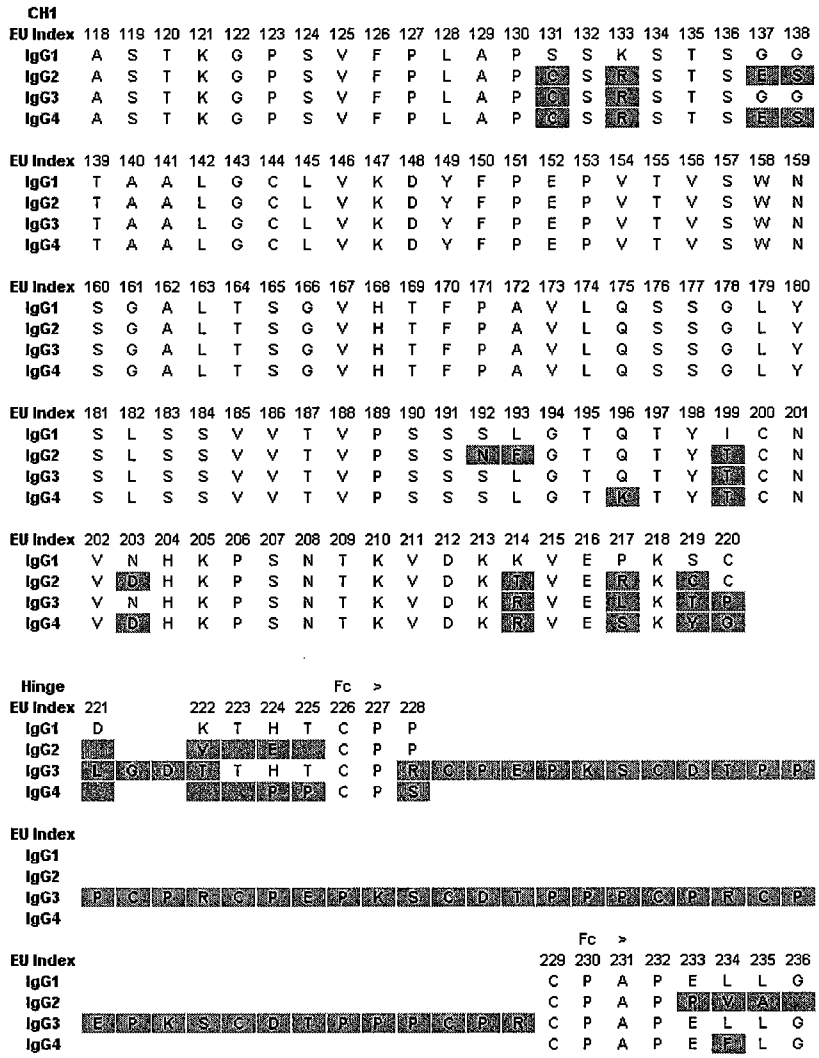
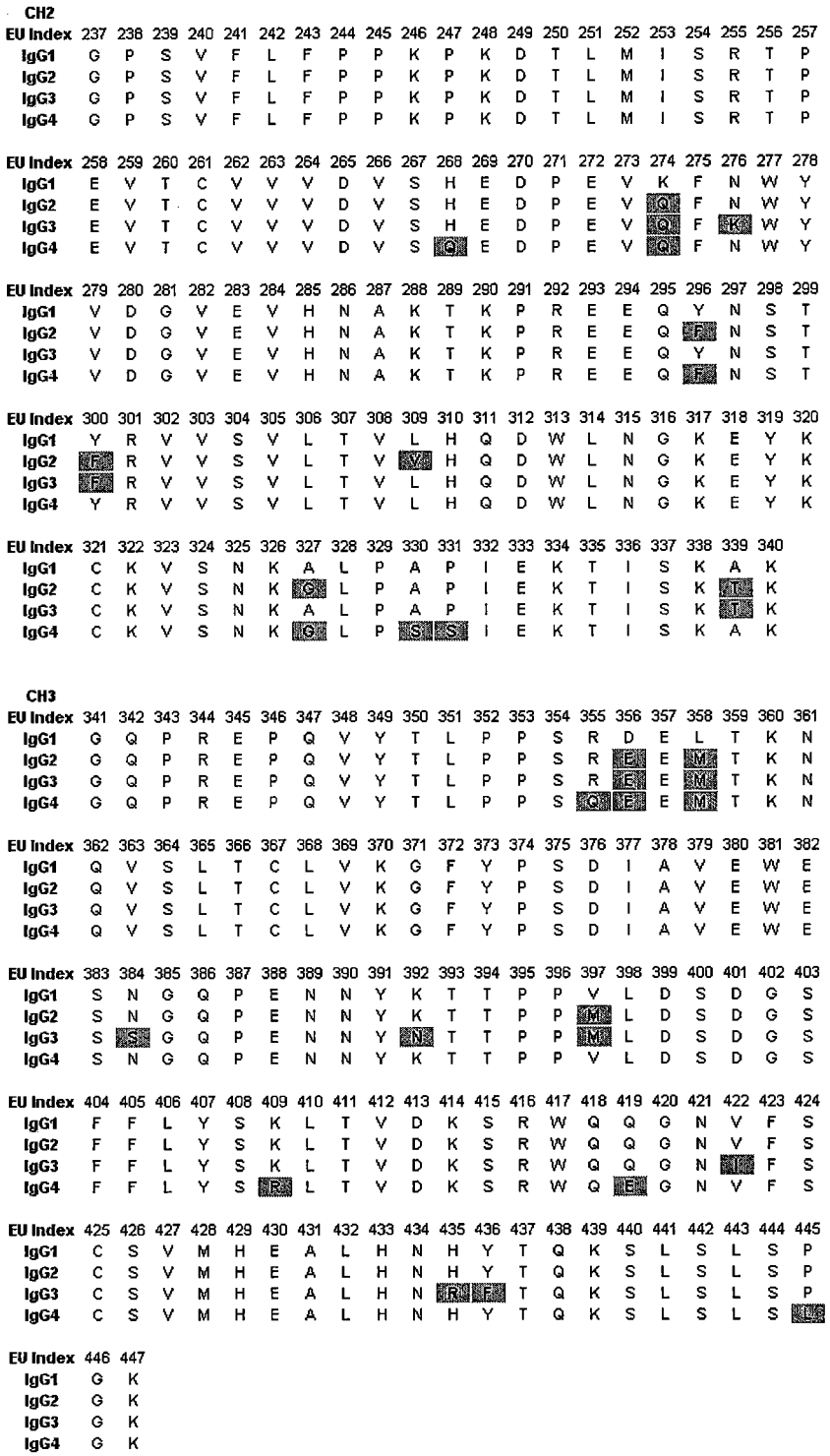

CH1
EU Index 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138
IgG1 A S T K G P S V F P L A P S S K S T S G G
IgG2 A S T K G P S V F P L A P C S R S T S E S
IgG3 A S T K G P S V F P L A P C S R S T S G G
IgG4 A S T K G P S V F P L A P C S R S T S E S
EU Index 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159
IgG1 T A A L G C L V K D Y F P E P V T V S W N
IgG2 T A A L G C L V K D Y F P E P V T V S W N
IgG3 T A A L G C L V K D Y F P E P V T V S W N
IgG4 T A A L G C L V K D Y F P E P V T V S W N
EU Index 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180
IgG1 S G A L T S G V H T F P A V L Q S S G L Y
IgG2 S G A L T S G V H T F P A V L Q S S G L Y
IgG3 S G A L T S G V H T F P A V L Q S S G L Y
IgG4 S G A L T S G V H T F P A V L Q S S G L Y
EU Index 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201
IgG1 S L S S V V T V P S S S L G T Q T Y I C N
IgG2 S L S S V V T V P S S N F G T Q T Y T C N
IgG3 S L S S V V T V P S S S L G T Q T Y T C N
IgG4 S L S S V V T V P S S S L G T K T Y T C N
EU Index 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220
IgG1 V N H K P S N T K V D K K V E P K S C
IgG2 V D H K P S N T K V D K T V E R K C C
IgG3 V N H K P S N T K V D K R V E L K T P
IgG4 V D H K P S N T K V D K R V E S K Y G
Hinge
Fc
EU Index 221 222 223 224 225 226 227 228
IgG1 D K T H T C P P
IgG2 V E C P P
IgG3 L E G D T T H T C P R C P E P K S C D T P P
IgG4 V E C P P S
EU Index
IgG1
IgG2
IgG3 P C P R C P E P K S C D T P P P C P R C P
IgG4
Fc
EU Index 229 230 231 232 233 234 235 236
IgG1 C P A P E L L G
IgG2 C P A P R V A A
IgG3 E P K S C D T P P P C P R C P E L L G
IgG4 C P A P E F L G

Figure 3b



CH2

EU Index	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257
IgG1	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P
IgG2	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P
IgG3	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P
IgG4	G	P	S	V	F	L	F	P	P	K	P	K	D	T	L	M	I	S	R	T	P

EU Index	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278
IgG1	E	V	T	C	V	V	V	D	V	S	H	E	D	P	E	V	K	F	N	W	Y
IgG2	E	V	T	C	V	V	V	D	V	S	H	E	D	P	E	V	Q	F	N	W	Y
IgG3	E	V	T	C	V	V	V	D	V	S	H	E	D	P	E	V	Q	F	K	W	Y
IgG4	E	V	T	C	V	V	V	D	V	S	Q	E	D	P	E	V	Q	F	N	W	Y

EU Index	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299
IgG1	V	D	G	V	E	V	H	N	A	K	T	K	P	R	E	E	Q	Y	N	S	T
IgG2	V	D	G	V	E	V	H	N	A	K	T	K	P	R	E	E	Q	F	N	S	T
IgG3	V	D	G	V	E	V	H	N	A	K	T	K	P	R	E	E	Q	Y	N	S	T
IgG4	V	D	G	V	E	V	H	N	A	K	T	K	P	R	E	E	Q	F	N	S	T

EU Index	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320
IgG1	Y	R	V	V	S	V	L	T	V	L	H	Q	D	W	L	N	G	K	E	Y	K
IgG2	F	R	V	V	S	V	L	T	V	V	H	Q	D	W	L	N	G	K	E	Y	K
IgG3	F	R	V	V	S	V	L	T	V	L	H	Q	D	W	L	N	G	K	E	Y	K
IgG4	Y	R	V	V	S	V	L	T	V	L	H	Q	D	W	L	N	G	K	E	Y	K

EU Index	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340
IgG1	C	K	V	S	N	K	A	L	P	A	P	I	E	K	T	I	S	K	A	K
IgG2	C	K	V	S	N	K	G	L	P	A	P	I	E	K	T	I	S	K	T	K
IgG3	C	K	V	S	N	K	A	L	P	A	P	I	E	K	T	I	S	K	T	K
IgG4	C	K	V	S	N	K	G	L	P	S	S	I	E	K	T	I	S	K	A	K

CH3

EU Index	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361
IgG1	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	D	E	L	T	K	N
IgG2	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	E	E	M	T	K	N
IgG3	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	R	E	E	M	T	K	N
IgG4	G	Q	P	R	E	P	Q	V	Y	T	L	P	P	S	Q	E	E	M	T	K	N

EU Index	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382
IgG1	Q	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E
IgG2	Q	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E
IgG3	Q	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E
IgG4	Q	V	S	L	T	C	L	V	K	G	F	Y	P	S	D	I	A	V	E	W	E

EU Index	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403
IgG1	S	N	G	Q	P	E	N	N	Y	K	T	T	P	P	V	L	D	S	D	G	S
IgG2	S	N	G	Q	P	E	N	N	Y	K	T	T	P	P	M	L	D	S	D	G	S
IgG3	S	S	G	Q	P	E	N	N	Y	N	T	T	P	P	M	L	D	S	D	G	S
IgG4	S	N	G	Q	P	E	N	N	Y	K	T	T	P	P	V	L	D	S	D	G	S

EU Index	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424
IgG1	F	F	L	Y	S	K	L	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S
IgG2	F	F	L	Y	S	K	L	T	V	D	K	S	R	W	Q	Q	G	N	V	F	S
IgG3	F	F	L	Y	S	K	L	T	V	D	K	S	R	W	Q	Q	G	N	H	F	S
IgG4	F	F	L	Y	S	R	L	T	V	D	K	S	R	W	Q	E	G	N	V	F	S

EU Index	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445
IgG1	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P
IgG2	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	P
IgG3	C	S	V	M	H	E	A	L	H	N	R	F	T	Q	K	S	L	S	L	S	P
IgG4	C	S	V	M	H	E	A	L	H	N	H	Y	T	Q	K	S	L	S	L	S	L

EU Index	446	447
IgG1	G	K
IgG2	G	K
IgG3	G	K
IgG4	G	K

Figure 4a

Allotype	Allotype	Position		
		214	356 358	431
G1m(1,17)	G1m(a,z)	K	D L	A
G1m(1,2,17)	G1m(a,x,z)	K	D L	G
G1m(3)	G1m(f)	R	E M	A
G1m(1,3)	G1m(a,f)	R	D L	A

Figure 4b

Allotype	Allotype	Position
		282
G2m(23)	G2m(n+)	V
	G2m(n-)	M

Figure 5

Receptor binding improvement	Receptor binding reduction	Cell activity	Therapeutic activity
Solely I	-	Enhance dendritic cell activity and uptake, and subsequent presentation of antigens; enhance monocyte and macrophage response to antibody	Enhance cell-based immune response against target
IIIa		Enhance ADCC and phagocytosis of broad range of cell types	Increased target cell lysis
IIIa	IIb	Enhance ADCC and phagocytosis of broad range of cell types	Increased target cell lysis
IIb, IIc		Reduction of activity of all FcR bearing cell types except NK cells and possible activation of NK cells via IIc receptor signaling	Enhancement of target cell lysis selective for NK cell accessible target cells
IIb, IIIa	-	Possible NK cell specific activation and enhancement of NK cell mediated ADCC	Enhancement of target cell lysis selective for NK cell accessible target cells
IIIb		Neutrophil mediated phagocytosis enhancement	Enhanced target cell destruction for neutrophil accessible cells
FcαR		Neutrophil mediated phagocytosis enhancement	Enhanced target cell destruction for neutrophil accessible cells
I,IIa,IIIa	IIb	Enhance dendritic cell activity and uptake, and subsequent presentation of antigens to T cells; enhance monocyte and macrophage response to antibody	Enhance cell-based immune response against target
IIb	IIIa,IIa,I	Reduction in activity of monocytes, macrophages, neutrophils, NK, dendritic and other gamma receptor bearing cells	Eliminate or reduce cell-mediated cytotoxicity against target bearing cells

Figure 6a

> H0 4G7 (SEQ ID NO:9)

EVQLQQSGPELIKPGASVKMSCKASGYTFTSYVMHWVKQKPGQGLEWIGYINPYNDGTKY
NEKFKGKATLTSDKSSSTAYMELSSLTSEDSAVYYCARGTYYYGSRVFDYWGQGTTLTVS
S

> L0 4G7 (SEQ ID NO:10)

DIVMTQAAPSIPVTPGESVSISCRSSKSLLNSNGNTYLYWFLQRPGQSPQLLIYRMSNLASG
VPDRFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEYPFTFGAGTKLELK

> H0 HD37 (SEQ ID NO:11)

QVQLQQSGAELVRPGSSVKISCKASGYAFSSYWMNWVKQRPGQGLEWIGQIWPGDGDT
NYNGKFKGKATLTADESSSTAYMQLSSLASEDSAVYFCARRETTTVGRYYYAMDYWGQG
TSVTVSS

> L0 HD37 (SEQ ID NO:12)

DILLTQTPASLAVSLGQRATISCKASQSVDYDGDSYLNWYQQIPGQPPKLLIYDASNLVSGIP
PRFSGSGSGTDFTLNIHPVEKVDAATYHCQQSTEDPWTFGGGTKLEIK

Figure 6b

> 4G7 VH CDR1 (SEQ ID NO:132):	SYVMH
> 4G7 VH CDR2 (SEQ ID NO:133):	YINPYNDGTKYNEKFKG
> 4G7 VH CDR3 (SEQ ID NO:134):	GTYYYGSRVFDY
> 4G7 VL CDR1 (SEQ ID NO:135):	RSSKSLLNSNGNTYLY
> 4G7 VL CDR2 (SEQ ID NO:136):	RMSNLAS
> 4G7 VL CDR3 (SEQ ID NO:137):	MQHLEYPFT
> HD37 VH CDR1 (SEQ ID NO:138):	SYWMN
> HD37 VH CDR2 (SEQ ID NO:139):	QIWPGDGDTNYNGKFKG
> HD37 VH CDR3 (SEQ ID NO:140):	RETTTVGRYYYAMDY
> HD37 VL CDR1 (SEQ ID NO:141):	KASQSVDYDGDSYLN
> HD37 VL CDR2 (SEQ ID NO:142):	DASNLVS
> HD37 VL CDR3 (SEQ ID NO:143):	QQSTEDPWT

Figure 7

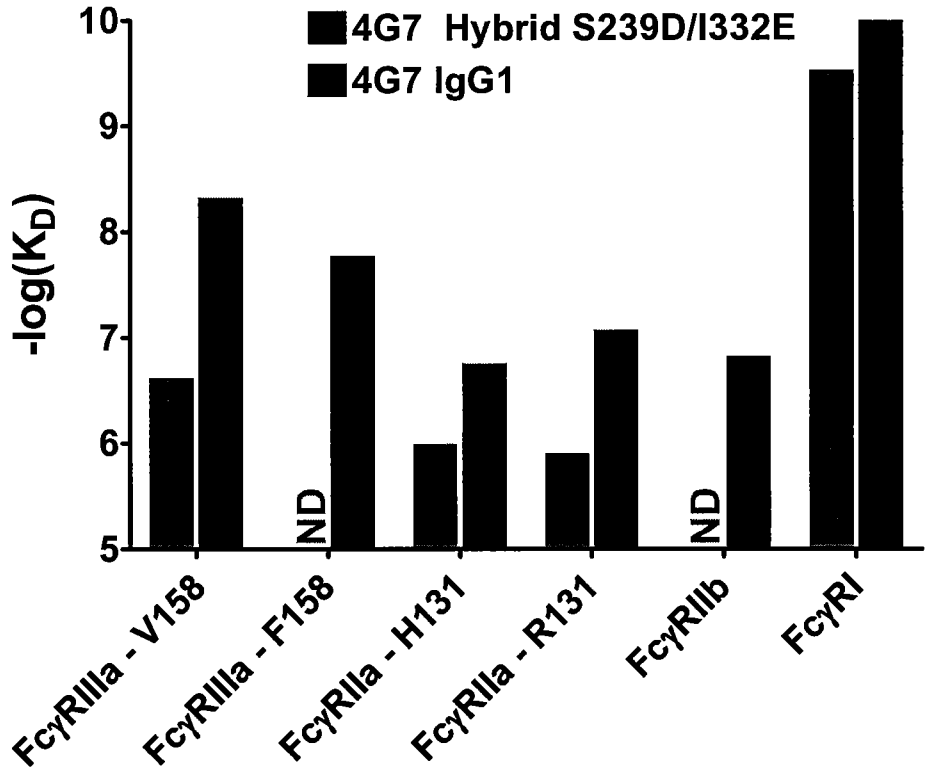

4G7 Hybrid S239D/I332E
4G7 IgG1
-log(K_D)
10
9
8
7
6
5
ND
ND
FcγRIIIa - V158
FcγRIIIa - F158
FcγRIIa - H131
FcγRIIa - R131
FcγRIIb
FcγRI

Figure 8a

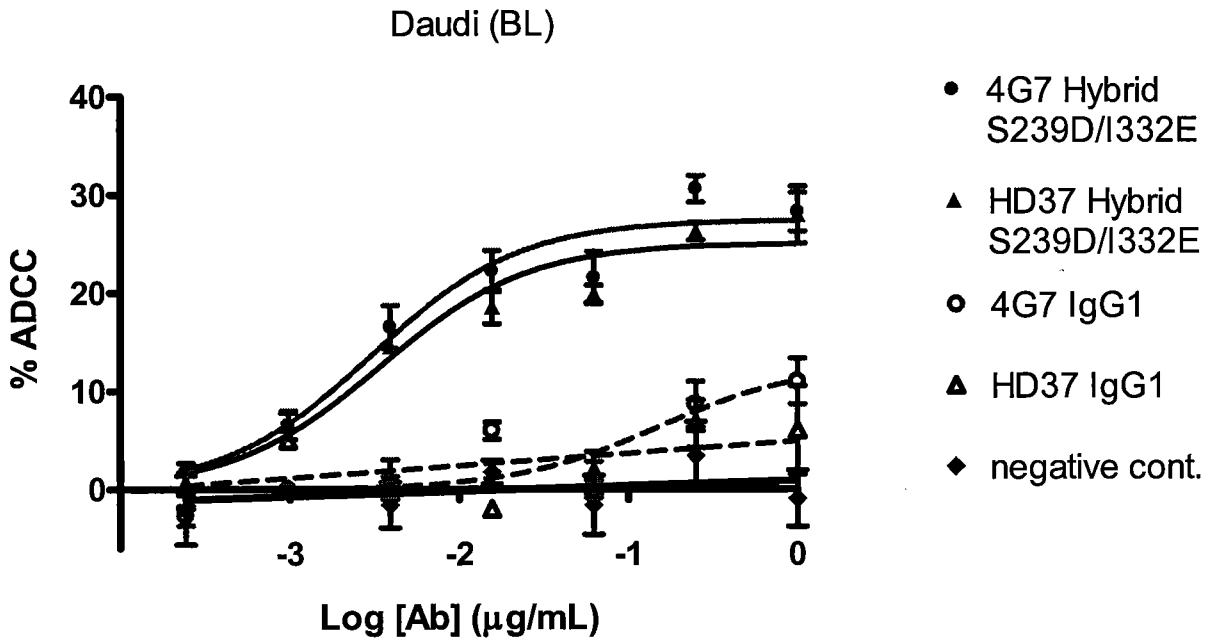

Daudi (BL)
% ADCC
40
30
20
10
0
-3
-2
-1
0
Log [Ab] (µg/mL)
4G7 Hybrid S239D/I332E
HD37 Hybrid S239D/I332E
4G7 IgG1
HD37 IgG1
negative cont.

Figure 8b

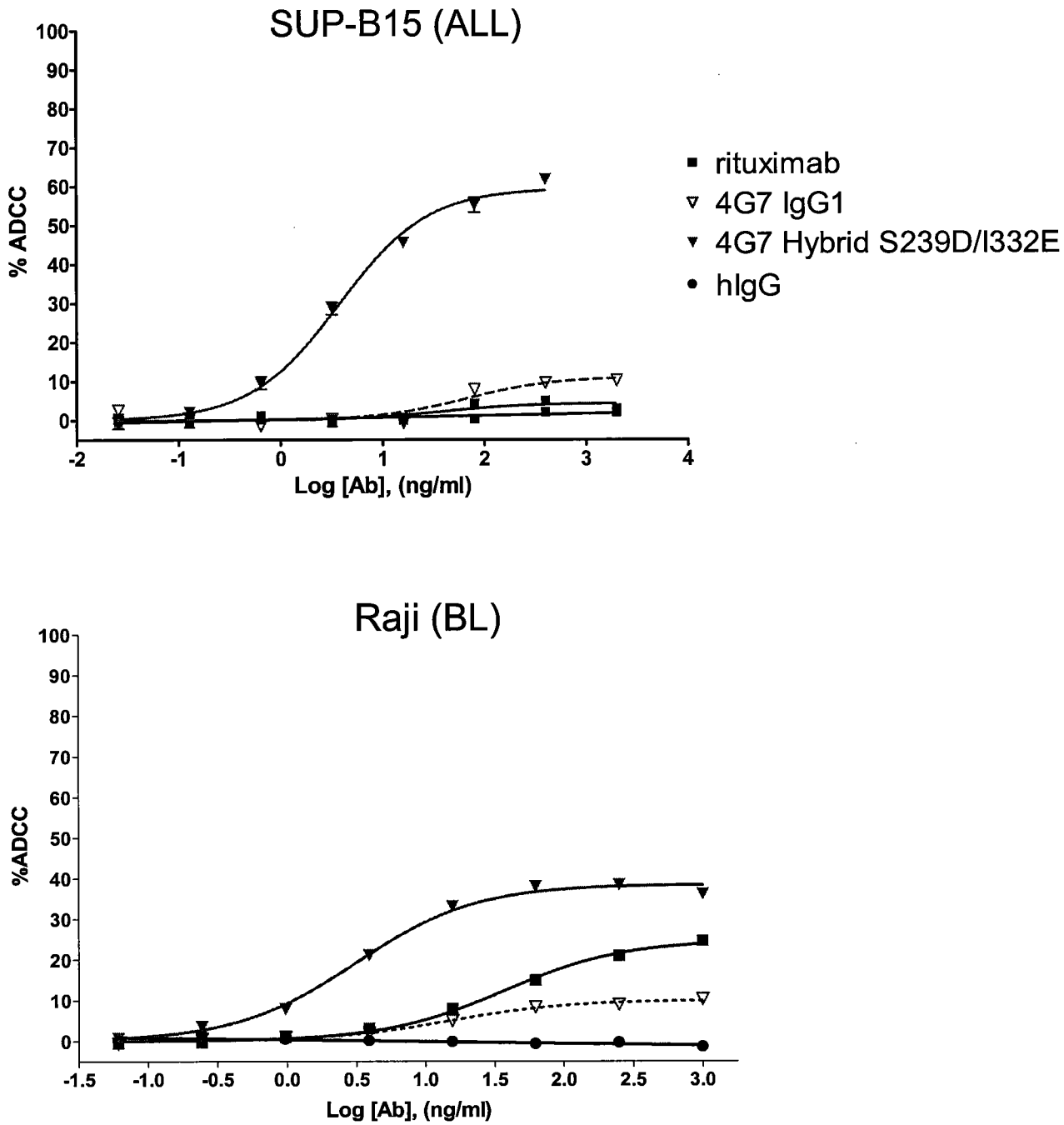

SUP-B15 (ALL)
% ADCC
Log [Ab], (ng/ml)
rituximab
4G7 IgG1
4G7 Hybrid S239D/I332E
hIgG
Raji (BL)
%ADCC
Log [Ab], (ng/ml)

Figure 9

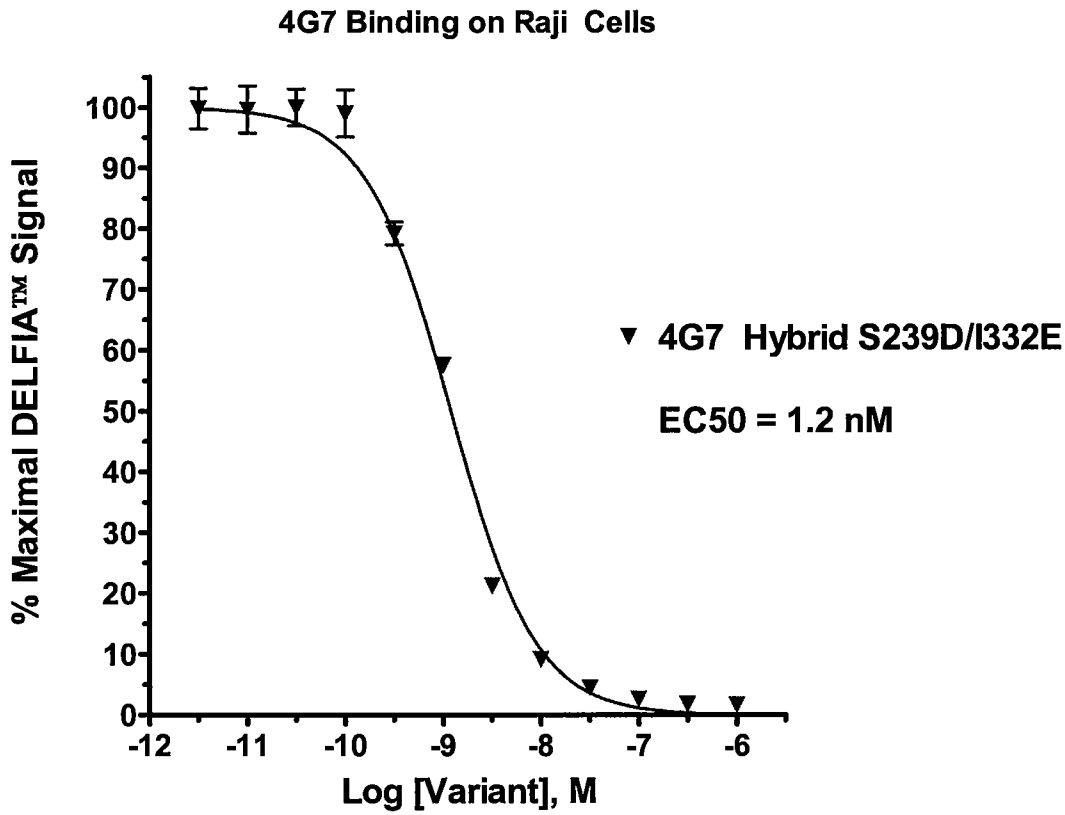

4G7 Binding on Raji Cells
% Maximal DELFIA™ Signal
100
90
80
70
60
50
40
30
20
10
0
-12
-11
-10
-9
-8
-7
-6
Log [Variant], M
4G7 Hybrid S239D/I332E
EC50 = 1.2 nM

Figure 10a

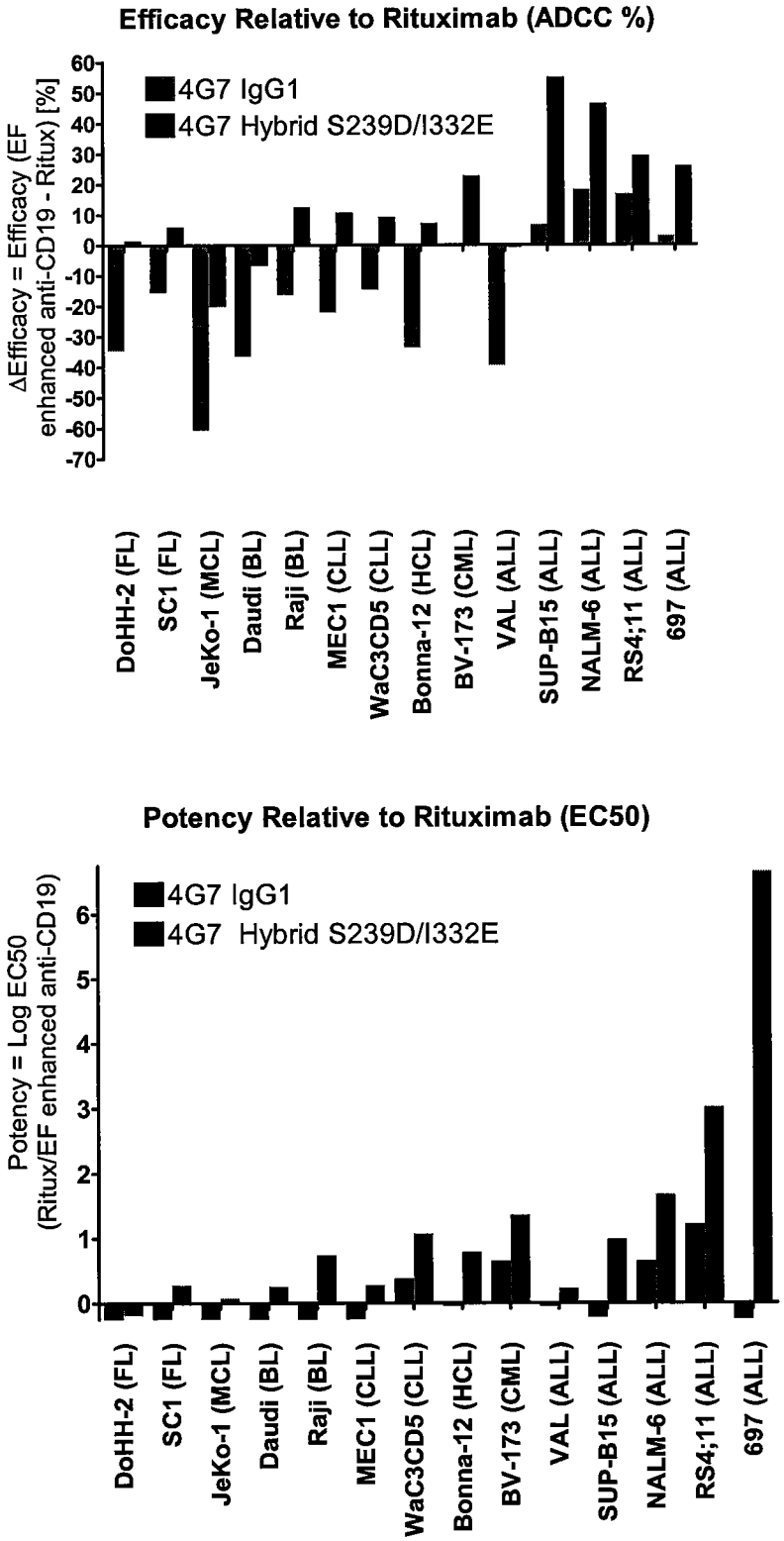

Efficacy Relative to Rituximab (ADCC %)
ΔEfficacy = Efficacy (EF enhanced anti-CD19 - Ritux) [%]
4G7 IgG1
4G7 Hybrid S239D/I332E
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
DoHH-2 (FL)
SC1 (FL)
JeKo-1 (MCL)
Daudi (BL)
Raji (BL)
MEC1 (CLL)
WaC3CD5 (CLL)
Bonna-12 (HCL)
BV-173 (CML)
VAL (ALL)
SUP-B15 (ALL)
NALM-6 (ALL)
RS4;11 (ALL)
697 (ALL)
Potency Relative to Rituximab (EC50)
Potency = Log EC50 (Ritux/EF enhanced anti-CD19)
4G7 IgG1
4G7 Hybrid S239D/I332E
6
5
4
3
2
1
0
DoHH-2 (FL)
SC1 (FL)
JeKo-1 (MCL)
Daudi (BL)
Raji (BL)
MEC1 (CLL)
WaC3CD5 (CLL)
Bonna-12 (HCL)
BV-173 (CML)
VAL (ALL)
SUP-B15 (ALL)
NALM-6 (ALL)
RS4;11 (ALL)
697 (ALL)

Figure 10b

Cancer cell type	Cell line
Hairy Cell Leukemia (HCL)	Bonna 12
Mantle Cell Lymphoma (MCL)	Jeko-1
Chronic Lymphocytic Leukemia (CLL)	Wac3CD5, MEC-1
Burkitt's Lymphoma (BL)	Daudi, Raji
Chronic Myelogenous Leukemia (CML)	BV-173
Follicular Lymphoma (FL)	DoHH-2, SC1
Acute Lymphoblastic Leukemia (ALL)	VAL, SUP-B15, NALM-6, RS4;11, 697

Figure 11

> H1 4G7 (SEQ ID NO:13)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGTKY
NEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGSRVFDYWGQGTLVTVSS

> H2 4G7 (SEQ ID NO:14)

QVQLQESGSGLVKPGGSLRLSCAASGYTFTSYVMHWVRQAPGKGLEWMGYINPYNDGTK
YNESLKSRVTISSDKSISTAYMELSSLRAEDTAVYYCARGTYYYGSRVFDYWGQGTLVTVS
S

> H3 4G7 (SEQ ID NO:15)

EVQLVESGGGLVQPGRSLRLSCAASGYTFTSYVMHWVRQMPGKGLEWMGYINPYNDGTK
YNEKFQGRVTITSDKSTSTAYMELSRLRSDDTAVYYCARGTYYYGSRVFDYWGQGTLVTV
SS

> H4 4G7 (SEQ ID NO:16)

EVQLQQSGPEVKKPGTSVKVSCKASGYTFTSYVMHWVRQAPGKGLVWVSYINPYNDGTK
YNESLKSRVTISSDKSISTAYLQMNSLRAEDTAVYYCARGTYYYGSRVFDYWGQGTLVTVS
S

Figure 12

> L1 4G7 (SEQ ID NO:17)

DIVMTQSPATLSLSPGERATLSCRSSKSLLNSNGNTYLYWFQQKPGQSPQLLIYRMSNLAS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPFTFGAGTKLEIK

> L2 4G7 (SEQ ID NO:18)

DIVMTQSPSSLSASVGDRVTISCRSSKSLLNSNGNTYLYWFQQKPGQSPQLLIYRMSNLAS
GVPDRFSGSGSGTEFTLTISSLQPEDVAVYYCMQHLEYPFTFGAGTKLEIK

> L3 4G7 (SEQ ID NO:19)

DIVMTQSPATLSVSPGERATISCRSSKSLLNSNGNTYLYWFLQKPGQSPQLLIYRMSNLASG
VPDRFSGSGSGTDFTLTISRVEAEDVGVYYCMQHLEYPFTFGAGTKLEIK

Figure 13

> H1 HD37 (SEQ ID NO:20)

TVQLVESGGGVVRPGGSLRLSCAASGYAFSSYWMNWVRQAPGKGLEWIGQIWPGDGDT
NYNGKFQDRVTITADESTSTAYMELRSLRSDDTAVYFCARRETTTVGRYYYAMDYWGQGT
LVTVSS

> H2 HD37 (SEQ ID NO:21)

QVQLVESGGGLVEPGGSLRLSCAASGYAFSSYWMNWVRQMPGKGLEWMGQIWPGDGD
TNYNPSLKSRVTITADESTSTAYMELSSLKAEDTAVYFCARRETTTVGRYYYAMDYWGQGT
LVTVSS

> H3 HD37 (SEQ ID NO:22)

QVQLQESGPGLVKPSQTLSLTCAASGYAFSSYWMNWVRQAPGKGLEWMGQIWPGDGDT
NYNGALKSRVTITADESTSTAYMELSSLRSEDTAVYFCARRETTTVGRYYYAMDYWGQGT
LVTVSS

> H4 HD37 (SEQ ID NO:23)

EVQLVQSGSELKKPGASVKVSCKASGYAFSSYWMNWVRQAPGKGLEWVAQIWPGDGDT
NYADSVKGRFTITADESTSTAYLQMNSLRAGDTAMYFCARRETTTVGRYYYAMDYWGQGT
LVTVSS

Figure 14

> L1 HD37 (SEQ ID NO:24)

DILLTQSPATLSLSPGERATLSCRASQSVDYDGDSYLNWYQQKPGQPPKLLIYDASNLVSGI
PPRFSGSGSGTDFTLTISRLEPEDFAVYHCQQSTEDPWTFGGGTKLEIK

> L2 HD37 (SEQ ID NO:25)

DILLTQSPSSLSVTPGEKVTITCRASQSVDYDGDSYLNWYQQKPGQPPKLLIYDASNLVSGI
PPRFSGSGSGTDFTLTINSLEAEDAATYHCQQSTEDPWTFGGGTKLEIK

> L3 HD37 (SEQ ID NO:26)

DILLTQTPLSLPVTPGEPASISCRASQSVDYDGDSYLNWYQQKPGQPPKLLIYDASNLVSGI
PPRFSGSGSGTDFTLKISRVEAEDVGVYHCQQSTEDPWTFGGGTKLEIK

Figure 15a

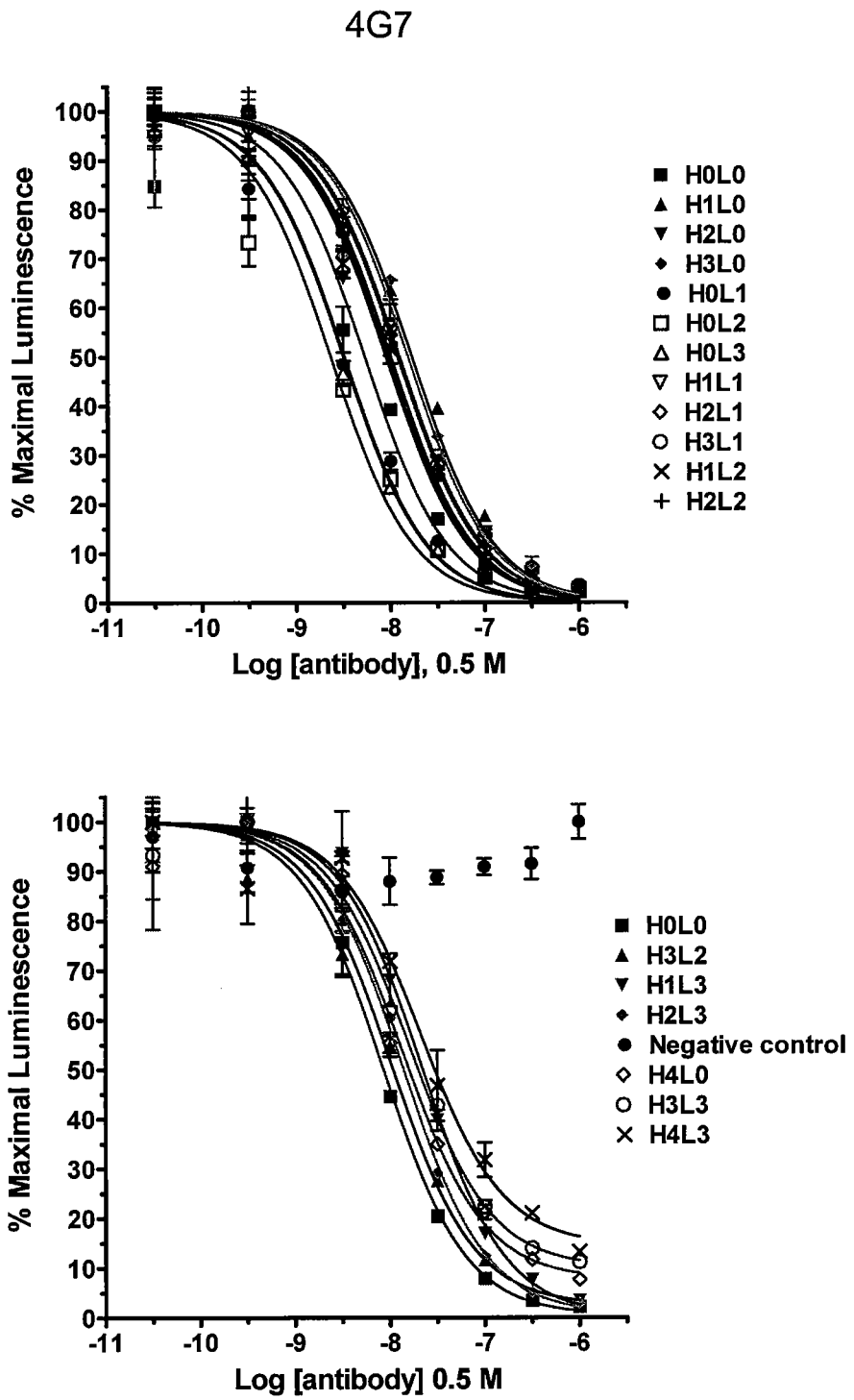

4G7
% Maximal Luminescence
Log [antibody], 0.5 M
H0L0
H1L0
H2L0
H3L0
H0L1
H0L2
H0L3
H1L1
H2L1
H3L1
H1L2
H2L2
% Maximal Luminescence
Log [antibody] 0.5 M
H0L0
H3L2
H1L3
H2L3
Negative control
H4L0
H3L3
H4L3

Figure 15b

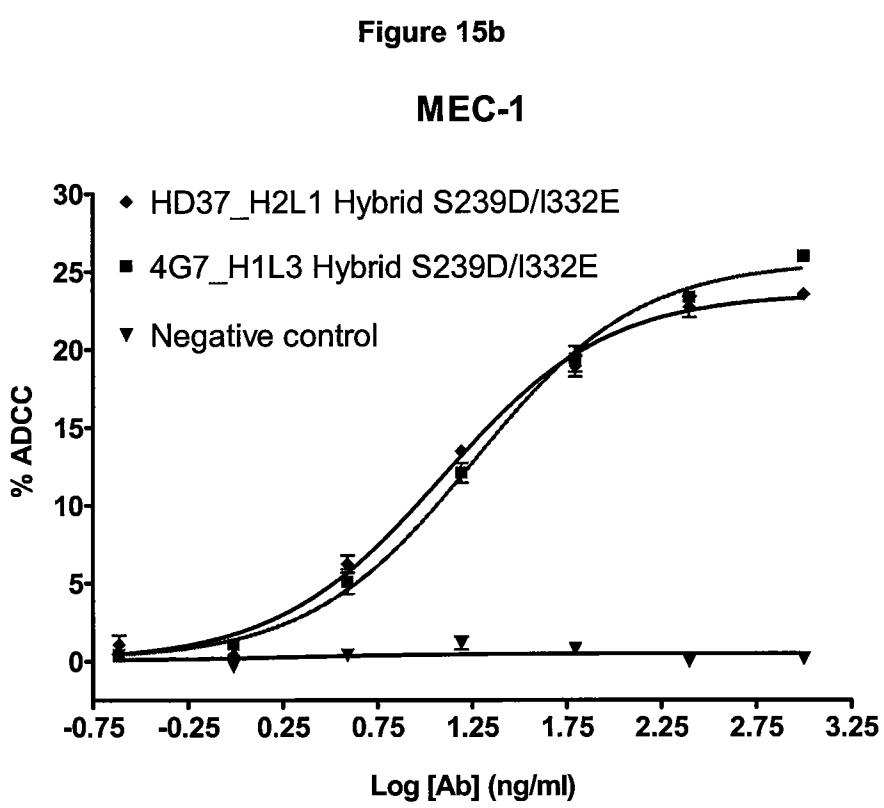


Figure 16

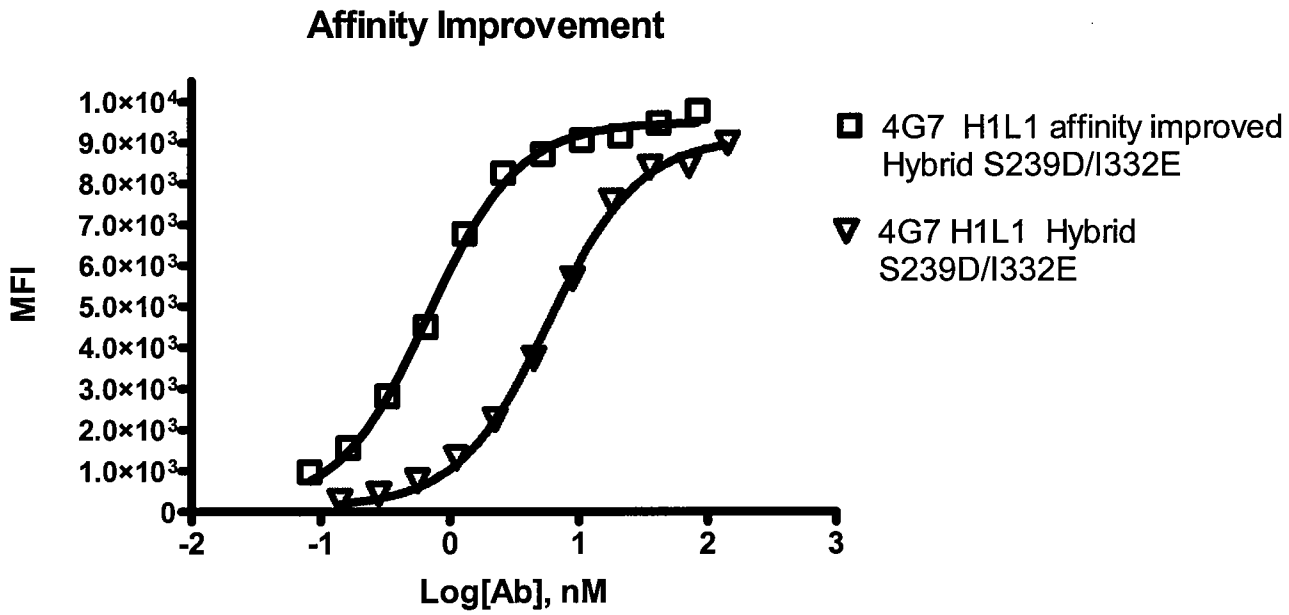


Figure 17

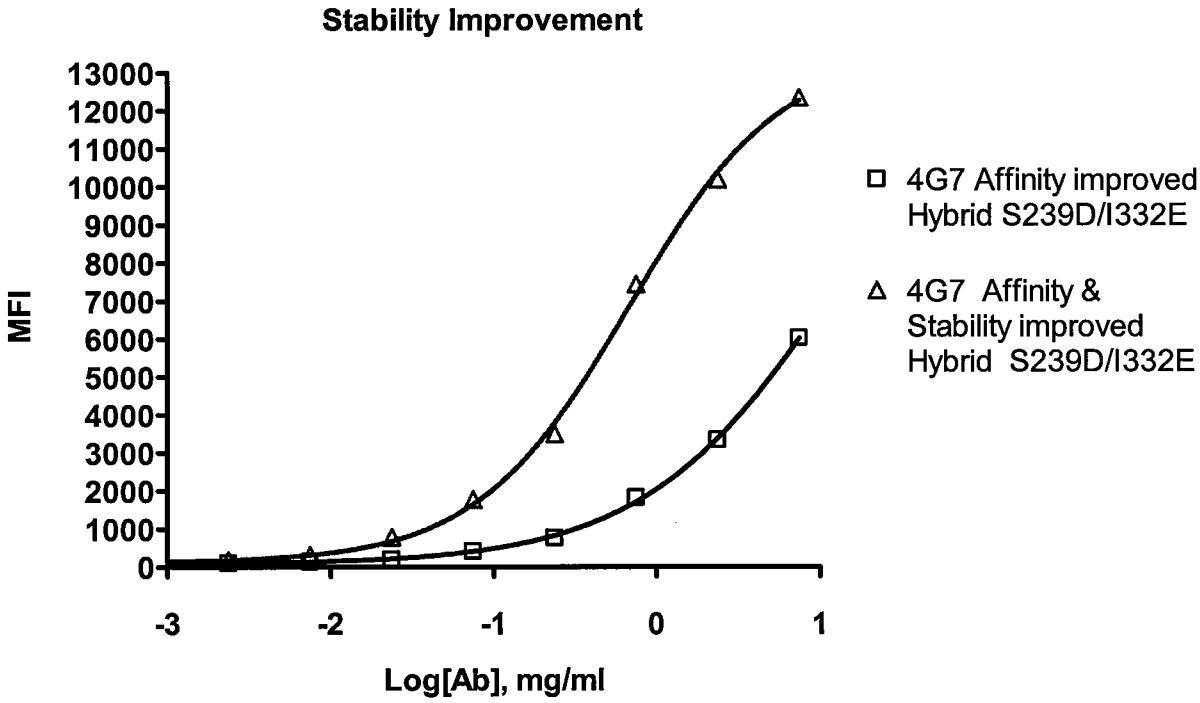

Stability Improvement
MFI
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-3
-2
-1
0
1
Log[Ab], mg/ml
4G7 Affinity improved Hybrid S239D/I332E
4G7 Affinity & Stability improved Hybrid S239D/I332E

Figure 18

> 4G7 H1.109 (SEQ ID NO:27)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGPKY
NEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGSRVFDYWGQGTLVTVSS

> 4G7 H1.113 (SEQ ID NO:28)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGHKY
NEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGSRVFDYWGQGTLVTVSS

> 4G7 H1.144 (SEQ ID NO:29)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGTKY
NEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGSRVFNYWGQGTLVTVSS

> 4G7 H1.146 (SEQ ID NO:30)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGTKY
NEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGSRVFHYWGQGTLVTVSS

> 4G7 H1.147 (SEQ ID NO:31)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGTKY
NEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGSRVFSYWGQGTLVTVSS

> 4G7 H1.191 (SEQ ID NO:32)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGTEY
NEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWGQGTLVTVSS

> 4G7 H1.192 (SEQ ID NO:33)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGPKY
NEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWGQGTLVTVSS

> 4G7 H1.196 (SEQ ID NO:34)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGPKY
NEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTSVFDYWGQGTLVTVSS

> 4G7 H1.199 (SEQ ID NO:35)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGPEY
NEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWGQGTLVTVSS

> 4G7 H1.201 (SEQ ID NO:36)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNSGTKY
NEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWGQGTLVTVSS

> 4G7 H1.202 (SEQ ID NO:37)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNEGTKY
NEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWGQGTLVTVSS

> 4G7 H1.203 (SEQ ID NO:38)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNSGTEY
NEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWGQGTLVTVSS

Figure 18 (continued)

> 4G7 H1.204 (SEQ ID NO:39)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNEGTEY
NEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWGQGTLVTVSS

> 4G7 H1.52 (SEQ ID NO:40)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGTKY
NEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGTRVFDYWGQGTLVTVSS

> 4G7 H1.60 (SEQ ID NO:41)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGTKY
NEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGLRVFDYWGQGTLVTVSS

> 4G7 H1.62 (SEQ ID NO:42)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGTKY
NEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGSEVFDYWGQGTLVTVSS

> 4G7 H1.65 (SEQ ID NO:43)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNDGTKY
NEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGSSVFDYWGQGTLVTVSS

> 4G7 H1.78 (SEQ ID NO:44)

EVQLVESGGGLVKPGGSLKLSCAASGYTFTSYVMHWVRQAPGKGLEWIGYINPYNAGTKY
NEKFQGRVTISSDKSISTAYMELSSLRSEDTAMYYCARGTYYYGSRVFDYWGQGTLVTVSS

Figure 19

> 4G7 L1.11 (SEQ ID NO:45)

DIVMTQSPATLSLSPGERATLSCRSSKSLLNSNGNTYLYWFQQKPGQSPQLLIYRMSNLAS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLNYPFTFGAGTKLEIK

> 4G7 L1.124 (SEQ ID NO:46)

DIVMTQSPATLSLSPGERATLSCRSSKSLLNSNGNTYLYWFQQKPGQSPQLLIYRMSNWAS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPFTFGAGTKLEIK

> 4G7 L1.138 (SEQ ID NO:47)

DIVMTQSPATLSLSPGERATLSCRSSKSLLNVNGNTYLYWFQQKPGQSPQLLIYRMSNLNS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPFTFGAGTKLEIK

> 4G7 L1.139 (SEQ ID NO:48)

DIVMTQSPATLSLSPGERATLSCRSSKSLLNVNGNTYLYWFQQKPGQSPQLLIYRMSNLAS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPNTFGAGTKLEIK

> 4G7 L1.141 (SEQ ID NO:49)

DIVMTQSPATLSLSPGERATLSCRSSKSLLNSNGNTYLYWFQQKPGQSPQLLIYRMSNLNS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPNTFGAGTKLEIK

> 4G7 L1.143 (SEQ ID NO:50)

DIVMTQSPATLSLSPGERATLSCRSSKSLQNSNGNTYLYWFQQKPGQSPQLLIYRMSNLNS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPFTFGAGTKLEIK

> 4G7 L1.144 (SEQ ID NO:51)

DIVMTQSPATLSLSPGERATLSCRSSKSLQNSNGNTYLYWFQQKPGQSPQLLIYRMSNLAS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPNTFGAGTKLEIK

> 4G7 L1.145 (SEQ ID NO:52)

DIVMTQSPATLSLSPGERATLSCRSSKSLQNSNGNTYLYWFQQKPGQSPQLLIYRMSNLAS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPITFGAGTKLEIK

> 4G7 L1.146 (SEQ ID NO:53)

DIVMTQSPATLSLSPGERATLSCRSSKSLLNVNGNTYLYWFQQKPGQSPQLLIYRMSNLNS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPNTFGAGTKLEIK

> 4G7 L1.148 (SEQ ID NO:54)

DIVMTQSPATLSLSPGERATLSCRSSKSLQNSNGNTYLYWFQQKPGQSPQLLIYRMSNLNS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPNTFGAGTKLEIK

> 4G7 L1.149 (SEQ ID NO:55)

DIVMTQSPATLSLSPGERATLSCRSSKSLQNSNGNTYLYWFQQKPGQSPQLLIYRMSNLNS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPITFGAGTKLEIK

> 4G7 L1.152 (SEQ ID NO:56)

DIVMTQSPATLSLSPGERATLSCRSSKSLQNVNGNTYLYWFQQKPGQSPQLLIYRMSNLAS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPNTFGAGTKLEIK

Figure 19 (continued)

> 4G7 L1.154 (SEQ ID NO:57)

DIVMTQSPATLSLSPGERATLSCRSSKSLQNVNGNTYLYWFQQKPGQSPQLLIYRMSNLNS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPNTFGAGTKLEIK

> 4G7 L1.155 (SEQ ID NO:58)

DIVMTQSPATLSLSPGERATLSCRSSKSLQNVNGNTYLYWFQQKPGQSPQLLIYRMSNLNS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPITFGAGTKLEIK

> 4G7 L1.160 (SEQ ID NO:59)

DIVMTQSPATLSLSPGERATLSCRSSKSLQNVNANTYLYWFQQKPGQSPQLLIYRMSNLNS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPITFGAGTKLEIK

> 4G7 L1.161 (SEQ ID NO:60)

DIVMTQSPATLSLSPGERATLSCRSSKSLQNVNSNTYLYWFQQKPGQSPQLLIYRMSNLNS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPITFGAGTKLEIK

> 4G7 L1.162 (SEQ ID NO:61)

DIVMTQSPATLSLSPGERATLSCRSSKSLQNANANTYLYWFQQKPGQSPQLLIYRMSNLNS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPITFGAGTKLEIK

> 4G7 L1.163 (SEQ ID NO:62)

DIVMTQSPATLSLSPGERATLSCRSSKSLQNANSNTYLYWFQQKPGQSPQLLIYRMSNLNS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPITFGAGTKLEIK

> 4G7 L1.164 (SEQ ID NO:63)

DIVMTQSPATLSLSPGERATLSCRSSKSLQNANGNTYLYWFQQKPGQSPQLLIYRMSNLNS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPITFGAGTKLEIK

> 4G7 L1.17 (SEQ ID NO:64)

DIVMTQSPATLSLSPGERATLSCRSSKSLLNSNGNTYLYWFQQKPGQSPQLLIYRMSNLAS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLTYPFTFGAGTKLEIK

> 4G7 L1.19 (SEQ ID NO:65)

DIVMTQSPATLSLSPGERATLSCRSSKSLLNSNGNTYLYWFQQKPGQSPQLLIYRMSNLAS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLYYPFTFGAGTKLEIK

> 4G7 L1.26 (SEQ ID NO:66)

DIVMTQSPATLSLSPGERATLSCRSSKSLQNSNGNTYLYWFQQKPGQSPQLLIYRMSNLAS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPFTFGAGTKLEIK

> 4G7 L1.3 (SEQ ID NO:67)

DIVMTQSPATLSLSPGERATLSCRSSKSLLNSNGNTYLYWFQQKPGQSPQLLIYRMQNLAS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPFTFGAGTKLEIK

> 4G7 L1.32 (SEQ ID NO:68)

DIVMTQSPATLSLSPGERATLSCRSSKSLLNVNGNTYLYWFQQKPGQSPQLLIYRMSNLAS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPFTFGAGTKLEIK

Figure 19 (continued)

> 4G7 L1.46 (SEQ ID NO:69)

DIVMTQSPATLSLSPGERATLSCRSSKSLLNSNGNTYLYWFQQKPGQSPQLLIYRMSHLAS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPFTFGAGTKLEIK

> 4G7 L1.54 (SEQ ID NO:70)

DIVMTQSPATLSLSPGERATLSCRSSKSLLNSNGNTYLYWFQQKPGQSPQLLIYRMSGLAS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPFTFGAGTKLEIK

> 4G7 L1.55 (SEQ ID NO:71)

DIVMTQSPATLSLSPGERATLSCRSSKSLLNSNGNTYLYWFQQKPGQSPQLLIYRMSYLAS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPFTFGAGTKLEIK

> 4G7 L1.64 (SEQ ID NO:72)

DIVMTQSPATLSLSPGERATLSCRSSKSLLNSNGNTYLYWFQQKPGQSPQLLIYRMSNLAS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPITFGAGTKLEIK

> 4G7 L1.67 (SEQ ID NO:73)

DIVMTQSPATLSLSPGERATLSCRSSKSLLNSNGNTYLYWFQQKPGQSPQLLIYRMSNLAS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPVTFGAGTKLEIK

> 4G7 L1.68 (SEQ ID NO:74)

DIVMTQSPATLSLSPGERATLSCRSSKSLLNSNGNTYLYWFQQKPGQSPQLLIYRMSNLAS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPNTFGAGTKLEIK

> 4G7 L1.8 (SEQ ID NO:75)

DIVMTQSPATLSLSPGERATLSCRSSKSLLNSNGNTYLYWFQQKPGQSPQLLIYRMKNLAS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPFTFGAGTKLEIK

> 4G7 L1.80 (SEQ ID NO:76)

DIVMTQSPATLSLSPGERATLSCRSSKSLLNSNGNTYLFWFQQKPGQSPQLLIYRMSNLAS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPFTFGAGTKLEIK

> 4G7 L1.9 (SEQ ID NO:77)

DIVMTQSPATLSLSPGERATLSCRSSKSLLNSNGNTYLYWFQQKPGQSPQLLIYRMLNLAS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPFTFGAGTKLEIK

> 4G7 L1.92 (SEQ ID NO:78)

DIVMTQSPATLSLSPGERATLSCRSSKSLLNSNGNTYLYWFQQKPGQSPQLLIYRMSNLLS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPFTFGAGTKLEIK

> 4G7 L1.96 (SEQ ID NO:79)

DIVMTQSPATLSLSPGERATLSCRSSKSLLNSNGNTYLYWFQQKPGQSPQLLIYRMSNLNS
GVPDRFSGSGSGTEFTLTISSLEPEDFAVYYCMQHLEYPFTFGAGTKLEIK

Figure 20

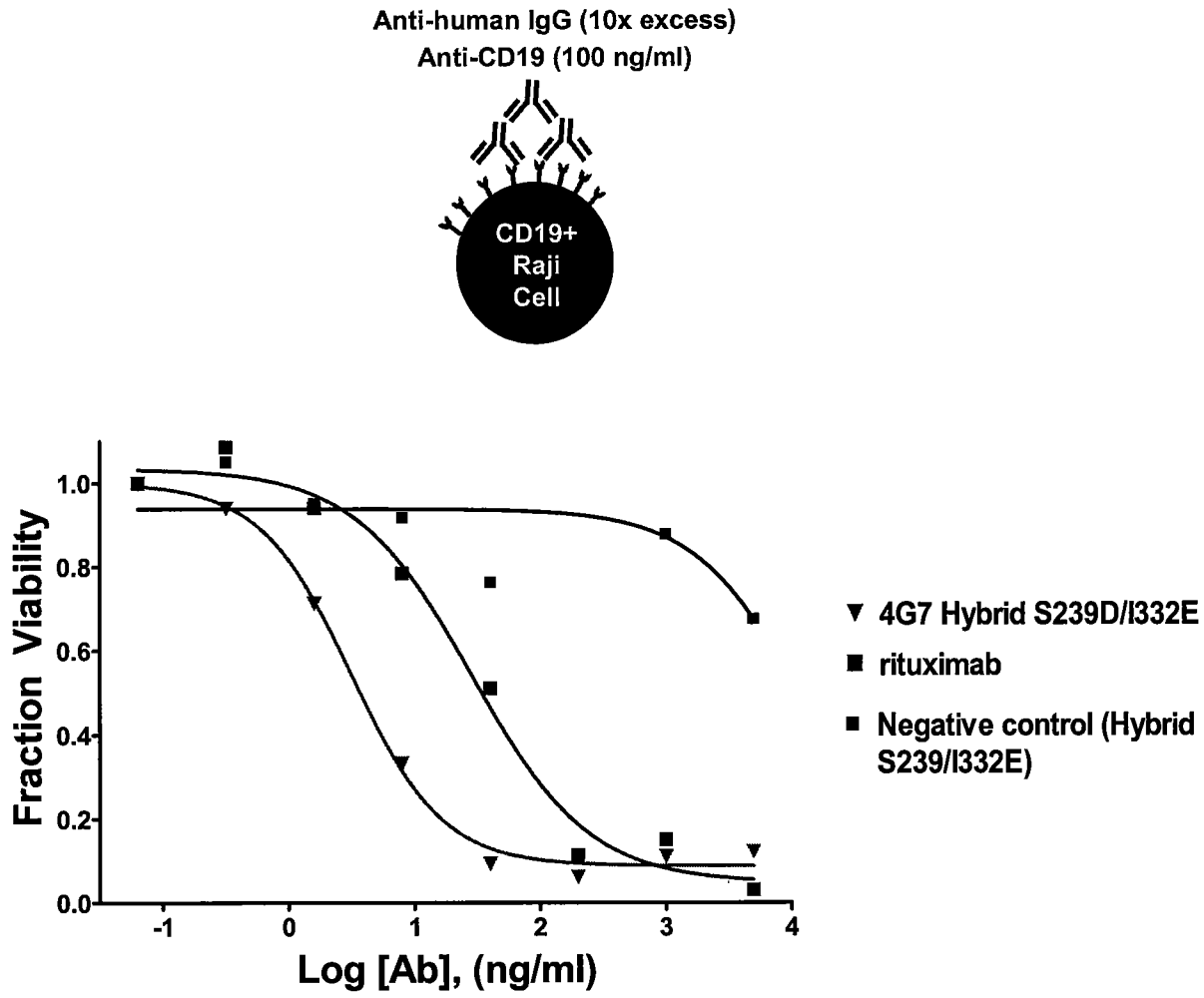
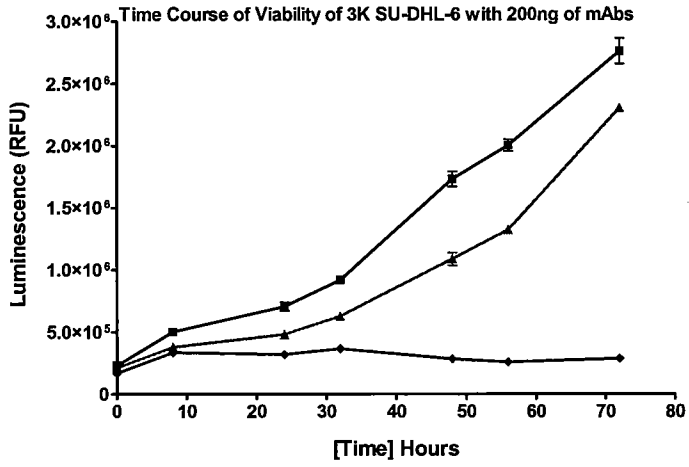
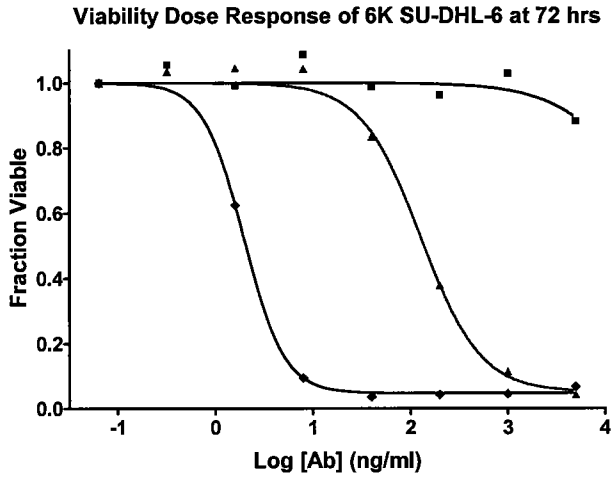

Anti-human IgG (10x excess)
Anti-CD19 (100 ng/ml)
CD19+
Raji
Cell
Fraction Viability
1.0
0.8
0.6
0.4
0.2
0.0
-1
0
1
2
3
4
Log [Ab], (ng/ml)
4G7 Hybrid S239D/I332E
rituximab
Negative control (Hybrid S239/I332E)

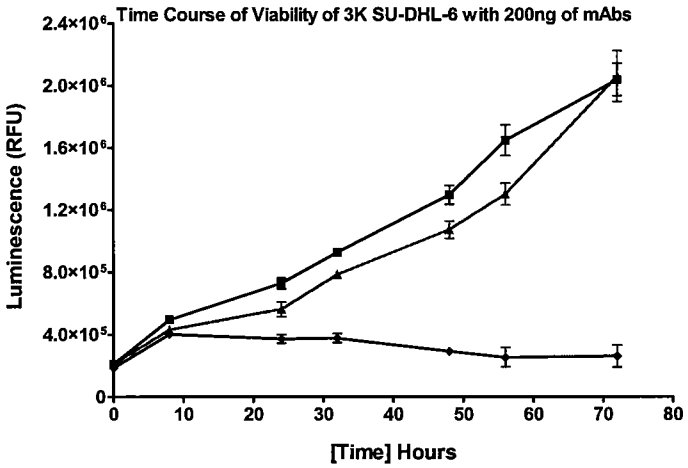
Figure 21

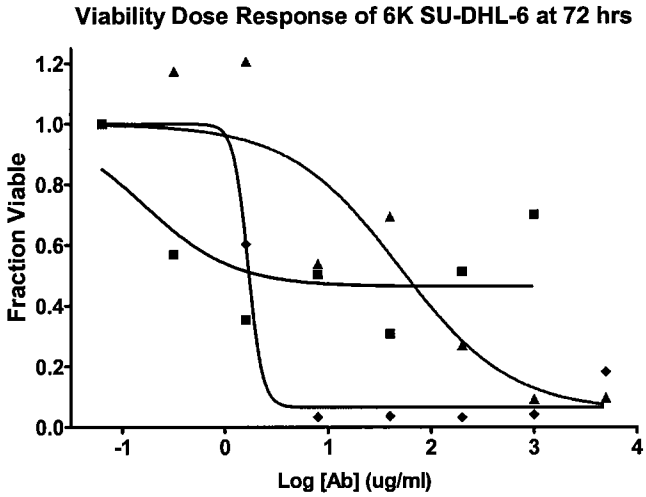
Crosslinked with a secondary anti-Fc mAb





Not crosslinked with a secondary anti-Fc mAb



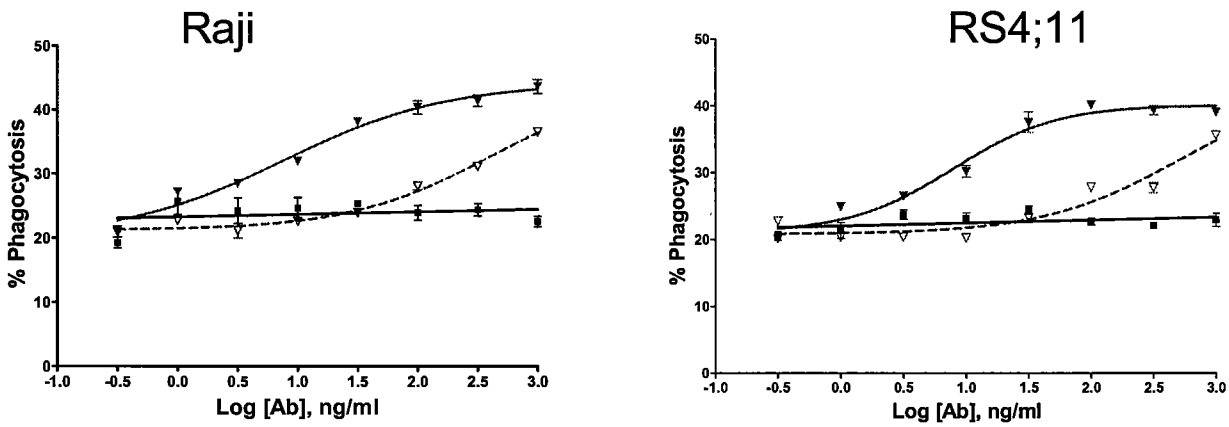


4G7 stability + affinity improved Hybrid S239D/I332E

rituximab

isotype control

Figure 22



▽ **4G7 IgG1**

▼ **4G7 stability + affinity improved Hybrid S239D/I332E**

▪ **Negative control**

Figure 23

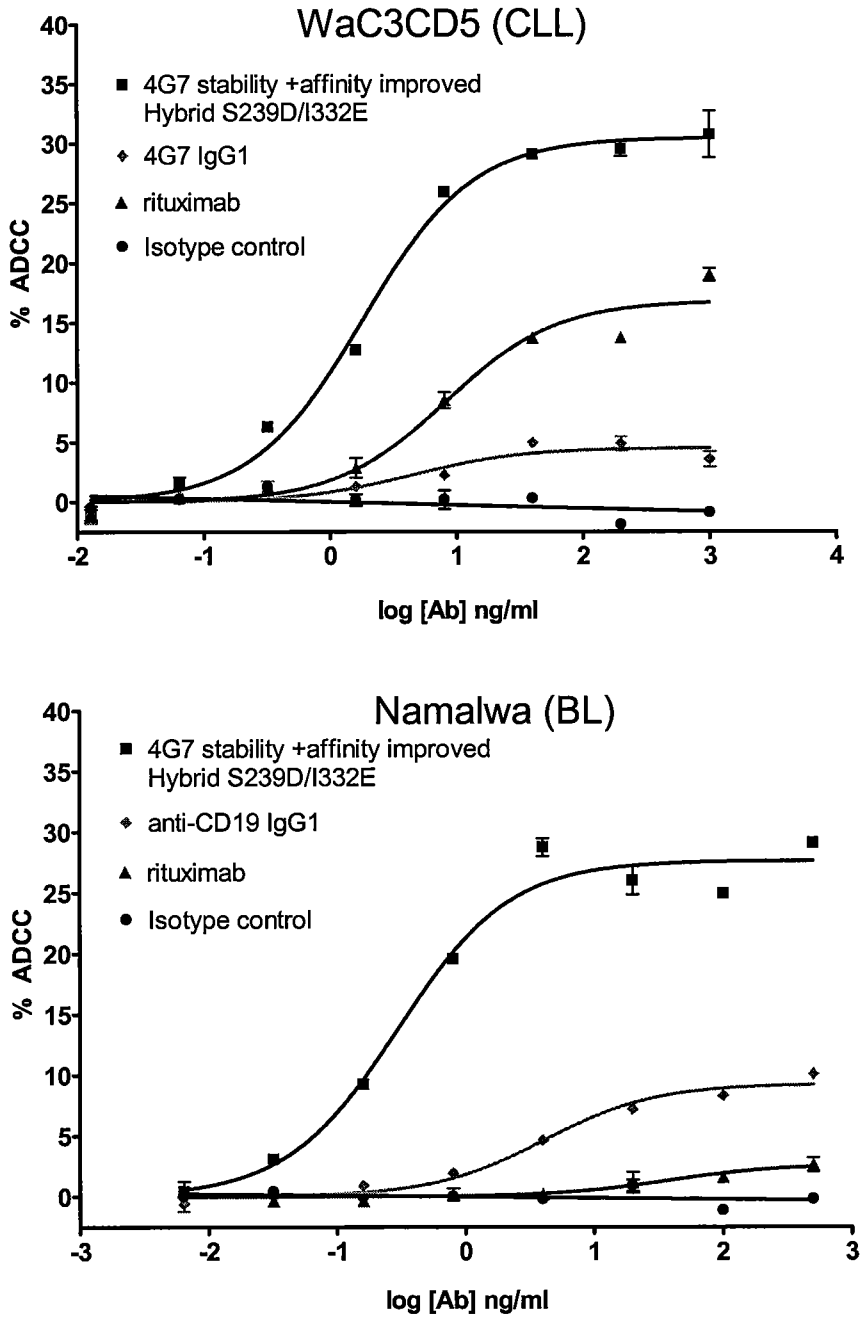

WaC3CD5 (CLL)
4G7 stability +affinity improved Hybrid S239D/I332E
4G7 IgG1
rituximab
Isotype control
% ADCC
log [Ab] ng/ml
Namalwa (BL)
4G7 stability +affinity improved Hybrid S239D/I332E
anti-CD19 IgG1
rituximab
Isotype control
% ADCC
log [Ab] ng/ml

Figure 23 – Continued

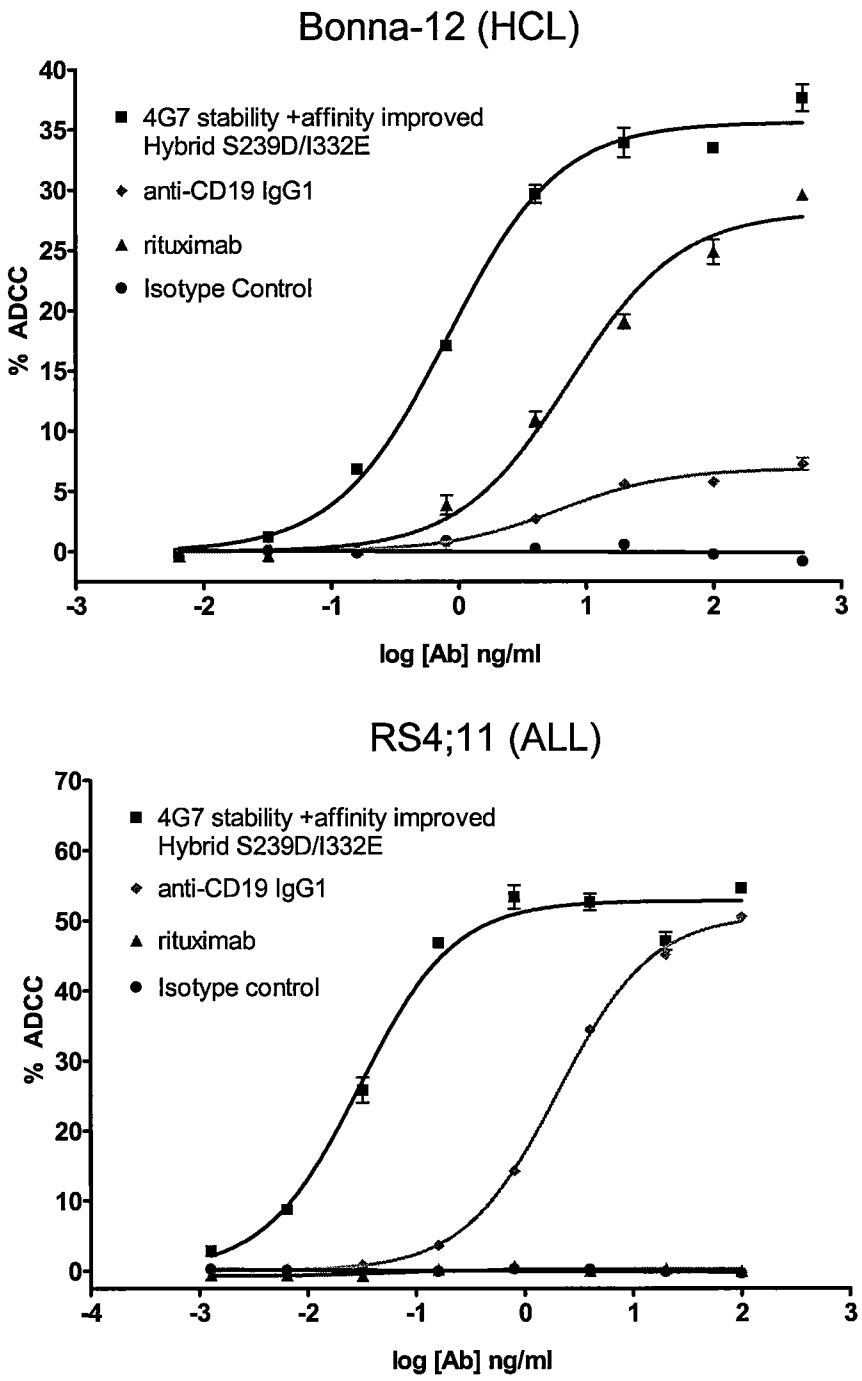


Figure 23 – Continued

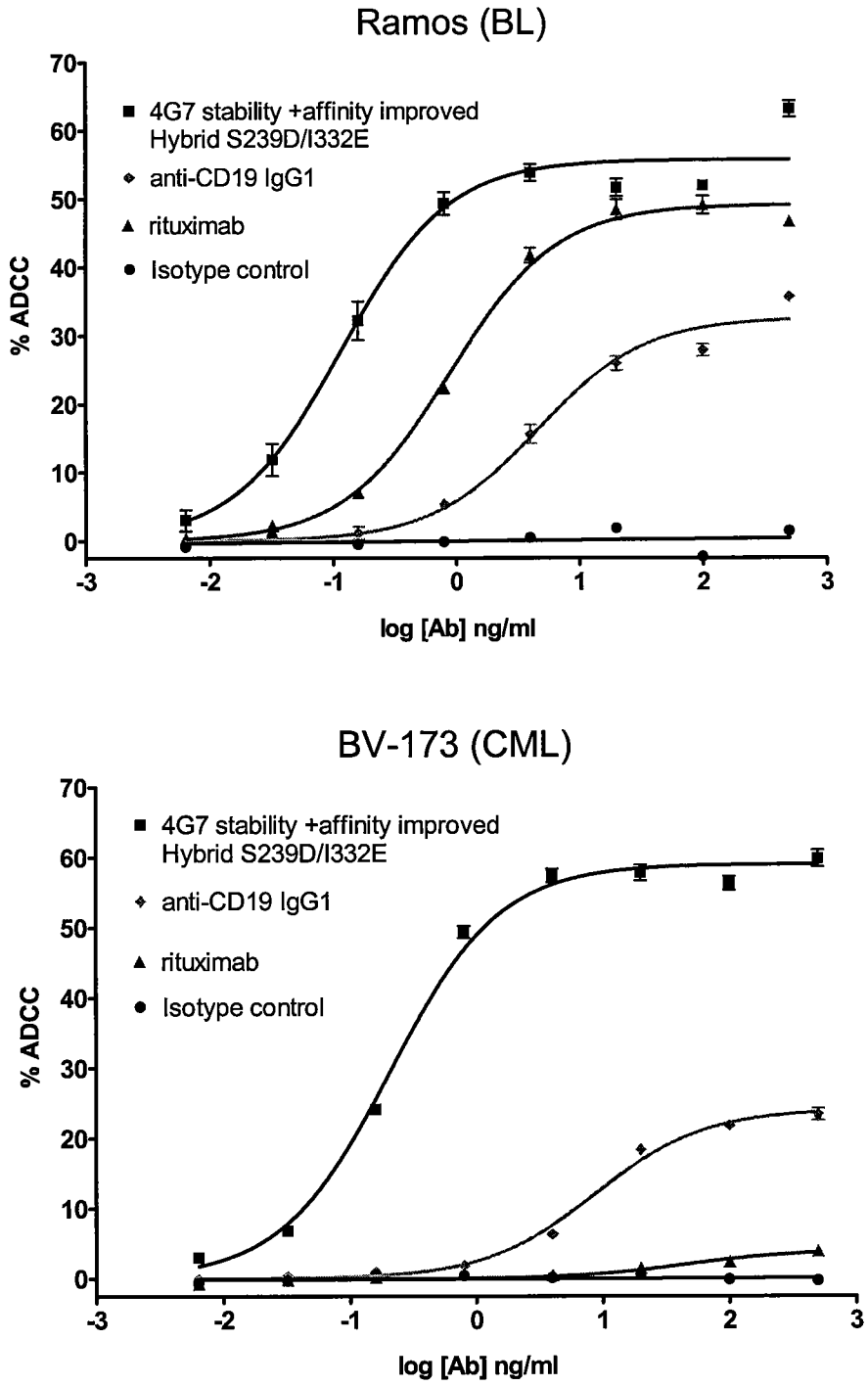


Figure 24

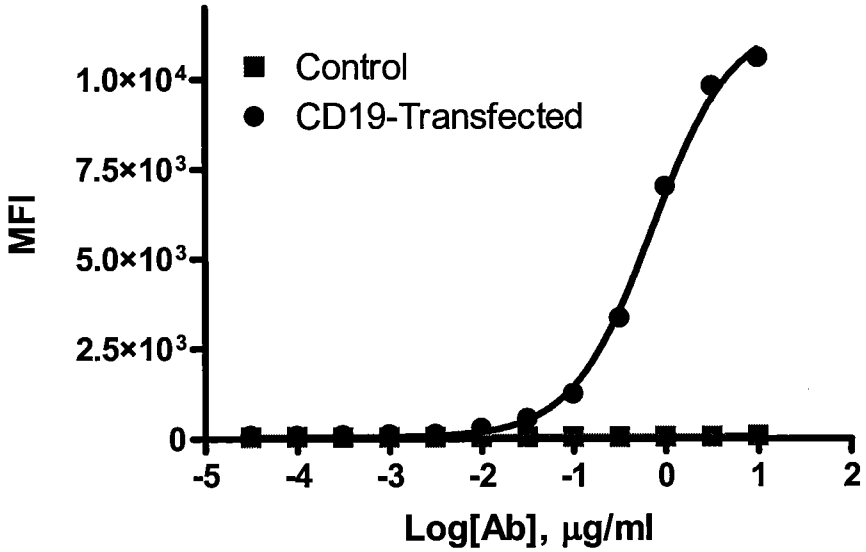

Control
CD19-Transfected
MFI
1.0×10⁴
7.5×10³
5.0×10³
2.5×10³
0
-5
-4
-3
-2
-1
0
1
2
Log[Ab], μg/ml

Figure 25

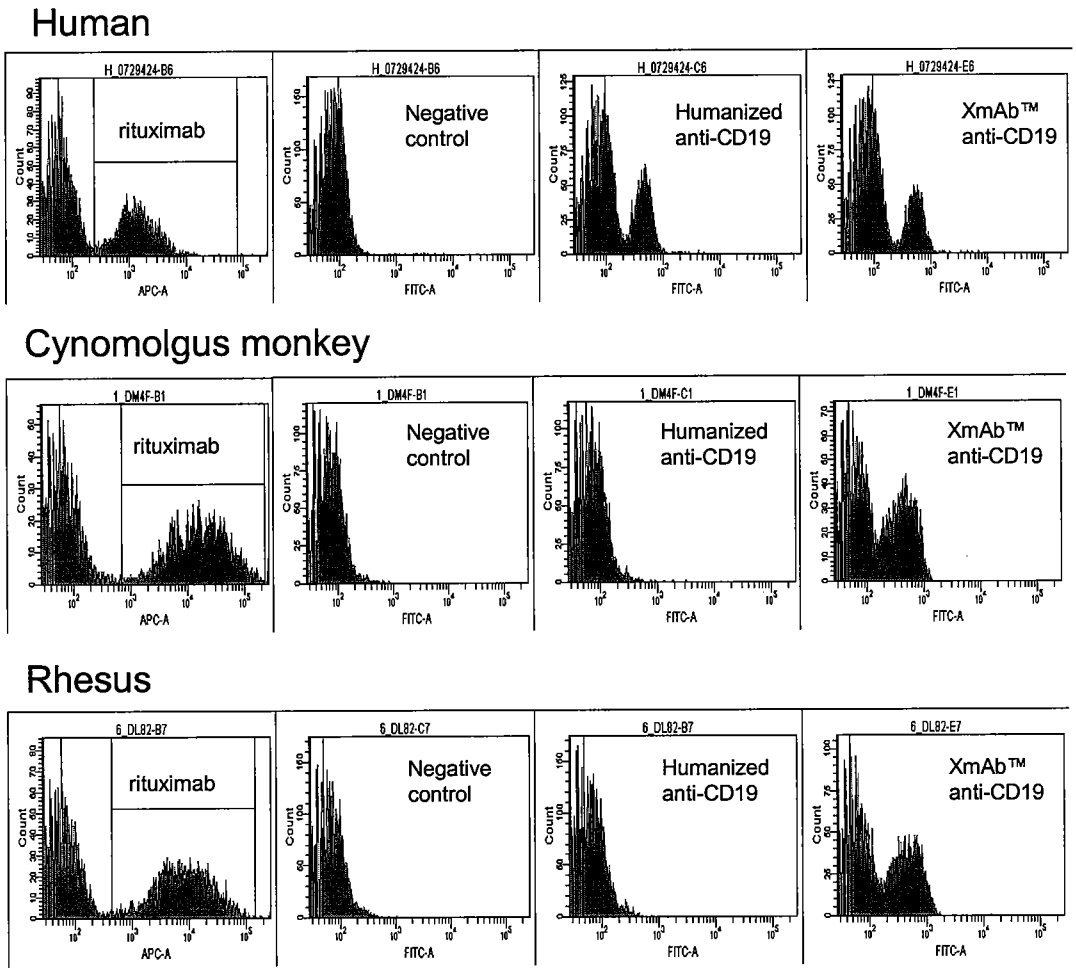

Human
H_0729424-B6
rituximab
H_0729424-B6
Negative control
H_0729424-C6
Humanized anti-CD19
H_0729424-E6
XmAb™ anti-CD19
Cynomolgus monkey
1_DM4F-B1
rituximab
1_DM4F-B1
Negative control
1_DM4F-C1
Humanized anti-CD19
1_DM4F-E1
XmAb™ anti-CD19
Rhesus
6_DL82-B7
rituximab
6_DL82-C7
Negative control
6_DL82-B7
Humanized anti-CD19
6_DL82-E7
XmAb™ anti-CD19
Count
APC-A
FITC-A

Figure 26

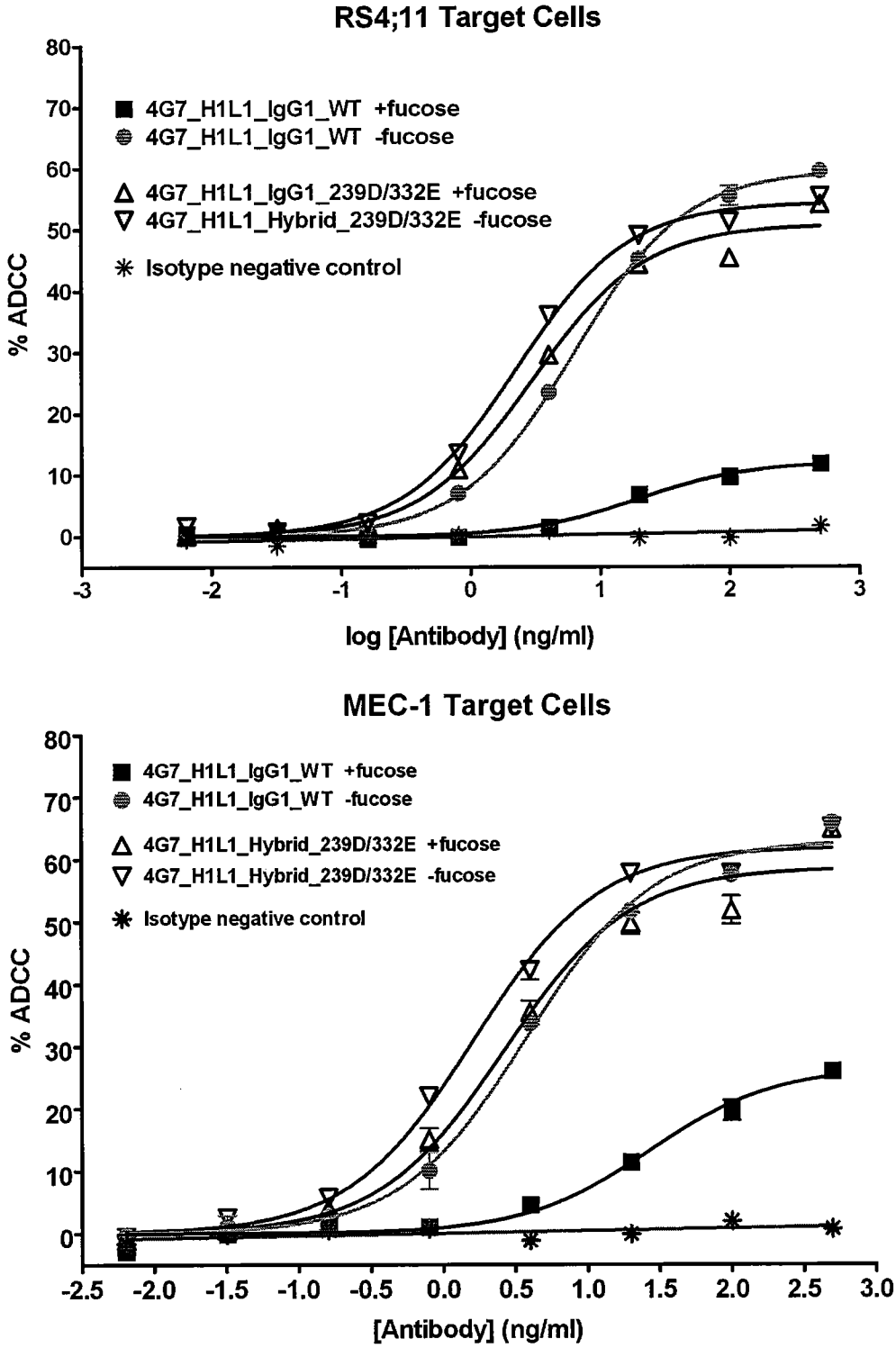

RS4;11 Target Cells
4G7_H1L1_IgG1_WT +fucose
4G7_H1L1_IgG1_WT -fucose
4G7_H1L1_IgG1_239D/332E +fucose
4G7_H1L1_Hybrid_239D/332E -fucose
Isotype negative control
% ADCC
log [Antibody] (ng/ml)
MEC-1 Target Cells
4G7_H1L1_IgG1_WT +fucose
4G7_H1L1_IgG1_WT -fucose
4G7_H1L1_Hybrid_239D/332E +fucose
4G7_H1L1_Hybrid_239D/332E -fucose
Isotype negative control
% ADCC
[Antibody] (ng/ml)

Figure 27

Heavy Chain

CDR-H1

Kabat	26	27	28	29	30	31	32	33	34	35	35a	35b
H1L1	G	Y	T	F	T	S			Y	V	M	H
Variants		F	A		A	A			F	I		N
			S		S	N			H	T		Y
					V	T			P	P		
					P	P			L	S		
					I	Q			W	L		
					Q	G			V	A		
					G	V			A	Y		
					H	H			N	F		
					N	E			Q	Q		
					Y	K			K	G		
					K	L				N		
						Y				K		

CDR-H2

Kabat	47	48	49	50	51	52	52a	52b	52c	53	54	55	56	57	58	59
H1L1	W	I	G	Y	I	N	P	Y			N	D	G	T	K	Y
Variants	F			F		D	S				D	E	A	A	R	F
				H		S	A				S	S	N	S	Q	H
							L				H	A	E	V	E	
							I				E	H	L	P	H	
							T				Q	Q	Q	I	S	
							V				P	R	H	Q	D	
							Y				R	P	P	G	P	
							Q				A	K	V	H	T	
							N				L	L	Y	N	Y	
							H				T	T	K	Y		
							G				G	G		K		
							F				Y	Y				
							K									

CDR-H3

Kabat	95	96	97	98	99	100	100a	100b	100c	100d	100e	100f	101	102
H1L1	G	T	Y	Y	Y			G	S	R	V	F	D	Y
Variants	A	A	F	F	F			A	A	K	I	L	N	F
	S	S	H	H	H			S	N	E			E	H
					P				T	D			H	P
					L				P	H			S	L
					W				Q	S			Q	W
					V				G	P			R	V
					A				V	Y			P	A
					S				H				K	N
					Q				E				A	Q
					K				K				L	K
									L				T	
									Y				G	
													Y	

Light Chain

CDR-L1

Kabat	24	25	26	27	27a	27b	27c	27d	27e	27f	28	29	30	31	32	33	34
H1L1	R	S	S	K	S	L	L	N	S		N	G	N	T	Y	L	Y
Variants				R	A		I	D	A		D	A	D	A	F		F
				Q	N		V	S	N		H	S	H	S	H		H
				E	T		P		T								P
				H	P		F		P								L
				S	Q		Y		Q								W
				D	G		W		G								V
				P	V		H		V								A
				T	H		S		H								N
				Y	E		Q		E								Q
					K		K		K								T
					L				Y								K
					Y												

CDR-L2

Kabat	49	50	51	52	53	54	55	56
H1L1	Y	R	M	S	N	L	A	S
Variants	F	K		A	D	V	G	A
	H	Q		N	S	I	V	T
	P			T	H	P	D	P
	L			P	E	F	L	Q
	W			Q	Q	Y	P	G
	V			G	P	W	H	N
	A			V	R	D	Q	V
	N			H	A	S	N	H
	Q			E	L	H	Y	E
	R			K	T	Q	K	K
				L	G	K		L
				Y	Y			Y

CDR-L3

Kabat	89	90	91	92	93	94	95	95a	95b	96	97
H1L1	M	Q	H	L	E	Y	P			F	T
Variants			N	I	D	F				L	
			Y	V	Q	H				Y	
				P	N	P				W	
				F	K	L				I	
				Y	S	W				H	
				W	H	V				P	
				N	R	A				V	
				S	P	N				N	
				H	T	Q				A	
				Q	G	R				Q	
				K	Y					K	

Figure 28

Template		Variants (Kabat numbering)								
		Heavy chain				Light chain				
Heavy chain	Light chain	1	2	3	4	1	2	3	4	5
H3.1	L0	Y27F								
H3.10	L0	Y34H								
H3.11	L0	V35I								
H3.12	L0	V35T								
H3.13	L0	H35bN								
H3.14	L0	H35bY								
H3.15	L0	W47F								
H3.16	L0	Y50F								
H3.17	L0	Y50H								
H3.18	L0	N52D								
H3.19	L0	N52S								
H3.2	L0	Y27H								
H3.20	L0	P52aS								
H3.21	L0	P52aA								
H3.22	L0	N54D								
H3.23	L0	N54S								
H3.24	L0	D55E								
H3.25	L0	D55S								
H3.26	L0	G56A								
H3.27	L0	G56N								
H3.28	L0	T57A								
H3.29	L0	T57S								
H3.3	L0	T28A								
H3.30	L0	K58R								
H3.31	L0	K58Q								
H3.32	L0	Y59F								
H3.33	L0	Y59H								
H3.34	L0	G95A								
H3.35	L0	G95S								
H3.36	L0	T96A								
H3.37	L0	T96S								
H3.38	L0	Y97F								
H3.39	L0	Y97H								
H3.4	L0	T28S								
H3.40	L0	Y98F								
H3.41	L0	Y98H								
H3.42	L0	Y99F								
H3.43	L0	Y99H								
H3.44	L0	G100bA								
H3.45	L0	G100bS								
H3.46	L0	S100cA								
H3.47	L0	S100cN								
H3.48	L0	R100dK								
H3.49	L0	R100dQ								
H3.5	L0	T30A								
H3.50	L0	V100eI								
H3.51	L0	F100fL								

Figure 28 - continued

Template		Variants (Kabat numbering) Heavy chain				Light chain				
Heavy chain	Light chain	1	2	3	4	1	2	3	4	5
H3.6	L0	T30S								
H3.7	L0	S31A								
H3.8	L0	S31N								
H3.9	L0	Y34F								
H1.100	L1	N54Q								
H1.101	L1	N54P								
H1.102	L1	N54R								
H1.103	L1	N54A								
H1.104	L1	N54L								
H1.105	L1	N54T								
H1.106	L1	N54G								
H1.107	L1	N54Y								
H1.108	L1	T57V								
H1.109	L1	T57P								
H1.110	L1	T57I								
H1.111	L1	T57Q								
H1.112	L1	T57G								
H1.113	L1	T57H								
H1.114	L1	T57N								
H1.115	L1	T57Y								
H1.116	L1	T57K								
H1.128	L1	K58E								
H1.128	L1	K58E								
H1.129	L1	K58H								
H1.130	L1	K58S								
H1.131	L1	K58D								
H1.132	L1	K58P								
H1.133	L1	K58T								
H1.134	L1	K58Y								
H1.135	L1	T30V								
H1.136	L1	T30P								
H1.137	L1	T30I								
H1.138	L1	T30Q								
H1.139	L1	T30G								
H1.140	L1	T30H								
H1.141	L1	T30N								
H1.142	L1	T30Y								
H1.143	L1	T30K								
H1.144	L1	D101N								
H1.145	L1	D101E								
H1.146	L1	D101H								
H1.147	L1	D101S								
H1.148	L1	D101Q								
H1.149	L1	D101R								
H1.150	L1	D101P								
H1.151	L1	D101K								
H1.152	L1	D101A								

Figure 28 - continued

Template		Variants (Kabat numbering) Heavy chain				Light chain				
Heavy chain	Light chain	1	2	3	4	1	2	3	4	5
H1.153	L1	D101L								
H1.154	L1	D101T								
H1.155	L1	D101G								
H1.156	L1	D101Y								
H1.157	L1	Y34P								
H1.158	L1	Y34L								
H1.159	L1	Y34W								
H1.160	L1	Y34V								
H1.161	L1	Y34A								
H1.162	L1	Y34N								
H1.163	L1	Y34Q								
H1.164	L1	Y34K								
H1.165	L1	Y99P								
H1.166	L1	Y99L								
H1.167	L1	Y99W								
H1.168	L1	Y99V								
H1.169	L1	Y99A								
H1.170	L1	Y99S								
H1.171	L1	Y99Q								
H1.172	L1	Y99K								
H1.173	L1	G56E								
H1.174	L1	G56L								
H1.175	L1	G56Q								
H1.176	L1	G56H								
H1.177	L1	G56P								
H1.178	L1	G56V								
H1.179	L1	G56Y								
H1.180	L1	G56K								
H1.181	L1	Y102F								
H1.182	L1	Y102H								
H1.183	L1	Y102P								
H1.184	L1	Y102L								
H1.185	L1	Y102W								
H1.186	L1	Y102V								
H1.187	L1	Y102A								
H1.188	L1	Y102N								
H1.189	L1	Y102Q								
H1.190	L1	Y102K								
H1.191	L1	K58E	S100cT							
H1.192	L1	T57P	S100cT							
H1.193	L1	K58E	R100dS							
H1.194	L1	T57P	R100dS							
H1.195	L1	S100cT	R100dS							
H1.196	L1	T57P	S100cT	R100dS						
H1.198	L1	T57P	K58E	S100cT	R100dS					
H1.199	L1	T57P	K58E	S100cT						
H1.200	L1	T57P	K58E							

Figure 28 - continued

Template		Variants (Kabat numbering)								
		Heavy chain				Light chain				
Heavy chain	Light chain	1	2	3	4	1	2	3	4	5
H1.52	L1	S100cT								
H1.53	L1	S100cP								
H1.54	L1	S100cQ								
H1.55	L1	S100cG								
H1.56	L1	S100cV								
H1.57	L1	S100cH								
H1.58	L1	S100cE								
H1.59	L1	S100cK								
H1.60	L1	S100cL								
H1.61	L1	S100cY								
H1.62	L1	R100dE								
H1.63	L1	R100dD								
H1.64	L1	R100dH								
H1.65	L1	R100dS								
H1.66	L1	R100dP								
H1.67	L1	R100dY								
H1.68	L1	S31T								
H1.69	L1	S31P								
H1.70	L1	S31Q								
H1.71	L1	S31G								
H1.72	L1	S31V								
H1.73	L1	S31H								
H1.74	L1	S31E								
H1.75	L1	S31K								
H1.76	L1	S31L								
H1.77	L1	S31Y								
H1.78	L1	D55A								
H1.79	L1	D55H								
H1.80	L1	D55Q								
H1.81	L1	D55R								
H1.82	L1	D55P								
H1.83	L1	D55K								
H1.84	L1	D55L								
H1.85	L1	D55T								
H1.86	L1	D55G								
H1.87	L1	D55Y								
H1.88	L1	V35P								
H1.89	L1	V35S								
H1.90	L1	V35L								
H1.91	L1	V35A								
H1.92	L1	V35Y								
H1.93	L1	V35F								
H1.94	L1	V35Q								
H1.95	L1	V35G								
H1.96	L1	V35N								
H1.97	L1	V35K								
H1.98	L1	N54H								

Figure 28 - continued

Template		Variants (Kabat numbering)								
		Heavy chain				Light chain				
Heavy chain	Light chain	1	2	3	4	1	2	3	4	5
H1.99	L1	N54E								
H3.117	L1	P52aL								
H3.118	L1	P52aI								
H3.119	L1	P52aT								
H3.120	L1	P52aV								
H3.121	L1	P52aY								
H3.122	L1	P52aQ								
H3.123	L1	P52aN								
H3.124	L1	P52aH								
H3.125	L1	P52aG								
H3.126	L1	P52aF								
H3.127	L1	P52aK								
H1	L1.1					S52T				
H1	L1.10					S52Y				
H1	L1.100					S56P				
H1	L1.101					S56Q				
H1	L1.102					S56G				
H1	L1.103					S56N				
H1	L1.104					S56V				
H1	L1.105					S56H				
H1	L1.106					S56E				
H1	L1.107					S56K				
H1	L1.108					S56L				
H1	L1.109					S56Y				
H1	L1.11					E93N				
H1	L1.110					S27aT				
H1	L1.111					S27aP				
H1	L1.112					S27aQ				
H1	L1.113					S27aG				
H1	L1.114					S27aV				
H1	L1.115					S27aH				
H1	L1.116					S27aE				
H1	L1.117					S27aK				
H1	L1.118					S27aL				
H1	L1.119					S27aY				
H1	L1.12					E93K				
H1	L1.120					L54I				
H1	L1.121					L54P				
H1	L1.122					L54F				
H1	L1.123					L54Y				
H1	L1.124					L54W				
H1	L1.125					L54D				
H1	L1.126					L54S				
H1	L1.127					L54H				
H1	L1.128					L54Q				
H1	L1.129					L54K				
H1	L1.13					E93S				

Figure 28 - continued

Template		Variants (Kabat numbering) Heavy chain				Light chain				
Heavy chain	Light chain	1	2	3	4	1	2	3	4	5
H1	L1.130					Y49P				
H1	L1.131					Y49L				
H1	L1.132					Y49W				
H1	L1.133					Y49V				
H1	L1.134					Y49A				
H1	L1.135					Y49N				
H1	L1.136					Y49Q				
H1	L1.137					Y49R				
H1	L1.138					S27eV	A55N			
H1.109	L1.138	T57P				S27eV	A55N			
H1.191	L1.138	K58E	S100cT			S27eV	A55N			
H1.192	L1.138	T57P	S100cT			S27eV	A55N			
H1.195	L1.138	S100cT	R100dS			S27eV	A55N			
H1.196	L1.138	T57P	S100cT	R100dS		S27eV	A55N			
H1.198	L1.138	T57P	K58E	S100cT	R100dS	S27eV	A55N			
H1.199	L1.138	T57P	K58E	S100cT		S27eV	A55N			
H1.52	L1.138	S100cT				S27eV	A55N			
H1	L1.139					S27eV	F96N			
H1.109	L1.139	T57P				S27eV	F96N			
H1.191	L1.139	K58E	S100cT			S27eV	F96N			
H1.192	L1.139	T57P	S100cT			S27eV	F96N			
H1.193	L1.139	K58E	R100dS			S27eV	F96N			
H1.194	L1.139	T57P	R100dS			S27eV	F96N			
H1.195	L1.139	S100cT	R100dS			S27eV	F96N			
H1.196	L1.139	T57P	S100cT	R100dS		S27eV	F96N			
H1.198	L1.139	T57P	K58E	S100cT	R100dS	S27eV	F96N			
H1.199	L1.139	T57P	K58E	S100cT		S27eV	F96N			
H1.52	L1.139	S100cT				S27eV	F96N			
H1	L1.14					E93H				
H1	L1.140					S27eV	F96I			
H1.109	L1.140	T57P				S27eV	F96I			
H1.191	L1.140	K58E	S100cT			S27eV	F96I			
H1.192	L1.140	T57P	S100cT			S27eV	F96I			
H1.52	L1.140	S100cT				S27eV	F96I			
H1	L1.141					A55N	F96N			
H1.109	L1.141	T57P				A55N	F96N			
H1.191	L1.141	K58E	S100cT			A55N	F96N			
H1.192	L1.141	T57P	S100cT			A55N	F96N			
H1.193	L1.141	K58E	R100dS			A55N	F96N			
H1.194	L1.141	T57P	R100dS			A55N	F96N			
H1.195	L1.141	S100cT	R100dS			A55N	F96N			
H1.196	L1.141	T57P	S100cT	R100dS		A55N	F96N			
H1.198	L1.141	T57P	K58E	S100cT	R100dS	A55N	F96N			
H1.199	L1.141	T57P	K58E	S100cT		A55N	F96N			
H1.52	L1.141	S100cT				A55N	F96N			
H1	L1.142					A55N	F96I			
H1.109	L1.142	T57P				A55N	F96I			

Figure 28 - continued

Template		Variants (Kabat numbering) Heavy chain				Light chain				
Heavy chain	Light chain	1	2	3	4	1	2	3	4	5
H1.191	L1.142	K58E	S100cT			A55N	F96I			
H1.192	L1.142	T57P	S100cT			A55N	F96I			
H1.193	L1.142	K58E	R100dS			A55N	F96I			
H1.194	L1.142	T57P	R100dS			A55N	F96I			
H1.52	L1.142	S100cT				A55N	F96I			
H1	L1.143					L27cQ	A55N			
H1.109	L1.143	T57P				L27cQ	A55N			
H1.191	L1.143	K58E	S100cT			L27cQ	A55N			
H1.192	L1.143	T57P	S100cT			L27cQ	A55N			
H1.195	L1.143	S100cT	R100dS			L27cQ	A55N			
H1.196	L1.143	T57P	S100cT	R100dS		L27cQ	A55N			
H1.198	L1.143	T57P	K58E	S100cT	R100dS	L27cQ	A55N			
H1.199	L1.143	T57P	K58E	S100cT		L27cQ	A55N			
H1.52	L1.143	S100cT				L27cQ	A55N			
H1	L1.144					L27cQ	F96N			
H1.109	L1.144	T57P				L27cQ	F96N			
H1.191	L1.144	K58E	S100cT			L27cQ	F96N			
H1.192	L1.144	T57P	S100cT			L27cQ	F96N			
H1.193	L1.144	K58E	R100dS			L27cQ	F96N			
H1.194	L1.144	T57P	R100dS			L27cQ	F96N			
H1.195	L1.144	S100cT	R100dS			L27cQ	F96N			
H1.196	L1.144	T57P	S100cT	R100dS		L27cQ	F96N			
H1.198	L1.144	T57P	K58E	S100cT	R100dS	L27cQ	F96N			
H1.199	L1.144	T57P	K58E	S100cT		L27cQ	F96N			
H1.52	L1.144	S100cT				L27cQ	F96N			
H1	L1.145					L27cQ	F96I			
H1.109	L1.145	T57P				L27cQ	F96I			
H1.191	L1.145	K58E	S100cT			L27cQ	F96I			
H1.192	L1.145	T57P	S100cT			L27cQ	F96I			
H1.52	L1.145	S100cT				L27cQ	F96I			
H1	L1.146					S27eV	A55N	F96N		
H1.109	L1.146	T57P				S27eV	A55N	F96N		
H1.191	L1.146	K58E	S100cT			S27eV	A55N	F96N		
H1.192	L1.146	T57P	S100cT			S27eV	A55N	F96N		
H1.193	L1.146	K58E	R100dS			S27eV	A55N	F96N		
H1.194	L1.146	T57P	R100dS			S27eV	A55N	F96N		
H1.195	L1.146	S100cT	R100dS			S27eV	A55N	F96N		
H1.196	L1.146	T57P	S100cT	R100dS		S27eV	A55N	F96N		
H1.198	L1.146	T57P	K58E	S100cT	R100dS	S27eV	A55N	F96N		
H1.199	L1.146	T57P	K58E	S100cT		S27eV	A55N	F96N		
H1.52	L1.146	S100cT				S27eV	A55N	F96N		
H1	L1.147					S27eV	A55N	F96I		
H1.109	L1.147	T57P				S27eV	A55N	F96I		
H1.191	L1.147	K58E	S100cT			S27eV	A55N	F96I		
H1.192	L1.147	T57P	S100cT			S27eV	A55N	F96I		
H1.193	L1.147	K58E	R100dS			S27eV	A55N	F96I		
H1.194	L1.147	T57P	R100dS			S27eV	A55N	F96I		

Figure 28 - continued

Variants (Kabat numbering)										
Template		Heavy chain				Light chain				
Heavy chain	Light chain	1	2	3	4	1	2	3	4	5
H1.52	L1.147	S100cT				S27eV	A55N	F96I		
H1	L1.148					L27cQ	A55N	F96N		
H1.109	L1.148	T57P				L27cQ	A55N	F96N		
H1.191	L1.148	K58E	S100cT			L27cQ	A55N	F96N		
H1.192	L1.148	T57P	S100cT			L27cQ	A55N	F96N		
H1.193	L1.148	K58E	R100dS			L27cQ	A55N	F96N		
H1.194	L1.148	T57P	R100dS			L27cQ	A55N	F96N		
H1.195	L1.148	S100cT	R100dS			L27cQ	A55N	F96N		
H1.196	L1.148	T57P	S100cT	R100dS		L27cQ	A55N	F96N		
H1.198	L1.148	T57P	K58E	S100cT	R100dS	L27cQ	A55N	F96N		
H1.199	L1.148	T57P	K58E	S100cT		L27cQ	A55N	F96N		
H1.52	L1.148	S100cT				L27cQ	A55N	F96N		
H1	L1.149					L27cQ	A55N	F96I		
H1.109	L1.149	T57P				L27cQ	A55N	F96I		
H1.191	L1.149	K58E	S100cT			L27cQ	A55N	F96I		
H1.192	L1.149	T57P	S100cT			L27cQ	A55N	F96I		
H1.193	L1.149	K58E	R100dS			L27cQ	A55N	F96I		
H1.194	L1.149	T57P	R100dS			L27cQ	A55N	F96I		
H1.52	L1.149	S100cT				L27cQ	A55N	F96I		
H1	L1.15					E93R				
H1	L1.150					L27cQ	S27eV			
H1	L1.151					L27cQ	S27eV	A55N		
H1.192	L1.151	T57P	S100cT			L27cQ	S27eV	A55N		
H1.195	L1.151	S100cT	R100dS			L27cQ	S27eV	A55N		
H1.196	L1.151	T57P	S100cT	R100dS		L27cQ	S27eV	A55N		
H1.198	L1.151	T57P	K58E	S100cT	R100dS	L27cQ	S27eV	A55N		
H1.199	L1.151	T57P	K58E	S100cT		L27cQ	S27eV	A55N		
H1.52	L1.151	S100cT				L27cQ	S27eV	A55N		
H1	L1.152					L27cQ	S27eV	F96N		
H1.192	L1.152	T57P	S100cT			L27cQ	S27eV	F96N		
H1.195	L1.152	S100cT	R100dS			L27cQ	S27eV	F96N		
H1.196	L1.152	T57P	S100cT	R100dS		L27cQ	S27eV	F96N		
H1.198	L1.152	T57P	K58E	S100cT	R100dS	L27cQ	S27eV	F96N		
H1.199	L1.152	T57P	K58E	S100cT		L27cQ	S27eV	F96N		
H1.52	L1.152	S100cT				L27cQ	S27eV	F96N		
H1	L1.153					L27cQ	S27eV	F96I		
H1	L1.154					L27cQ	S27eV	A55N	F96N	
H1.192	L1.154	T57P	S100cT			L27cQ	S27eV	A55N	F96N	
H1.195	L1.154	S100cT	R100dS			L27cQ	S27eV	A55N	F96N	
H1.196	L1.154	T57P	S100cT	R100dS		L27cQ	S27eV	A55N	F96N	
H1.198	L1.154	T57P	K58E	S100cT	R100dS	L27cQ	S27eV	A55N	F96N	
H1.199	L1.154	T57P	K58E	S100cT		L27cQ	S27eV	A55N	F96N	
H1.52	L1.154	S100cT				L27cQ	S27eV	A55N	F96N	
H1	L1.155					L27cQ	S27eV	A55N	F96I	
H1.191	L1.155	K58E	S100cT			L27cQ	S27eV	A55N	F96I	
H1.201	L1.155	D55S	S100cT			L27cQ	S27eV	A55N	F96I	
H1.203	L1.155	D55S	K58E	S100cT		L27cQ	S27eV	A55N	F96I	

Figure 28 - continued

Template		Variants (Kabat numbering) Heavy chain				Light chain				
Heavy chain	Light chain	1	2	3	4	1	2	3	4	5
H1.52	L1.155	S100cT				L27cQ	S27eV	A55N	F96I	
H1	L1.16					E93P				
H1.191	L1.160	K58E	S100cT			L27cQ	S27eV	G29A	A55N	F96I
H1.201	L1.160	D55S	S100cT			L27cQ	S27eV	G29A	A55N	F96I
H1.202	L1.160	D55E	S100cT			L27cQ	S27eV	G29A	A55N	F96I
H1.203	L1.160	D55S	K58E	S100cT		L27cQ	S27eV	G29A	A55N	F96I
H1.204	L1.160	D55E	K58E	S100cT		L27cQ	S27eV	G29A	A55N	F96I
H1.52	L1.160	S100cT				L27cQ	S27eV	G29A	A55N	F96I
H1.191	L1.161	K58E	S100cT			L27cQ	S27eV	G29S	A55N	F96I
H1.201	L1.161	D55S	S100cT			L27cQ	S27eV	G29S	A55N	F96I
H1.202	L1.161	D55E	S100cT			L27cQ	S27eV	G29S	A55N	F96I
H1.203	L1.161	D55S	K58E	S100cT		L27cQ	S27eV	G29S	A55N	F96I
H1.204	L1.161	D55E	K58E	S100cT		L27cQ	S27eV	G29S	A55N	F96I
H1.52	L1.161	S100cT				L27cQ	S27eV	G29S	A55N	F96I
H1.191	L1.162	K58E	S100cT			L27cQ	S27eA	G29A	A55N	F96I
H1.201	L1.162	D55S	S100cT			L27cQ	S27eA	G29A	A55N	F96I
H1.202	L1.162	D55E	S100cT			L27cQ	S27eA	G29A	A55N	F96I
H1.203	L1.162	D55S	K58E	S100cT		L27cQ	S27eA	G29A	A55N	F96I
H1.204	L1.162	D55E	K58E	S100cT		L27cQ	S27eA	G29A	A55N	F96I
H1.52	L1.162	S100cT				L27cQ	S27eA	G29A	A55N	F96I
H1.191	L1.163	K58E	S100cT			L27cQ	S27eA	G29S	A55N	F96I
H1.201	L1.163	D55S	S100cT			L27cQ	S27eA	G29S	A55N	F96I
H1.202	L1.163	D55E	S100cT			L27cQ	S27eA	G29S	A55N	F96I
H1.203	L1.163	D55S	K58E	S100cT		L27cQ	S27eA	G29S	A55N	F96I
H1.204	L1.163	D55E	K58E	S100cT		L27cQ	S27eA	G29S	A55N	F96I
H1.52	L1.163	S100cT				L27cQ	S27eA	G29S	A55N	F96I
H1.201	L1.164	D55S	S100cT			L27cQ	S27eA	A55N	F96I	
H1.203	L1.164	D55S	K58E	S100cT		L27cQ	S27eA	A55N	F96I	
H1	L1.17					E93T				
H1	L1.18					E93G				
H1	L1.19					E93Y				
H1	L1.2					S52P				
H1	L1.20					L27cP				
H1	L1.21					L27cF				
H1	L1.22					L27cY				
H1	L1.23					L27cW				
H1	L1.24					L27cH				
H1	L1.25					L27cS				
H1	L1.26					L27cQ				
H1	L1.27					L27cK				
H1	L1.28					S27eT				
H1	L1.29					S27eP				
H1	L1.3					S52Q				
H1	L1.30					S27eQ				
H1	L1.31					S27eG				
H1	L1.32					S27eV				
H1.109	L1.32	T57P								

Figure 28 - continued

Template		Variants (Kabat numbering) Heavy chain				Light chain				
Heavy chain	Light chain	1	2	3	4	1	2	3	4	5
H1.52	L1.32	S100cT				S27eV				
H1	L1.33					S27eH				
H1	L1.34					S27eE				
H1	L1.35					S27eK				
H1	L1.36					S27eY				
H1	L1.37					L92P				
H1	L1.38					L92F				
H1	L1.39					L92Y				
H1	L1.4					S52G				
H1	L1.40					L92W				
H1	L1.41					L92N				
H1	L1.42					L92S				
H1	L1.43					L92H				
H1	L1.44					L92Q				
H1	L1.45					L92K				
H1	L1.46					N53H				
H1	L1.47					N53E				
H1	L1.48					N53Q				
H1	L1.49					N53P				
H1	L1.5					S52V				
H1	L1.50					N53R				
H1	L1.51					N53A				
H1	L1.52					N53L				
H1	L1.53					N53T				
H1	L1.54					N53G				
H1	L1.55					N53Y				
H1	L1.56					K27E				
H1	L1.57					K27H				
H1	L1.58					K27S				
H1	L1.59					K27D				
H1	L1.6					S52H				
H1	L1.60					K27P				
H1	L1.61					K27T				
H1	L1.62					K27Y				
H1	L1.63					F96W				
H1	L1.64					F96I				
H1.191	L1.64	K58E	S100cT			F96I				
H1.192	L1.64	T57P	S100cT			F96I				
H1	L1.65					F96H				
H1	L1.66					F96P				
H1	L1.67					F96V				
H1	L1.68					F96N				
H1.109	L1.68	T57P								
H1.191	L1.68	K58E	S100cT			F96N				
H1.192	L1.68	T57P	S100cT			F96N				
H1.195	L1.68	S100cT	R100dS			F96N				
H1.196	L1.68	T57P	S100cT	R100dS		F96N				

Figure 28 - continued

Template		Variants (Kabat numbering) Heavy chain				Light chain				
Heavy chain	Light chain	1	2	3	4	1	2	3	4	5
H1.198	L1.68	T57P	K58E	S100cT	R100dS	F96N				
H1.199	L1.68	T57P	K58E	S100cT		F96N				
H1.52	L1.68	S100cT				F96N				
H1	L1.69					F96A				
H1	L1.7					S52E				
H1	L1.70					F96Q				
H1	L1.71					F96K				
H1	L1.72					Y94P				
H1	L1.73					Y94L				
H1	L1.74					Y94W				
H1	L1.75					Y94V				
H1	L1.76					Y94A				
H1	L1.77					Y94N				
H1	L1.78					Y94Q				
H1	L1.79					Y94R				
H1	L1.8					S52K				
H1	L1.80					Y34F				
H1	L1.81					Y34H				
H1	L1.82					Y34P				
H1	L1.83					Y34L				
H1	L1.84					Y34W				
H1	L1.85					Y34V				
H1	L1.86					Y34A				
H1	L1.87					Y34N				
H1	L1.88					Y34Q				
H1	L1.89					Y34T				
H1	L1.9					S52L				
H1	L1.90					Y34K				
H1	L1.91					A55D				
H1	L1.92					A55L				
H1	L1.93					A55P				
H1	L1.94					A55H				
H1	L1.95					A55Q				
H1	L1.96					A55N				
H1.191	L1.96	K58E	S100cT			A55N				
H1.192	L1.96	T57P	S100cT			A55N				
H1.198	L1.96	T57P	K58E	S100cT	R100dS	A55N				
H1.199	L1.96	T57P	K58E	S100cT		A55N				
H1	L1.97					A55Y				
H1	L1.98					A55K				
H1	L1.99					S56T				
H3	L2.1					K27R				
H3	L2.10					S27eN				
H3	L2.11					N28D				
H3	L2.12					N28H				
H3	L2.13					G39A				
H3	L2.14					G39S				

Figure 28 - continued

		Variants (Kabat numbering)								
Template		Heavy chain				Light chain				
Heavy chain	Light chain	1	2	3	4	1	2	3	4	5
H3	L2.15					N30D				
H3	L2.16					N30H				
H3	L2.17					T31A				
H3	L2.18					T31S				
H3	L2.19					Y32F				
H3	L2.2					K27Q				
H3	L2.20					Y32H				
H3	L2.21					Y34F				
H3	L2.22					Y49F				
H3	L2.23					Y49H				
H3	L2.24					R50K				
H3	L2.25					R50Q				
H3	L2.26					S52A				
H3	L2.27					S52N				
H3	L2.28					N53D				
H3	L2.29					N53S				
H3	L2.3					S27aA				
H3	L2.30					L54V				
H3	L2.31					A55G				
H3	L2.32					A55V				
H3	L2.33					S56A				
H3	L2.34					H91N				
H3	L2.35					N91Y				
H3	L2.36					L92I				
H3	L2.37					L92V				
H3	L2.38					E93D				
H3	L2.39					E93Q				
H3	L2.4					S27aN				
H3	L2.40					Y94F				
H3	L2.41					Y94H				
H3	L2.42					F96L				
H3	L2.43					F96Y				
H3	L2.5					L27cI				
H3	L2.6					L27cV				
H3	L2.7					N27dD				
H3	L2.8					N27dS				
H3	L2.9					S27eA				

Figure 29

Variants (Kabat numbering)

Template		Heavy chain				Light chain					Fold change
Heavy chain	Light chain	1	2	3	4	1	2	3	4	5	binding
H1.109	L1	T57P									1.52
H1.113	L1	T57H									1.29
H1.144	L1	D101N									2.26
H1.146	L1	D101H									2.45
H1.147	L1	D101S									2.65
H1.196	L1	T57P	S100cT	R100dS							3.42
H1.52	L1	S100cT									3.22
H1.60	L1	S100cL									1.56
H1.62	L1	R100dE									1.65
H1.65	L1	R100dS									1.41
H1.78	L1	D55A									1.14
H1	L1.11					E93N					1.59
H1	L1.124					L54W					1.47
H1.52	L1.138	S100cT				S27eV	A55N				3.93
H1.199	L1.139	T57P	K58E	S100cT		S27eV	F96N				2.36
H1.52	L1.139	S100cT				S27eV	F96N				1.80
H1.192	L1.141	T57P	S100cT			A55N	F96N				2.57
H1.199	L1.141	T57P	K58E	S100cT		A55N	F96N				4.02
H1.52	L1.143	S100cT				L27cQ	A55N				3.83
H1.192	L1.144	T57P	S100cT			L27cQ	F96N				3.63
H1.52	L1.145	S100cT				L27cQ	F96I				6.41
H1.192	L1.146	T57P	S100cT			S27eV	A55N	F96N			3.19
H1.196	L1.146	T57P	S100cT	R100dS		S27eV	A55N	F96N			3.57
H1.199	L1.146	T57P	K58E	S100cT		S27eV	A55N	F96N			4.11
H1.192	L1.148	T57P	S100cT			L27cQ	A55N	F96N			4.63
H1.199	L1.148	T57P	K58E	S100cT		L27cQ	A55N	F96N			4.34
H1.191	L1.149	K58E	S100cT			L27cQ	A55N	F96I			6.02
H1.52	L1.149	S100cT				L27cQ	A55N	F96I			8.60
H1.192	L1.152	T57P	S100cT			L27cQ	S27eV	F96N			2.98
H1.196	L1.152	T57P	S100cT	R100dS		L27cQ	S27eV	F96N			4.36
H1.199	L1.152	T57P	K58E	S100cT		L27cQ	S27eV	F96N			3.55
H1.192	L1.154	T57P	S100cT			L27cQ	S27eV	A55N	F96N		5.12
H1.196	L1.154	T57P	S100cT	R100dS		L27cQ	S27eV	A55N	F96N		4.44
H1.191	L1.155	K58E	S100cT			L27cQ	S27eV	A55N	F96I		4.56
H1.201	L1.155	D55S	S100cT			L27cQ	S27eV	A55N	F96I		4.48
H1.203	L1.155	D55S	K58E	S100cT		L27cQ	S27eV	A55N	F96I		4.73
H1.52	L1.155	S100cT				L27cQ	S27eV	A55N	F96I		6.16
H1.191	L1.160	K58E	S100cT			L27cQ	S27eV	G29A	A55N	F96I	2.91
H1.201	L1.160	D55S	S100cT			L27cQ	S27eV	G29A	A55N	F96I	4.51
H1.202	L1.160	D55E	S100cT			L27cQ	S27eV	G29A	A55N	F96I	3.96
H1.203	L1.160	D55S	K58E	S100cT		L27cQ	S27eV	G29A	A55N	F96I	1.76
H1.204	L1.160	D55E	K58E	S100cT		L27cQ	S27eV	G29A	A55N	F96I	3.46
H1.52	L1.160	S100cT				L27cQ	S27eV	G29A	A55N	F96I	4.18
H1.191	L1.161	K58E	S100cT			L27cQ	S27eV	G29S	A55N	F96I	3.97
H1.201	L1.161	D55S	S100cT			L27cQ	S27eV	G29S	A55N	F96I	3.89
H1.202	L1.161	D55E	S100cT			L27cQ	S27eV	G29S	A55N	F96I	4.30
H1.203	L1.161	D55S	K58E	S100cT		L27cQ	S27eV	G29S	A55N	F96I	2.92

Figure 29 - continued

Template		Variants (Kabat numbering) Heavy chain				Light chain					Fold change
Heavy chain	Light chain	1	2	3	4	1	2	3	4	5	binding
H1.204	L1.161	D55E	K58E	S100cT		L27cQ	S27eV	G29S	A55N	F96I	3.27
H1.52	L1.161	S100cT				L27cQ	S27eV	G29S	A55N	F96I	3.18
H1.191	L1.162	K58E	S100cT			L27cQ	S27eA	G29A	A55N	F96I	3.35
H1.201	L1.162	D55S	S100cT			L27cQ	S27eA	G29A	A55N	F96I	3.97
H1.202	L1.162	D55E	S100cT			L27cQ	S27eA	G29A	A55N	F96I	4.32
H1.203	L1.162	D55S	K58E	S100cT		L27cQ	S27eA	G29A	A55N	F96I	3.25
H1.204	L1.162	D55E	K58E	S100cT		L27cQ	S27eA	G29A	A55N	F96I	4.07
H1.52	L1.162	S100cT				L27cQ	S27eA	G29A	A55N	F96I	5.57
H1.191	L1.163	K58E	S100cT			L27cQ	S27eA	G29S	A55N	F96I	5.32
H1.201	L1.163	D55S	S100cT			L27cQ	S27eA	G29S	A55N	F96I	3.87
H1.202	L1.163	D55E	S100cT			L27cQ	S27eA	G29S	A55N	F96I	3.57
H1.203	L1.163	D55S	K58E	S100cT		L27cQ	S27eA	G29S	A55N	F96I	3.20
H1.204	L1.163	D55E	K58E	S100cT		L27cQ	S27eA	G29S	A55N	F96I	4.73
H1.52	L1.163	S100cT				L27cQ	S27eA	G29S	A55N	F96I	3.74
H1.201	L1.164	D55S	S100cT			L27cQ	S27eA	A55N	F96I		4.41
H1.203	L1.164	D55S	K58E	S100cT		L27cQ	S27eA	A55N	F96I		5.58
H1	L1.17					E93T					1.57
H1	L1.19					E93Y					1.76
H1	L1.26					L27cQ					1.73
H1	L1.3					S52Q					1.85
H1	L1.32					S27eV					2.61
H1	L1.46					N53H					1.75
H1	L1.54					N53G					1.50
H1	L1.55					N53Y					1.58
H1	L1.64					F96I					1.80
H1	L1.67					F96V					1.73
H1	L1.68					F96N					2.12
H1	L1.8					S52K					2.18
H1	L1.80					Y34F					1.62
H1	L1.9					S52L					1.84
H1	L1.92					A55L					1.54
H1	L1.96					A55N					1.58

Figure 30a

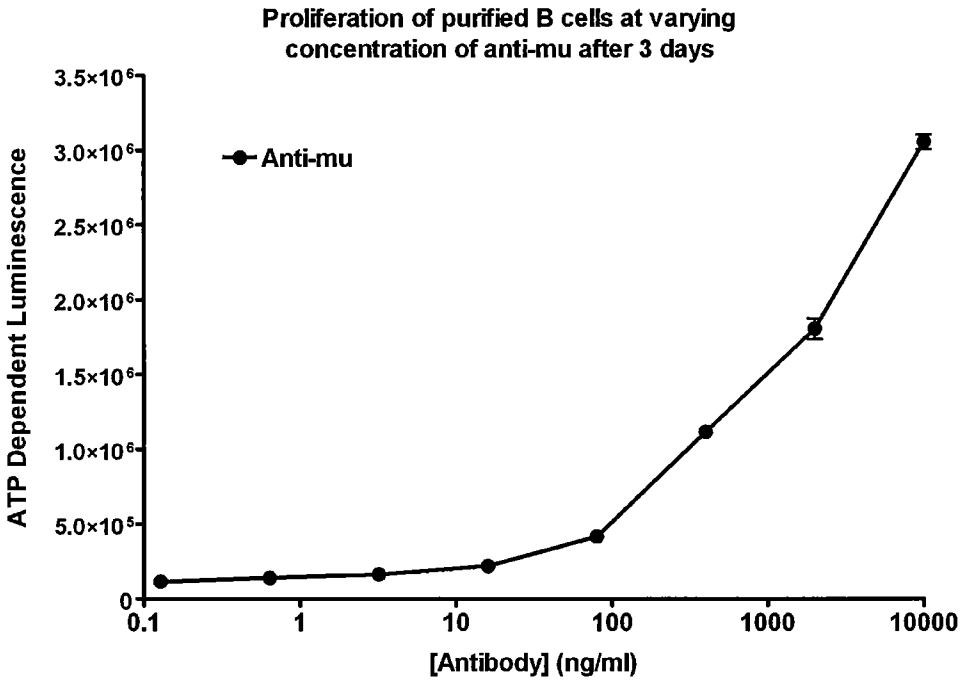


Figure 30b