HEPATOCYTE GROWTH FACTOR (HGF) BINDING PROTEINS

RELATED APPLICATIONS

[0001] This application claims the benefit and priority to U.S. Provisional Application Nos. 60/810,714, filed June 2, 2006, and 60/860,509, filed November 21, 2006, the disclosures of which are incorporated by reference herein.

FIELD OF THE INVENTION

[0002] The field of the invention is molecular biology, immunology and oncology. More particularly, the field is antibody-based binding proteins that bind human hepatocyte growth factor (HGF).

BACKGROUND

[0003] Hepatocyte Growth Factor (HGF), also known as Scatter Factor (SF), is a multi-functional heterodimeric protein produced predominantly by mesenchymal cells, and is an effector of cells expressing the Met tyrosine kinase receptor (Bottaro *et al.* (1991) SCIENCE 251: 802-804, Rubin *et al.* (1993) BIOCHIM. BIOPHYS. ACTA 1155: 357-371). The human Met receptor is also known as "c-Met." Mature HGF contains two polypeptide chains, the α-chain and the β-chain. Published studies suggest it is the α-chain that contains HGF's c-Met receptor binding domain.

[0004] When it binds to its cognate receptor, HGF mediates a number of cellular activities. The HGF-Met signaling pathway plays a role in liver regeneration, wound healing, neural regeneration, angiogenesis and malignancies. See, e.g., Cao *et al.* (2001) PROC. NATL. ACAD. SCI. USA 98: 7443-7448, Burgess *et al.* (2006) CANCER RES. 66: 1721-1729, and U.S. Patent Nos. 5,997,868 and 5,707,624. Investigators have been developing a number of HGF modulators, including antibodies, to treat various disorders that involve HGF activity, for example, certain HGF responsive cancers. See, e.g., International Application Publication No. WO 2005/017107.

[0005] The basic structure common to all antibodies is shown schematically in Figure 1. Antibodies are multimeric proteins that contain four polypeptide chains. Two of the polypeptide chains are called heavy or H chains and two of the polypeptide chains are called light or L chains. The immunoglobulin heavy and light chains are connected by an interchain

disulfide bond. The immunoglobulin heavy chains are connected by a number of interchain disulfide bonds. A light chain is composed of one variable region (V_L in Figure 1) and one constant region (C_L in Figure 1), while the heavy chain is composed of one variable region (V_H in Figure 1) and at least three constant regions (CH_1, CH_2 and CH_3 in Figure 1). The variable regions determine the specificity of the antibody and the constant regions have other functions.

[0006] Amino acid and structural information indicate that each variable region comprises three hypervariable regions (also known as complementarity determining regions or CDRs) flanked by four relatively conserved framework regions or FRs. The three CDRs, referred to as CDR_1, CDR_2, and CDR_3, are responsible for the binding specificity of individual antibodies. When antibodies are to be used as diagnostic and therapeutic agents, typically it is desirable to create antibodies that have the highest binding specificity and affinity to the target molecule. It is believed that differences in the variable regions can have profound effects on the specificity and affinity of the antibody.

[0007] U.S. Patent No. 5,707,624 describes the use of anti-HGF antibodies in the treatment of Kaposi's sarcoma. Similarly, U.S. Patent No. 5,997,868 describes treating a tumor by administering an anti-HGF antibody to the patient to be treated so as to block the ability of endogeneous HGF to promote angiogenesis in the tumor. More recently, investigators propose that antibodies that bind the β-chain of HGF may have potential as therapeutic agents in patients with HGF-dependent tumors (Burgess (2006) *supra*).

[0008] Notwithstanding, there is still a need for additional HGF modulators that can be used as therapeutic and diagnostic agents.

SUMMARY OF THE INVENTION

[0009] The invention is based, in part, upon the discovery of a family of binding proteins that specifically bind HGF, in particular, human HGF. The binding proteins are antibody-based in so far as they contain antigen (i.e., HGF) binding sites based on the CDRs of a family of antibodies that specifically bind HGF. The CDRs confer the binding specificity of the binding proteins to HGF. The binding proteins can be used as diagnostic and therapeutic agents. When used as a therapeutic agent, the binding proteins are engineered (e.g., humanized) so as to reduce or eliminate the risk of inducing an immune response against the binding protein when administered to the recipient (e.g., a human).

[0010] The binding proteins neutralize the activity of HGF and, therefore, can be used as a therapeutic agent. In certain embodiments, the binding proteins prevent HGF from binding to its cognate receptor, c-Met, thereby neutralizing HGF activity. In other embodiments, the binding proteins bind to HGF and neutralize its biological activity but without preventing HGF from binding to the c-Met receptor. Because HGF has been implicated in the growth and proliferation of cancer cells, the binding proteins can be used to inhibit the proliferation of cancer cells. Furthermore, when administered to a mammal, the binding proteins can inhibit or reduce tumor growth in the mammal.

[0011] These and other aspects and advantages of the invention will become apparent upon consideration of the following figures, detailed description, and claims.

DESCRIPTION OF THE DRAWINGS

[0012] The invention can be more completely understood with reference to the following drawings.

[0013] Figure 1 is a schematic representation of a typical antibody.

[0014] Figure 2 is a schematic diagram showing the amino acid sequence defining the complete immunoglobulin heavy chain variable region of the antibodies denoted as 1A3, 1D3, 1F3, 2B8, 2F8, 3A12, 3B6 and 3D11. The amino acid sequences for each antibody are aligned against one another and the regions defining the signal peptide, CDR_1, CDR_2, and CDR_3 are identified in boxes. The unboxed sequences represent FR sequences.

[0015] Figure 3 is a schematic diagram showing the CDR_1, CDR_2, and CDR_3 sequences for each of the immunoglobulin heavy chain variable region sequences presented in Figure 2.

[0016] Figure 4 is a schematic diagram showing the amino acid sequence defining the complete immunoglobulin light chain variable region of the antibodies 1A3, 1D3, 1F3, 2B8, 2F8, 3A12, 3B6, and 3D11. The amino acid sequences for each antibody are aligned against one another and the regions defining the signal peptide, CDR_1, CDR_2, and CDR_3 are identified in boxes. The unboxed sequences represent FR sequences.

[0017] Figure 5 is a schematic diagram showing the CDR_1, CDR_2, and CDR_3 sequences for each of the immunoglobulin light chain variable region sequences presented in Figure 4.

[0018] Figure 6 is a graph summarizing results from an experiment to measure tumor inhibitory activity of anti-HGF antibodies 1D3, 1F3, 1A3 and 2B8 in a U87MG xenograft model. Diamonds correspond to PBS; triangles correspond to anti-HGF antibody 1A3; X corresponds to anti-HGF antibody 1D3; squares correspond to anti-HGF antibody 1F3, and circles correspond to anti-HGF antibody 2B8.

[0019] Figure 7 is a graph summarizing results from an experiment to measure tumor inhibitory activity of anti-HGF antibodies 1D3, 1F3, 1A3 and 2B8 in a U118 xenograft model. Diamonds correspond to IgG; squares correspond to anti-HGF antibody 1F3, triangles to anti-HGF antibody 1D3; X corresponds to anti-HGF antibody 1A3; and circles correspond to anti-HGF antibody 2B8.

[0020] Figure 8 is a table summarizing surface plasmon resonance data on antigen-binding affinity and kinetics of interaction between human HGF and chimeric, chimeric/humanized, or humanized 2B8 antibodies. The table lists the pairs of Kappa light chain and IgG1 heavy chain tested. Those antibodies with standard deviations (STDEV) listed were analyzed in three independent experiments.

[0021] Figure 9 is a bar chart summarizing experimental data indicating that Hu2B8 binds an epitope mutually exclusive to murine monoclonal antibody 2B8. Humanized or chimeric 2B8 was captured on an anti-human Fc chip. HGF then was bound to the humanized or chimeric 2B8. The ability of mouse 2B8 or the control antibody (polyclonal goat anti-HGF antibody) to bind the captured HGF was measured. Both humanized 2B8 antibodies and chimeric 2B8 prevent murine 2B8 from binding HGF. White bars correspond to the chimeric 2B8 antibody; gray bars correspond to the humanized Hu2B8 antibody (kappa variable region Kv1-39.1 and heavy chain variable region Hv5-51.1); black bars correspond to the humanized Hu2B8 antibody (kappa variable region Kv3-15.1 and heavy chain variable region Hv5-51.1).

DETAILED DESCRIPTION OF THE INVENTION

[0022] The invention is based, in part, upon the discovery of a family of binding proteins that specifically bind, and neutralize the activity of, HGF, in particular, human HGF. The binding proteins can be used in a variety of diagnostic and therapeutic applications. The binding proteins are based upon the antigen binding sites of certain monoclonal antibodies that have been selected for their ability to bind, and neutralize the activity of, HGF. In particular,

the binding proteins contain immunoglobulin variable region CDR sequences that together define a binding site for HGF.

[0023] In view of the neutralizing activity of these antibodies, they are particularly useful in modulating the growth and/or proliferation of HGF responsive cells, for example, cancer cells. When used as a therapeutic agent, the binding proteins can be engineered so as to minimize or eliminate the risk of inducing an immune response against the binding proteins when administered to the recipient. Furthermore, depending upon the particular application, it is contemplated that the binding proteins can be conjugated to other moieties, for example, detectable labels, for example, radiolabels, and effector molecules, for example, other protein and small molecule-based therapeutics. Each of these features and aspects of the invention are discussed in more detail below.

I – Binding Proteins That Bind HGF

[0024] In one aspect, the invention provides an isolated binding protein that binds human HGF. The binding protein comprises (i) an immunoglobulin light chain variable region comprising the structure CDR_{L1}-CDR_{L2}-CDR_{L3}, and (ii) an immunoglobulin heavy chain variable region comprising three complementarity determining regions (CDRs), wherein the immunoglobulin light chain variable region and the immunoglobulin heavy chain variable region together define a single binding site for binding human HGF. CDR_{L1} comprises the amino acid sequence X_1 X_2 Ser X_4 X_5 X_6 X_7 X_8 X_9 X_{10} X_{11} X_{12} X_{13} X_{14} X_{15}, wherein amino acid X_1 is Arg, Lys, or Ser, X_2 is Ala or Thr, X_4 is Glu, Gln, or Ser, X_5 is Asn, Asp, or Ser, X_6 is Ile or Val, X_7 is Asp, Lys, Ser, Val, or Tyr, X_8 is a peptide bond or Tyr, X_9 is a peptide bond or Asp, X_{10} is a peptide bond or Gly, X_{11} is a peptide bond or Asn, X_{12} is a peptide bond, Ile, or Ser, X_{13} is Asn or Tyr, X_{14} is Ile, Leu, Met, or Val, X_{15} is Ala, Asn, His, or Ser. CDR_{L2} comprises the amino acid sequence X_{16} X_{17} X_{18} X_{19} X_{20} X_{21} X_{22}, wherein amino acid X_{16} is Ala, Asp, Arg, Gly, or Val, X_{17} is Ala, Thr, or Val, X_{18} is Asn, Ser, or Thr, X_{19} is Arg, Asn, Lys, or His, X_{20} is Leu or Arg, X_{21} is Ala, Asn, Glu, Val, or Pro, X_{22} is Asp, Ser, or Thr. CDR_{L3} comprises the amino acid sequence X_{23} X_{24} X_{25} X_{26} X_{27} X_{28} Pro X_{30} Thr, wherein amino acid X_{23} is Leu, Gly, or Gln, X_{24} is His or Gln, X_{25} is Phe, Ser, Trp, or Tyr, X_{26} is Asp, Ile, Ser, Trp, or Tyr, X_{27} is Gly, Glu, Asn, or Ser, X_{28} is Asp, Asn, Phe, Thr, or Tyr, X_{30} is Leu, Phe, Pro, or Tyr.

[0025] In another aspect, the invention provides an isolated binding protein that binds human HGF comprising (i) an immunoglobulin heavy chain variable region comprising the structure CDR_{H1}-CDR_{H2}-CDR_{H3} and (ii) an immunoglobulin light chain variable region comprising three complementarity determining regions (CDRs), wherein the immunoglobulin heavy chain variable region and the immunoglobulin light chain variable region together define a single binding site for binding human HGF. CDR_{H1} comprises the amino acid sequence X_1 Tyr X_3 X_4 X_5, wherein amino acid X_1 is Asp, Asn, Ser, or Thr, X_3 is Phe, Ser, Trp, or Tyr, X_4 is Ile, Leu, or Met, X_5 is Asn, His, or Ser. CDR_{H2} comprises the amino acid sequence X_6 Ile X_8 X_9 X_{10} X_{11} Gly X_{13} X_{14} X_{15} Tyr X_{17} X_{18} X_{19} X_{20} X_{21} X_{22}, wherein amino acid X_6 is Lys, Gln, Glu, Val, or Tyr, X_8 is Asn, Gly, Ser, Trp, or Tyr, X_9 is Ala, Pro or Ser, X_{10} is Gly or Thr, X_{11} is a peptide bond, Asp, Asn, Gly, or Ser, X_{13} is Asp, Asn, His, or Ser, X_{14} is Ser or Thr, X_{15} is Asn or Tyr, X_{17} is Asn or Pro, X_{18} is Ala, Asp, Gly, Gln, Glu, Pro, or Ser, X_{19} is Asn, Lys, Met, or Ser, X_{20} is Leu, Phe or Val, X_{21} is Lys, Met, or Gln, X_{22} is Asp, Gly or Ser. CDR_{H3} comprises the amino acid sequence X_{23} X_{24} X_{25} X_{26} X_{27} X_{28} X_{29} X_{30} X_{31} X_{32} X_{33} X_{34} Tyr, wherein amino acid X_{23} is Arg, Asn, Gln, or Glu, X_{24} is Gly, Leu, Arg, or Tyr, X_{25} is a peptide bond, Asp, or Gly, X_{26} is a peptide bond or Gly, X_{27} is a peptide bond or Tyr, X_{28} is a peptide bond, Leu, or Tyr, X_{29} is a peptide bond, Gly, Leu, Arg, or Val, X_{30} is a peptide bond, Asp, Gly, or Glu, X_{31} is a peptide bond, Asn, Arg, Ser, or Tyr, X_{32} is peptide bond, Ala, Gly, Ile, or Tyr, X_{33} is Met or Phe, X_{34} is Ala or Asp.

[0026] It is understood that the binding protein can comprise both the immunoglobulin light chain and the immunoglobulin heavy chain sequences or the fragments thereof, noted above. Furthermore, it is understood that the binding protein can be an intact antibody or an antigen binding fragment thereof, or a biosynthetic antibody site.

[0027] In certain embodiments, the CDR sequences of the immunoglobulin light chain and the immunoglobulin heavy chain are interposed with framework regions (FR).

[0028] In certain other embodiments, the CDR sequences of the immunoglobulin light chain and the immunoglobulin heavy chain are interposed between human or humanized framework regions.

[0029] In another aspect, the invention provides an isolated binding protein that specifically binds human HGF. The binding protein comprises: (a) an immunoglobulin light chain variable region comprising the structure CDR_{L1}-CDR_{L2}-CDR_{L3} and (b) immunoglobulin heavy chain

variable region, wherein the immunoglobulin light chain variable region and the immunoglobulin heavy chain variable region together define a single binding site for binding human HGF. The CDR_{L1} comprises a sequence selected from the group consisting of SEQ ID NO. 8 **(1A3)**, SEQ ID NO. 18 **(2B8)**, SEQ ID NO. 28 **(2F8)**, SEQ ID NO. 38 **(3B6)**, SEQ ID NO. 48 **(3D11)**, SEQ ID NO. 58 **(1D3)**, SEQ ID NO. 68 **(1F3)**, and SEQ ID NO. 78 **(3A12)**. The CDR_{L2} comprises a sequence selected from the group consisting of SEQ ID NO. 9 **(1A3)**, SEQ ID NO. 19 **(2B8)**, SEQ ID NO. 29 **(2F8)**, SEQ ID NO. 39 **(3B6)**, SEQ ID NO. 49 **(3D11)**, SEQ ID NO. 59 **(1D3)**, SEQ ID NO. 69 **(1F3)**, SEQ ID NO. 79 **(3A12)** and SEQ ID NO. 206 **(LRMR2B8LC)**. The CDR_{L3} comprises a sequence selected from the group consisting of SEQ ID NO. 10 **(1A3)**, SEQ ID NO. 20 **(2B8)**, SEQ ID NO. 30 **(2F8)**, SEQ ID NO. 40 **(3B6)**, SEQ ID NO. 50 **(3D11)**, SEQ ID NO. 60 **(1D3)**, SEQ ID NO. 70 **(1F3)**, and SEQ ID NO. 80 **(3A12)**. Throughout the specification and claims, the sequences denoted by a particular SEQ ID NO. are followed in parentheses by the antibody that was the origin of the particular sequence. By way of example, SEQ ID NO. 8 **(1A3)** indicates that the sequence of SEQ ID NO. 8 is based upon the sequence present in antibody 1A3.

[0030] In one embodiment, the binding protein comprises an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the sequence of SEQ ID NO. 8 **(1A3)**, a CDR_{L2} comprising the sequence of SEQ ID NO. 9 **(1A3)**, and a CDR_{L3} comprising the sequence of SEQ ID NO. 10 **(1A3)**.

[0031] In another embodiment, the binding protein comprises an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the sequence of SEQ ID NO. 18 **(2B8)**, a CDR_{L2} comprising the sequence of SEQ ID NO. 19 **(2B8)** or SEQ ID NO. 206 **(LRMR2B8LC)**, and a CDR_{L3} comprising the sequence of SEQ ID NO. 20 **(2B8)**.

[0032] In another embodiment, the binding protein comprises an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the sequence of SEQ ID NO. 28 **(2F8)**, a CDR_{L2} comprising the sequence of SEQ ID NO. 29 **(2F8)**, and a CDR_{L3} comprising the sequence of SEQ ID NO. 30 **(2F8)**.

[0033] In another embodiment, the binding protein comprises an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the sequence of SEQ ID NO. 38 **(3B6)**, a CDR_{L2} comprising the sequence of SEQ ID NO. 39 **(3B6)**, and a CDR_{L3} comprising the sequence of SEQ ID NO. 40 **(3B6)**.

[0034] In another embodiment, the binding protein comprises an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the sequence of SEQ ID NO. 48 **(3D11)**, a CDR_{L2} comprising the sequence of SEQ ID NO. 49 **(3D11)**, and a CDR_{L3} comprising the sequence of SEQ ID NO. 50 **(3D11)**.

[0035] In another embodiment, the binding protein comprises an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the sequence of SEQ ID NO. 58 **(1D3)**, a CDR_{L2} comprising the sequence of SEQ ID NO. 59 **(1D3)**, and a CDR_{L3} comprising the sequence of SEQ ID NO. 60 **(1D3)**.

[0036] In another embodiment, the binding protein comprises an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the sequence of SEQ ID NO. 68 **(1F3)**, a CDR_{L2} comprising the sequence of SEQ ID NO. 69 **(1F3)**, and a CDR_{L3} comprising the sequence of SEQ ID NO. 70 **(1F3)**.

[0037] In another embodiment, the binding protein comprises an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the sequence of SEQ ID NO. 78 **(3A12)**, a CDR_{L2} comprising the sequence of SEQ ID NO. 79 **(3A12)**, and a CDR_{L3} comprising the sequence of SEQ ID NO. 80 **(3A12)**.

[0038] In each of the foregoing embodiments, the CDR_{L1}, CDR_{L2}, and CDR_{L3} sequences preferably are interposed between human or humanized immunoglobulin FRs. It is understood that the binding protein can be an intact antibody, an antigen binding fragment thereof, or a biosynthetic antibody site.

[0039] In another aspect, the invention provides an isolated binding protein that binds human HGF. The binding protein comprises (a) an immunoglobulin heavy chain variable region comprising the structure CDR_{H1}-CDR_{H2}-CDR_{H3}, and (b) an immunoglobulin light chain variable region, wherein the immunoglobulin heavy chain variable region and the immunoglobulin light chain variable region together define a single binding site for binding human HGF. The CDR_{H1} comprises a sequence selected from the group consisting of SEQ ID NO. 5 **(1A3)**, SEQ ID NO. 15 **(2B8)**, SEQ ID NO. 25 **(2F8)**, SEQ ID NO. 35 **(3B6)**, SEQ ID NO. 45 **(3D11)**, SEQ ID NO. 55 **(1D3)**, SEQ ID NO. 65 **(1F3)**, and SEQ ID NO. 75 **(3A12)**; the CDR_{H2} comprises a sequence selected from the group consisting of SEQ ID NO. 6 **(1A3)**, SEQ ID NO. 16 **(2B8)**, SEQ ID NO. 26 **(2F8)**, SEQ ID NO. 36 **(3B6)**, SEQ ID NO. 46 **(3D11)**, SEQ ID NO. 56 **(1D3)**, SEQ ID NO. 66 **(1F3)**, SEQ ID NO. 76 **(3A12)**, SEQ ID NO. 202

(Hu2B8 Hv1f.1), SEQ ID NO. 203 **(Hu2B8 Hv5a.1 or Hu2B8 Hv5-51.1)**, SEQ ID NO. 204 **(LR2B8HC)** and SEQ ID NO. 205 **(LRMR2B8HC)**; and the CDR_{H3} comprises a sequence selected from the group consisting of SEQ ID NO. 7 **(1A3)**, SEQ ID NO. 17 **(2B8)**, SEQ ID NO. 27 **(2F8)**, SEQ ID NO. 37 **(3B6)**, SEQ ID NO. 47 **(3D11)**, SEQ ID NO. 57 **(1D3)**, SEQ ID NO. 67 **(1F3)**, and SEQ ID NO. 77 **(3A12)**.

[0040] In one embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising: a CDR_{H1} comprising the sequence of SEQ ID NO. 5 **(1A3)**; a CDR_{H2} comprising the sequence of SEQ ID NO. 6 **(1A3)**; and a CDR_{H3} comprising the sequence of SEQ ID NO. 7 **(1A3)**.

[0041] In another embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising: a CDR_{H1} comprising the sequence of SEQ ID NO. 15 **(2B8)**; a CDR_{H2} comprising the sequence of SEQ ID NO. 16 **(2B8)**, SEQ ID NO. 202 **(Hu2B8 Hv1f.1)**, SEQ ID NO. 203 **(Hu2B8 Hv5a.1 or Hu2B8 Hv5-51.1)**, SEQ ID NO. 204 **(LR2B8HC)** or SEQ ID NO. 205 **(LRMR2B8HC)**; and a CDR_{H3} comprising the sequence of SEQ ID NO. 17 **(2B8)**.

[0042] In another embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising: a CDR_{H1} comprising the sequence of SEQ ID NO. 25 **(2F8)**; a CDR_{H2} comprising the sequence of SEQ ID NO. 26 **(2F8)**; and a CDR_{H3} comprising the sequence of SEQ ID NO. 27 **(2F8)**.

[0043] In another embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising the sequence of SEQ ID NO. 35 **(3B6)**; a CDR_{H2} comprising the sequence of SEQ ID NO. 36 **(3B6)**; and a CDR_{H3} comprising the sequence of SEQ ID NO. 37 **(3B6)**.

[0044] In another embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising: a CDR_{H1} comprising the sequence of SEQ ID NO. 45 **(3D11)**; a CDR_{H2} comprising the sequence of SEQ ID NO. 46 **(3D11)**; and a CDR_{H3} comprising the sequence of SEQ ID NO. 47 **(3D11)**.

[0045] In another embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising: a CDR_{H1} comprising the sequence of SEQ ID NO. 55 **(1D3)**;

a CDR_{H2} comprising the sequence of SEQ ID NO. 56 **(1D3)**; and a CDR_{H3} comprising the sequence of SEQ ID NO. 57 **(1D3)**.

[0046] In another embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising: a CDR_{H1} comprising the sequence of SEQ ID NO. 65 **(1F3)**; a CDR_{H2} comprising the sequence of SEQ ID NO. 66 **(1F3)**; and a CDR_{H3} comprising the sequence of SEQ ID NO. 67 **(1F3)**.

[0047] In another embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising: a CDR_{H1} comprising the sequence of SEQ ID NO. 75 **(3A12)**; a CDR_{H2} comprising the sequence of SEQ ID NO. 76 **(3A12)**; and a CDR_{H3} comprising the sequence of SEQ ID NO. 77 **(3A12)**.

[0048] In each of the foregoing embodiments, the CDR_{H1}, CDR_{H2}, and CDR_{H3} sequences preferably are interposed between human or humanized immunoglobulin FRs. It is understood that the binding protein can be an intact antibody, an antigen binding fragment thereof, or a biosynthetic antibody site.

[0049] In another aspect, the invention provides a binding protein that binds human HGF. The binding protein comprises an immunoglobulin heavy chain variable region selected from the group consisting of residues 20-141 of SEQ ID NO. 2 **(1A3)**, residues 20-137 of SEQ ID NO. 12 **(2B8)**, residues 20-137 of SEQ ID NO. 22 **(2F8)**, residues 20-139 of SEQ ID NO. 32 **(3B6)**, residues 20-132 of SEQ ID NO. 42 **(3D11)**, residues 20-141 of SEQ ID NO. 52 **(1D3)**, residues 20-141 of SEQ ID NO. 62 **(1F3)**, and residues 20-141 of SEQ ID NO. 72 **(3A12)** and an immunoglobulin light chain variable region selected from the group consisting of residues 21-127 of SEQ ID NO. 4 **(1A3)**, residues 21-127 of SEQ ID NO. 14 **(2B8)**, residues 20-131 of SEQ ID NO. 24 **(2F8)**, residues 23-129 of SEQ ID NO. 34 **(3B6)**, residues 23-128 of SEQ ID NO. 44 **(3D11)**, residues 21-127 of SEQ ID NO. 54 **(1D3)**, residues 21-127 of SEQ ID NO. 64 **(1F3)**, and residues 21-127 of SEQ ID NO. 74 **(3A12)**.

[0050] In another embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of residues 20-141 of SEQ ID NO. 2 **(1A3)**, and an immunoglobulin light chain variable region comprising the amino acid sequence of residues 21-127 of SEQ ID NO. 4 **(1A3)**.

[0051] In one embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of residues 20-137 of SEQ ID NO. 12 **(2B8)**, and an immunoglobulin light chain variable region comprising the amino acid sequence of residues 21-127 of SEQ ID NO. 14 **(2B8)**.

[0052] In another embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of residues 20-137 of SEQ ID NO. 22 **(2F8)**, and an immunoglobulin light chain variable region comprising the amino acid sequence of residues 20-131 of SEQ ID NO. 24 **(2F8)**.

[0053] In another embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of residues 20-139 of SEQ ID NO. 32 **(3B6)**, and an immunoglobulin light chain variable region comprising the amino acid sequence of residues 23-129 of SEQ ID NO. 34 **(3B6)**.

[0054] In another embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of residues 20-132 of SEQ ID NO. 42 **(3D11)**, and an immunoglobulin light chain variable region comprising the amino acid sequence of residues 23-128 of SEQ ID NO. 44 **(3D11)**.

[0055] In another embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of residues 20-141 of SEQ ID NO. 52 **(1D3)**, and an immunoglobulin light chain variable region comprising the amino acid sequence of residues 21-127 of SEQ ID NO. 54 **(1D3)**.

[0056] In another embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of residues 20-141 of SEQ ID NO. 62 **(1F3)**, and an immunoglobulin light chain variable region comprising the amino acid sequence of residues 21-127 of SEQ ID NO. 64 **(1F3)**.

[0057] In another embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of residues 20-141 of SEQ ID NO. 72 **(3A12)**, and an immunoglobulin light chain variable region comprising the amino acid sequence of residues 21-127 of SEQ ID NO. 74 **(3A12)**.

[0058] In each of the foregoing embodiments, the binding protein can be an intact antibody, an antigen binding fragment thereof, or a biosynthetic antibody site.

[0059] In another aspect, the invention provides an isolated binding protein that binds human HGF. The binding protein comprises (i) an immunoglobulin light chain variable region selected from the group consisting of SEQ ID NO. 173 **(Hu2B8 Kv1-39.1 light chain variable region)**, SEQ ID NO. 179 **(Hu2B8 Kv3-15.1 light chain variable region)**, SEQ ID NO. 193 **(LR2B8LC light chain variable region)**, and SEQ ID NO. 199 **(LRMR2B8LC light chain variable region)**; and (ii) an immunoglobulin heavy chain variable region selected from the group consisting of SEQ ID NO. 159 **(Hu2B8 Hv1f.1 heavy chain variable region)**, SEQ ID NO. 165 **(Hu2B8 Hv5a.1 heavy chain variable region)**, SEQ ID NO. 169 **(Hu2B8 Hv5-51.1 heavy chain variable region)**, SEQ ID NO. 183 **(LR2B8HC heavy chain variable region)**, and SEQ ID NO. 189 **(LRMR2B8LC light chain variable region)**. The binding protein can be an intact antibody, an antigen binding fragment thereof, or a biosynthetic antibody site.

[0060] In another aspect, the invention provides an isolated binding protein that binds human HGF. The binding protein comprises (i) an immunoglobulin light chain selected from the group consisting of SEQ ID NO. 177 **(Hu2B8 Kv1-39.1 + kappa constant (Km(3) allotype (allele 2))**, SEQ ID NO. 181 **(Hu2B8 Kv3-15.1 + Kappa constant (Km(3) allotype (allele 2))**, SEQ ID NO. 197 **(LR2B8LC + Kappa constant (Km(3) allotype (allele 1))**, and SEQ ID NO. 201 **(LRMR2B8LC + Kappa constant (Km(3) allotype (allele 1))**; and (ii) an immunoglobulin heavy chain selected from the group consisting of SEQ ID NO. 163 **(Hu2B8 Hv1f.1 + IgG1 Constant (G1m(17,1) allotype))**, SEQ ID NO. 167 **(Hu2B8 Hv5a.1 + IgG1 Constant (G1m(17,1) allotype))**, SEQ ID NO. 171 **(Hu2B8 Hv5-51.1 + IgG1 Constant (G1m(17,1) allotype))**, SEQ ID NO. 187 **(LR2B8HC + IgG1 Constant (G1m(3) allotype) (allele 1))**, and SEQ ID NO. 191 **(LRMR2B8HC +IgG1 Constant (G1m(3) allotype) (allele 1))**. The binding protein can be an intact antibody, an antigen binding fragment thereof, or a biosynthetic antibody site.

[0061] In another aspect, the invention provides an isolated binding protein that binds reduced human HGF. The binding protein comprises (i) an immunoglobulin light chain variable region comprising three CDRs, and (ii) an immunoglobulin heavy chain variable region comprising three CDRs. The CDRs typically are interposed between FRs. The CDRs of the immunoglobulin light chain and the immunoglobulin heavy chain together define a binding site that binds reduced human HGF, for example, the α-chain of reduced HGF. Reduced HGF refers to HGF treated with an amount of reducing agent, for example,

dithiothreitol (DTT), 2-mercaptoethanol, or glutathione sufficient to reduce the disulfide linkage between the α-chain and the β-chain. Exemplary concentrations include, for example, 100 mM DTT and 5% 2-mercaptoethanol.

[0062] In certain embodiments, the binding protein comprises an immunoglobulin light chain variable region comprising at least one CDR selected from the group consisting of CDR_{L1}, CDR_{L2} and CDR_{L3}. Optionally, the binding protein comprises two CDRs, for example, CDR_{L1} and CDR_{L2}, or CDR_{L1} and CDR_{L3}, or CDR_{L1} and CDR_{L3}. Optionally, the binding protein comprises all three CDRs, i.e., CDR_{L1}, CDR_{L2} and CDR_{L3}. CDR_{L1} comprises the amino acid sequence X_1 X_2 Ser X_4 X_5 X_6 X_7 X_8 X_9 X_{10} X_{11} X_{12} X_{13} X_{14} X_{15}, wherein amino acid X_1 is Arg or Lys, X_2 is Ala or Thr, X_4 is Glu or Gln, X_5 is Asn, Ser, or Asp, X_6 is Ile or Val, X_7 is Tyr, Asp, or Lys, X_8 is a peptide bond or Tyr, X_9 is a peptide bond or Asp, X_{10} is a peptide bond or Gly, X_{11} is a peptide bond or Asn, X_{12} is a peptide bond or Ser, X_{13} is Asn or Tyr, X_{14} is Ile or Leu, X_{15} is Ala, Asn, or Ser. CDR_{L2} comprises the amino acid sequence X_{16} X_{17} X_{18} X_{19} Leu X_{21} X_{22}, wherein amino acid X_{16} is Ala, Asp, Val, or Arg, X_{17} is Ala or Val, X_{18} is Asn, Ser, or Thr, X_{19} is Arg, Asn, or His, X_{21} is Ala, Glu, Val, or Pro, X_{22} is Asp or Ser. CDR_{L3} comprises the amino acid sequence X_{23} X_{24} X_{25} X_{26} X_{27} X_{28} Pro X_{30} Thr, wherein amino acid X_{23} is Leu or Gln, X_{24} is His or Gln, X_{25} is Phe, Ser, or Tyr, X_{26} is Asp, Ile, or Trp, X_{27} is Gly or Glu, X_{28} is Asp, Phe, or Thr, X_{30} is Phe, Pro, or Tyr.

[0063] In another embodiment, the binding protein comprises an immunoglobulin heavy chain variable region comprising at least one CDR selected from the group consisting of CDR_{H1}, CDR_{H2}, and CDR_{H3}. Optionally, the binding protein comprises two CDRs, for example, CDR_{H1} and CDR_{H2}, or CDR_{H1} and CDR_{H3}, or CDR_{H1} and CDR_{H3}. Optionally, the binding protein comprises all three CDRs, i.e., CDR_{H1}, CDR_{H2} and CDR_{H3}. CDR_{H1} comprises the amino acid sequence X_1 Tyr X_3 X_4 X_5, wherein amino acid X_1 is Asp, Asn, Ser, or Thr, X_3 is Phe, Trp, or Tyr, X_4 is Ile or Met, X_5 is Asn, His, or Ser. CDR_{H2} comprises the amino acid sequence X_6 Ile X_8 X_9 Gly X_{11} Gly X_{13} X_{14} X_{15} Tyr X_{17} X_{18} X_{19} X_{20} Lys X_{22}, wherein amino acid X_6 is Lys, Gln, or Tyr, X_8 is Gly, Ser, or Tyr, X_9 is Pro or Ser, X_{11} is Asp, Gly, or Ser, X_{13} is Asp or Ser, X_{14} is Ser or Thr, X_{15} is Asn or Tyr, X_{17} is Asn or Pro, X_{18} is Ala, Asp, Gly, or Glu, X_{19} is Asn, Met, or Ser, X_{20} is Phe or Val, X_{22} is Asp or Gly. CDR_{H3} comprises the amino acid sequence X_{23} X_{24} X_{25} X_{26} X_{27} X_{28} X_{29} X_{30} X_{31} X_{32} X_{33} Asp Tyr, wherein amino acid X_{23} is Arg or Gln, X_{24} is Gly or Leu, X_{25} is Asp, Gly, or a peptide bond, X_{26} is Gly or a peptide bond,

X_{27} is a peptide bond or Tyr, X_{28} is Leu, a peptide bond or Tyr, X_{29} is a Gly, Arg or Leu, X_{30} is Asp, Gly or Glu, X_{31} is a Tyr, Arg or Asn, X_{32} is Ala, Gly or Tyr, X_{33} is Met or Phe.

[0064] It is understood that the binding protein can comprise both the immunoglobulin heavy chain and the immunoglobulin light chain sequences or the fragments thereof, noted above. Furthermore, it is understood that the binding protein can be an intact antibody or an antigen binding fragment thereof, or a biosynthetic antibody site.

[0065] In certain embodiments, the binding protein comprises an immunoglobulin light chain variable region comprising (i) a CDR_{L1} having a sequence selected from the group consisting of SEQ ID NO. 8 **(1A3)**, SEQ ID NO. 28 **(2F8)**, SEQ ID NO. 38 **(3B6)**, SEQ ID NO. 58 **(1D3)**, and SEQ ID NO. 68 **(1F3)**, (ii) a CDR_{L2} having a sequence selected from the group consisting of SEQ ID NO. 9 **(1A3)**, SEQ ID NO. 29 **(2F8)**, SEQ ID NO. 39 **(3B6)**, SEQ ID NO. 59 **(1D3)**, and SEQ ID NO. 69 **(1F3)**, and (iii) a CDR_{L3} having a sequence selected from the group consisting of SEQ ID NO. 10 **(1A3)**, SEQ ID NO. 30 **(2F8)**, SEQ ID NO. 40 **(3B6)**, SEQ ID NO. 60 **(1D3)**, and SEQ ID NO. 70 **(1F3)**. The CDR sequences can be interposed between human or humanized FRs. In other embodiments, the binding protein comprises an immunoglobulin light chain variable region comprising an amino acid sequence selected from the group consisting of residues 21-127 of SEQ ID NO. 4 **(1A3)**, residues 20-131 of SEQ ID NO. 24 **(2F8)**, residues 23-129 of SEQ ID NO. 34 **(3B6)**, residues 21-127 of SEQ ID NO. 54 **(1D3)**, and residues 21-127 of SEQ ID NO. 64 **(1F3)**.

[0066] In certain other embodiments, the binding protein comprises an immunoglobulin heavy chain variable region comprising (i) a CDR_{H1} having a sequence selected from the group consisting of SEQ ID NO. 5 **(1A3)**, SEQ ID NO. 25 **(2F8)**, SEQ ID NO. 35 **(3B6)**, SEQ ID NO. 55 **(1D3)**, and SEQ ID NO. 65 **(1F3)**, (ii) a CDR_{H2} having a sequence selected from the group consisting of SEQ ID NO. 6 **(1A3)**, SEQ ID NO. 26 **(2F8)**, SEQ ID NO. 36 **(3B6)**, SEQ ID NO. 56 **(1D3)**, and SEQ ID NO. 66 **(1F3)**, and (iii) a CDR_{H3} having a sequence selected from the group consisting of SEQ ID NO. 7 **(1A3)**, SEQ ID NO. 27 **(2F8)**, SEQ ID NO. 37 **(3B6)**, SEQ ID NO. 57 **(1D3)**, and SEQ ID NO. 67 **(1F3)**. The CDR sequences can be interposed between human or humanized FRs. In another embodiment, the immunoglobulin heavy chain variable region comprises an amino acid sequence selected from the group consisting of residues 20-141 of SEQ ID NO. 2 **(1A3)**, residues 20-137 of SEQ ID NO. 22

(2F8), residues 20-139 of SEQ ID NO. 32 **(3B6)**, residues 20-141 of SEQ ID NO. 52 **(1D3)**, and residues 20-141 of SEQ ID NO. 62 **(1F3)**.

[0067] In another aspect, the invention provides an isolated binding protein that binds human HGF and comprises an immunoglobulin light chain variable region and an immunoglobulin heavy chain variable region. The isolated binding protein competes for binding to HGF with at least one reference antibody selected from the group consisting of (i) an antibody having an immunoglobulin light chain variable region of residues 20-131 of SEQ ID NO. 24 **(2F8)**, and an immunoglobulin heavy chain variable region of residues 20-137 of SEQ ID NO. 22 **(2F8)**, (ii) an antibody having an immunoglobulin light chain variable region of residues 23-129 of SEQ ID NO. 34 **(3B6)**, and an immunoglobulin heavy chain variable region of residues 20-139 of SEQ ID NO. 32 **(3B6)**, and (iii) an antibody having an immunoglobulin light chain variable region of residues 23-128 of SEQ ID NO. 44 **(3D11)**, and an immunoglobulin heavy chain variable region of residues 20-132 of SEQ ID NO. 42 **(3D11)**. Under certain circumstances, the binding protein binds the same epitope of HGF as one of the reference antibodies.

[0068] It is understood that each of the binding proteins discussed above can be an intact antibody, for example, a monoclonal antibody. Alternatively, the binding protein can be an antigen binding fragment of an antibody, or can be a biosynthetic antibody binding site. Antibody fragments include Fab, Fab', $(Fab')_2$ or Fv fragments. Techniques for making such antibody fragments are known to those skilled in the art. A number of biosynthetic antibody binding sites are known in the art and include, for example, single Fv or sFv molecules, described, for example, in U.S. Patent Nos. 5,476,786. Other biosynthetic antibody binding sites include bispecific or bifunctional binding proteins, for example, bispecific or bifunctional antibodies, which are antibodies or antibody fragments that bind at least two different antigens. For example, bispecific binding proteins can bind HGF, for example, human HGF, and another antigen of interest. Methods for making bispecific antibodies are known in art and, include, for example, by fusing hybridomas or by linking Fab' fragments. See, e.g., Songsivilai *et al.* (1990) CLIN. EXP. IMMUNOL. 79: 315-325; Kostelny *et al.* (1992) J. IMMUNOL. 148: 1547-1553.

[0069] The binding proteins of the invention can bind hHGF containing a cysteine to arginine substitution at position 561 or a glycine to glutamate substitution at position 555.

[0070] In another aspect, the invention provides an isolated binding protein that binds human HGF with a k_d of $4.0x10^{-5}$ s^{-1} or lower, $3.0x10^{-5}$ s^{-1} or lower, or $2.0x10^{-5}$ s^{-1} or lower. The isolated binding proteins can bind human HGF with a k_d from $5.0x10^{-5}$ s^{-1} to $0.5x10^{-5}$ s^{-1}, or from $4.0x10^{-5}$ s^{-1} to $1.0x10^{-5}$ s^{-1}, or from $3.0x10^{-5}$ s^{-1} to $1.5x10^{-5}$ s^{-1}. In another aspect, the invention provides an isolated binding protein that binds human HGF with a K_D of 100 pM or lower, or 20 pM or lower, or 10 pM or lower, or 5 pM or lower. The isolated binding proteins can bind human HGF with a K_D from 100 pM to 5 pM, or from 20 pM to 5 pM, or from 15 pM to 10 pM, or from 20 pM to 10 pM, or from 15 pM to 5 pM. Unless otherwise specified, K_D values are determined by the methods, and under the conditions, described in Example 6.

[0071] In another aspect, the invention provides an isolated binding protein that binds human HGF, wherein the antibody binds to human HGF with lower K_D at 37°C than at 25°C. The binding protein binding optionally binds human HGF with a K_D less than 5 pM at 37°C.

[0072] In other aspects and embodiments, the binding proteins can inhibit hHGF from binding to c-Met. For example, the binding proteins can have an IC_{50} (concentration at 50% of maximum inhibition) of at least about 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, and 7.0 nM when assayed using the protocol described in Example 7(a). In certain other embodiments, the binding proteins can neutralize HGF BrdU incorporation in 4 MBr-5 cells (ATCC, Catalog No. CCL208) using the method described in Example 7(b).

[0073] The binding proteins have an IC_{50} of 50 nM or lower, preferably 45, 40, 35, 30, 25, 20, 15, 10, 5, 1, 0.5 nM or lower, when assayed using the protocol described in Example 7(b). In certain other embodiments, the binding proteins can be used to inhibit HGF stimulated c-Met phosphorylation in PC-3 cells (ATCC, Manassus, VA Catalog No. CRL-1435) using the assay described in Example 9. The binding proteins inhibit HGF-stimulated (1.25 nM) c-Met phosphorylation in PC-3 cells with an IC_{50} of 2 nM or less (Table 8), using the assay described in Example 9.

II – Production of Binding Proteins

[0074] Binding proteins of the invention can be produced in various ways using approaches know in the art. For example, DNA molecules encoding light chain variable regions and heavy chain variable regions can be chemically synthesized, using a commercial synthesizer and sequence information provided herein. Such synthetic DNA molecules can be ligated to other appropriate nucleotide sequences, including, e.g., constant region coding sequences, and

expression control sequences, to produce conventional gene expression constructs encoding the desired binding proteins. Production of defined gene constructs is within routine skill in the art. Alternatively, the sequences provided herein can be cloned out of hybridomas by conventional hybridization techniques or PCR techniques, using synthetic nucleic acid probes whose sequences are based on sequence information provided herein or prior art sequence information regarding genes encoding the heavy and light chains of murine antibodies in hybridoma cells. Production and use of such probes is within ordinary skill in the art.

[0075] The nucleic acids encoding the desired binding proteins can be introduced (ligated) into expression vectors, which can be introduced into a host cell via standard transfection or transformation techniques known in the art. Exemplary host cells include, for example, *E. coli* cells, Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), and myeloma cells that do not otherwise produce immunoglobulin protein. Transfected host cells can be grown under conditions that permit the host cells to express the genes of interest, for example, the genes that encode the immunoglobulin light or heavy chain variable regions. The resulting expression products can be harvested using techniques known in the art.

[0076] The particular expression and purification conditions will vary depending upon what expression system is employed. For example, if the gene is to be expressed in *E. coli*, it is first cloned into an expression vector. This is accomplished by positioning the engineered gene downstream from a suitable bacterial promoter, e.g., Trp or Tac, and a signal sequence, e.g., a sequence encoding fragment B of protein A (FB). The resulting expressed fusion protein typically accumulates in refractile or inclusion bodies in the cytoplasm of the cells, and may be harvested after disruption of the cells by French press or sonication. The refractile bodies then are solubilized, and the expressed proteins refolded and cleaved by the methods already established for many other recombinant proteins.

[0077] If the engineered gene is to be expressed in eukayotic host cells, for example, myeloma cells or CHO cells, it is first inserted into an expression vector containing a suitable eukaryotic promoter, a secretion signal, immunoglobulin enhancers, and various introns. This expression vector optionally can contain sequences encoding all or part of a constant region, enabling an entire, or a part of, a heavy or light chain to be expressed. The gene construct can be transfected into myeloma cells or CHO cells using established transfection protocols. Such

transfected cells can express V_L or V_H fragments, V_L-V_H heterodimers, V_H-V_L or V_L-V_H single chain polypeptides, complete heavy or light immunoglobulin chains, or portions thereof, each of which may be attached to a protein domain having another function (e.g., cytotoxicity).

III – Modifications to the Binding Proteins

[0078] It is understood that the binding proteins can be modified to optimize performance depending upon the intended use of the binding proteins. For example, when the binding protein is being used as a therapeutic agent, the binding protein can be modified to reduce its immunogenicity in the intended recipient. Alternatively or in addition, the binding protein can be fused or coupled to another protein or peptide, for example, a growth factor, cytokine, or cytotoxin. Such modifications can be achieved by using routine gene manipulation techniques known in the art.

[0079] Various techniques for reducing the antigenicity of antibodies and antibody fragments are known in the art. These techniques can be used to reduce or eliminate the antigenicity of the binding proteins of the invention. For example, when the binding proteins are to be administered to a human, the binding proteins preferably are engineered to reduce their antigenicity in humans. This process often is referred to as humanization. Preferably, the humanized binding proteins have the same or substantially the same affinity for the antigen as the original non-humanized binding protein it was derived from.

[0080] In one well known humanization approach, chimeric proteins are created in which immunoglobulin constant regions of antibodies from one species, e.g., mouse, are replaced with immunoglobulin constant regions from a second, different species, e.g., a human. In this example, the resulting antibody is a mouse-human chimera, where the human constant region sequences, in principle, are less immunogenic than the counterpart murine sequences. This type of antibody engineering is described, for example, Morrison, *et al.* (1984) PROC. NAT. ACAD. SCI. 81: 6851-6855, Neuberger *et al.* (1984) NATURE 312: 604-608; U.S. Patent Nos. 6,893,625 (Robinson); 5,500,362 (Robinson); and 4,816,567 (Cabilly).

[0081] In another approach, known as CDR grafting, the CDRs of the light and heavy chain variable regions of an antibody of interest are grafted into frameworks (FRs) from another species. For example, murine CDRs can be grafted into human FR sequences. In some embodiments, the CDRs of the light and heavy chain variable regions of an anti-HGF antibody are grafted into human FRs or consensus human FRs. In order to create consensus human FRs,

FRs from several human heavy chain or light chain amino acid sequences are aligned to identify a consensus amino acid sequence. CDR grafting is described, for example, in U.S. Patent Nos. 7,022,500 (Queen); 6,982,321 (Winter); 6,180,370 (Queen); 6,054,297 (Carter); 5,693,762 (Queen); 5,859,205 (Adair); 5,693,761 (Queen); 5,565,332 (Hoogenboom); 5,585,089 (Queen); 5,530,101 (Queen); Jones *et al.* (1986) NATURE 321: 522-525; Riechmann *et al.* (1988) NATURE 332: 323-327; Verhoeyen *et al.* (1988) SCIENCE 239: 1534-1536; and Winter (1998) FEBS LETT 430: 92-94.

[0082] In an approach called "superhumanization," antibodies in which human immunogenicity is reduced or eliminated are created by an alternative form of grafting. In superhumanization, human FR sequences are chosen from a set of human germline genes based on the structural similarity of the human CDRs to those of the mouse antibody to be humanized. This approach is described, for example, in U.S. Patent No. 6,881,557 (Foote) and in Tan *et al.* (2002) J. IMMUNOL 169:1119-1125.

[0083] Other approaches to reduce immunogenicity include, techniques are known as "reshaping," "hyperchimerization," or "veneering/resurfacing" to produce humanized antibodies. See, e.g., Vaswami *et al.* (1998) ANNALS OF ALLERGY, ASTHMA, & IMMUNOL. 81: 105; Roguska *et al.* (1996) PROT. ENGINEER 9: 895-904; and U.S. Patent No. 6,072,035 (Hardman). In the veneering/resurfacing approach, the surface accessible amino acid residues in the murine antibody are replaced by amino acid residues more frequently found at the same positions in a human antibody. This type of antibody resurfacing is described, for example, in U.S. Patent No. 5,639,641 (Pedersen).

[0084] One exemplary approach for converting a mouse antibody into a form suitable for medical use in humans is known as ACTIVMAB™ technology (Vaccinex, Inc., Rochester, NY), which involves a vaccinia virus-based vector to express antibodies in mammalian cells. High levels of combinatorial diversity of immunoglobulin heavy and light chains are said to be produced. See, e.g., U.S. Patent Nos. 6,706,477 (Zauderer); 6,800,442 (Zauderer); and 6,872,518 (Zauderer).

[0085] Another exemplary approach for converting a mouse antibody into a form suitable for use in humans is technology practiced commercially by KaloBios Pharmaceuticals, Inc. (Palo Alto, CA). This technology involves the use of a proprietary human "acceptor" library to produce an "epitope focused" library for antibody selection.

[0086] Another exemplary approach for modifying a mouse antibody into a form suitable for medical use in humans is HUMAN ENGINEERING™ (HE™) technology, which is practiced commercially by XOMA (US) LLC. See, e.g., International Application Publication No. WO 93/11794 and U.S. Patent Nos. 5,766,886; 5,770,196; 5,821,123; and 5,869,619.

[0087] Any suitable approach, including any of the above approaches, can be used to reduce or eliminate human immunogenicity of a binding protein of interest.

[0088] In addition, it is possible to create fully human antibodies in mice. In this approach, human antibodies are prepared using a transgenic mouse in which the mouse's antibody-producing genes have been replaced by a substantial portion of the human antibody producing genes. Such mice produce human immunoglobulin instead of murine immunoglobulin molecules. See, e.g., WO 98/24893 (Jacobovitz et al.) and Mendez *et al.* (1997) NATURE GENETICS 15: 146-156. Fully human anti-HGF monoclonal antibodies can be produced using the following approach. Transgenic mice containing human immunoglobulin genes are immunized with the antigen of interest, e.g., HGF. Lymphatic cells from the mice then are obtained from the mice, which are then fused with a myeloid-type cell line to prepare immortal hybridoma cell lines. The hybridoma cell lines are screened and selected to identify hybridoma cell lines that produce antibodies specific to HGF.

[0089] Binding proteins of the invention can be conjugated with other molecules, depending upon their intended use. For example, if the binding protein is going to be used as a therapeutic, then the binding protein can be conjugated with another agent, for example, an effector molecule that modulates or otherwise promotes the therapy. To the extent that the effector is non-protein based agent, for example, a small molecule drug, a radiolabel or toxin, then, the agent can be chemically coupled to the binding protein using standard *in vitro* coupling chemistries. If, on the other hand, the effector molecule is a protein or peptide, for example, an enzyme, receptor, toxin, growth factor, cytokine or other immunomodulator, then the binding protein can either be chemically coupled to the effector using *in vitro* coupling chemistries or can be coupled to the effector as a fusion protein. Fusion proteins can be constructed and expressed using the techniques similar to those discussed in section II.

IV – Use of Binding Proteins

[0090] The binding proteins described herein can be used as a diagnostic agent or a therapeutic agent.

(1) Therapeutic Applications

[0091] Because the binding proteins of the invention neutralize the activity of HGF, they can be used in various therapeutic applications. For example, certain binding proteins of the invention are useful in the prevention or treatment of hyperproliferative diseases or disorders, e.g., various forms of cancer.

[0092] The binding proteins can be used to inhibit or reduce the proliferation of tumor cells. In such an approach, the tumor cells are exposed to a therapeutically effective amount of the binding protein so as to inhibit or reduce proliferation of the tumor cell. In certain embodiments, the binding proteins inhibit tumor cell proliferation by at least 50%, 60%, 70%, 80%, 90%, 95% or 100%.

[0093] In certain embodiments, the binding protein is used to inhibit or reduce proliferation of a tumor cell wherein the binding protein reduces the ability of hHGF to bind to c-Met. In other embodiments, the binding protein is used to inhibit or reduce the proliferation of a tumor cell even when the binding protein binds hHGF but does not substantially inhibit hHGF binding to c-Met, as shown by antibody 3B6 in Tables 5 and 6.

[0094] In addition, the binding protein can be used to inhibit, or slow down tumor growth or development in a mammal. In such a method, an effective amount of the binding protein is administered to the mammal so as to inhibit or slow down tumor growth in the mammal. Accordingly, the binding proteins can be used to treat tumors, for example, in a mammal. The method comprises administering to the mammal a therapeutically effective amount of the binding protein. The binding protein can be administered alone or in combination with another pharmaceutically active molecule, so as to treat the tumor.

[0095] It is contemplated that the binding proteins of the invention can be used in the treatment of a variety of HGF responsive disorders, including, for example, HGF responsive tumor cells in lung cancer, breast cancer, colon cancer, prostate cancer, ovarian cancer, head and neck cancer, ovarian cancer, multiple myeloma, liver cancer, gastric cancer, esophageal cancer, kidney cancer, nasopharangeal cancer, pancreatic cancer, mesothelioma, melanoma and glioblastoma.

[0096] As used herein, "treat, "treating" and "treatment" refer to the treatment of a disease-state in a mammal, particularly in a human, and include: (a) preventing the disease-state from

occurring in a mammal, in particular, when such mammal is predisposed to the disease-state but has not yet been diagnosed as having it; (b) inhibiting the disease-state, i.e., arresting its development; and/or (c) relieving the disease-state, i.e., causing regression of the disease state.

[0097] Generally, a therapeutically effective amount of active component will be in the range of from about 0.1 mg/kg to about 100 mg/kg, optionally from about 1 mg/kg to about 100 mg/kg, optionally from about 1 mg/kg to 10 mg/kg. The amount administered will depend on variables such as the type and extent of disease or indication to be treated, the overall health status of the particular patient, the relative biological efficacy of the binding protein delivered, the formulation of the binding protein, the presence and types of excipients in the formulation, and the route of administration. The initial dosage administered may be increased beyond the upper level in order to rapidly achieve the desired blood-level or tissue level, or the initial dosage may be smaller than the optimum and the daily dosage may be progressively increased during the course of treatment depending on the particular situation. Human dosage can be optimized, e.g., in a conventional Phase I dose escalation study designed to run from 0.5 mg/kg to 20 mg/kg. Dosing frequency can vary, depending on factors such as route of administration, dosage amount and the disease condition being treated. Exemplary dosing frequencies are once per day, once per week and once every two weeks. A preferred route of administration is parenteral, e.g., intravenous infusion. Formulation of monoclonal antibody-based drugs is within ordinary skill in the art. In some embodiments of the invention, the binding protein, e.g., monoclonal antibody, is lyophilized and reconstituted in buffered saline at the time of administration.

[0098] The binding proteins may be administered either alone or in combination with other pharmaceutically active ingredients. The other active ingredients, e.g., immunomodulators, can be administered together with the binding protein, or can be administered before or after the binding protein.

[0099] Formulations containing the binding proteins for therapeutic use, typically include the binding proteins combined with a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" means buffers, carriers, and excipients, that are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. The carrier(s) should be

"acceptable" in the sense of being compatible with the other ingredients of the formulations and not deleterious to the recipient. Pharmaceutically acceptable carriers, in this regard, are intended to include any and all buffers, solvents, dispersion media, coatings, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is known in the art.

[0100] The formulations can be conveniently presented in a dosage unit form and can be prepared by any suitable method, including any of the methods well known in the pharmacy art. A pharmaceutical composition of the invention should be formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral administration or non-parenteral administration, for example, intravenous, intradermal, inhalation, transdermal (topical), transmucosal, and rectal administration. Useful solutions for oral or parenteral administration can be prepared by any of the methods well known in the pharmaceutical art, described, for example, in *Remington's Pharmaceutical Sciences*, 18th ed. (Mack Publishing Company, 1990).

[0101] Formulations suitable for oral administration can be in the form of: discrete units such as injectables, capsules, gelatin capsules, sachets, tablets, troches, or lozenges, each containing a predetermined amount of the binding protein; a powder or granular composition; a solution or a suspension in an aqueous liquid or non-aqueous liquid; or an oil-in-water emulsion or a water-in-oil emulsion.

[0102] Formulations suitable for parenteral administration include, for example, the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[0103] In general, compositions suitable for injectable use include aqueous solutions (where water soluble) or dispersions and powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include

physiological saline, bacteriostatic water, Cremophor ELTM (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). It should be stable under the conditions of manufacture and storage and should be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol), and suitable mixtures thereof.

[0104] Pharmaceutical formulations preferably are sterile. Sterilization can be accomplished, for example, by filtration through sterile filtration membranes. Where the composition is lyophilized, sterilization using this method can be conducted prior to or following lyophilization and reconstitution. Once the pharmaceutical composition has been formulated, it can be stored, for example, in vials as a solution, suspension, gel, emulsion, solid, or as a dehydrated or lyophilized powder.

(2) Diagnostic Applications

[0105] Whenever the binding proteins are used for diagnostic purposes, either *in vitro* or *in vivo*, the binding proteins typically are labeled either directly or indirectly with a detectable moiety. The detectable moiety can be any moiety which is capable of producing, either directly or indirectly, a detectable signal. For example, the detectable moiety may be a radioisotope, such as ^{3}Hydrogen (^{3}H), ^{14}Carbon (^{14}C), ^{32}Phosphorus (^{32}P), ^{35}Sulfur (^{35}S), or ^{125}Iodine (^{125}I); a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin; an enzyme, such as alkaline phosphatase, beta-galactosidase, or horseradish peroxidase; a spin probe, such as a spin label; or a colored particle, for example, a latex or gold particle. It is understood that the binding protein can be conjugated to the detectable moiety using a number of approaches known in the art, for example, as described in Hunter *et al.* (1962) NATURE 144: 945; David *et al.* (1974) BIOCHEMISTRY 13: 1014; Pain *et al.* (1981) J. IMMUNOL. METH. 40: 219; and Nygren (1982) J. HISTOCHEM. AND CYTOCHEM. 30: 407. The labels may be detected, e.g., visually or with the aid of a spectrophotometer or other detector.

[0106] The binding proteins can be employed in a wide range of immunoassay techniques available in the art. Exemplary immunoassays include, for example, sandwich immunoassays, competitive immunoassays, immunohistochemical procedures.

[0107] In a sandwich immunoassay, two antibodies that bind an analyte or antigen of interest are used, *e.g.*, one immobilized onto a solid support, and one free in solution and

labeled with a detectable moiety. When a sample containing the antigen is introduced into this system, the antigen binds to both the immobilized antibody and the labeled antibody, to form a "sandwich" immune complex on the surface of the support. The complexed protein is detected by washing away non-bound sample components and excess labeled antibody, and measuring the amount of labeled antibody complexed to protein on the support's surface. Alternatively, the antibody free in solution can be detected by a third antibody labeled with a detectable moiety which binds the free antibody. A detailed review of immunological assay design, theory and protocols can be found in numerous texts, including Butt, ed., (1984) PRACTICAL IMMUNOLOGY, Marcel Dekker, New York; Harlow *et al.* eds. (1988) ANTIBODIES, A LABORATORY APPROACH, Cold Spring Harbor Laboratory; and Diamandis *et al.*, eds. (1996) IMMUNOASSAY, Academic Press, Boston.

[0108] It is contemplated that the labeled binding proteins are useful as *in vivo* imaging agents, whereby the binding proteins can target the imaging agents to particular tissues of interest in the recipient. A preferred remotely detectable moiety for *in vivo* imaging includes the radioactive atom Technetium^{-99m} (^{99m}Tc), a gamma emitter with a half-life of about six hours. Non-radioactive moieties also useful in *in vivo* imaging include nitroxide spin labels as well as lanthanide and transition metal ions all of which induce proton relaxation *in situ*. In addition to immunoimaging, the complexed radioactive moieties may be used in standard radioimmunotherapy protocols to destroy the targeted cell. Preferred nucleotides for high dose radioimmunotherapy include the radioactive atoms ^{90}Yttrium (^{90}Yt), ^{131}Iodine (^{131}I) and ^{111}Indium (^{111}In). The binding protein can be labeled with ^{131}I, ^{111}In and ^{99m}TC using coupling techniques known in the imaging arts. Similarly, procedures for preparing and administering the imaging agent as well as capturing and processing images are well known in the imaging art and so are not discussed in detail herein. Similarly, methods for performing antibody-based immunotherapies are well known in the art. See, for example, U.S. Patent No. 5,534,254.

[0109] Throughout the description, where compositions are described as having, including, or comprising specific components, it is contemplated that compositions also consist essentially of, or consist of, the recited components. Similarly, where processes are described as having, including, or comprising specific process steps, the processes also consist essentially of, or consist of, the recited processing steps. Except where indicated otherwise, the order of steps or order for performing certain actions are immaterial so long as the invention remains operable.

Moreover, unless otherwise noted, two or more steps or actions may be conducted simultaneously.

EXAMPLES

[0110] The following Examples discuss the production and characterization of a number of anti-hHGF monoclonal antibodies.

Example 1 – Production of Anti-hHGF Monoclonal Antibodies

[0111] This Example describes the production of a number of anti-hHGF monoclonal antibodies.

[0112] Immunizations, fusions, and primary screens were conducted at MBS Inc. (Portland, ME), following the Repetitive Immunization Multiple Sites (RIMMS) protocol. Five AJ mice and Five Balb/c mice were immunized with recombinant human HGF (R&D Systems, Minneapolis, MN; Catalog No. 294-HGN-025). Two mice with sera displaying highest anti-HGF activity by Enzyme Linked Immunosorbent Assay (ELISA) were chosen for subsequent fusion. Spleens and lymph nodes from the appropriate mice were harvested. B-cells then were harvested and fused with an myeloma line. Fusion products were serially diluted on one or more plates to near clonality. Supernatants from the resulting fusions were screened for their binding to hHGF by ELISA. Supernatants identified as containing antibodies to HGF were further characterized by *in vitro* functional testing as discussed in the following examples. A panel of hybridomas was selected and the hybridomas were subcloned and expanded. The monoclonal antibodies then were purified by affinity chromatography on Protein A/G resin under standard conditions.

Example 2 – Sequence Analysis of anti-hHGF Monoclonal Antibodies

[0113] This Example describes isotype and sequence analyses of the anti-hHGF monoclonal antibodies produced in Example 1.

a. Determination of HGF Murine Monoclonal Antibody Isotypes

[0114] The light-chain type and heavy chain isotype of each monoclonal antibody were determined using the IsoStrip Mouse Monoclonal Antibody Isotyping Kit in accordance the manufacturer's instructions (Roche Applied Science).

[0115] All the antibodies were determined to contain a Kappa immunoglobulin light chain and an IgG1 immunoglobulin heavy chain.

b. Determination of Nucleotide Sequences Encoding Immunoglobulin Heavy and Light Chain Variable Regions

[0116] Total RNA was extracted from each monoclonal hybridoma cell line using the RNeasy Miniprep kit according to the manufacturer's instructions (Qiagen Venlo, The Netherlands). Full-length first strand cDNA was generated using the BD SMART™ RACE cDNA Amplification Kit according to the manufacturer's instructions (Clontech) using the oligonucleotide primers BD SMART II A (5' aagcagtggtatcaacgcagagtacgcggg 3') (SEQ ID NO. 85) and 5'-RACE CDS Primer (5' ttttttttttttttttttttttttttvn 3', where v = a, g, or c and n = a, g, c, or t) (SEQ ID NO. 86) for the purpose of 5' RACE (Rapid Amplification of cDNA Ends).

[0117] The variable regions of the Kappa and Heavy (IgG1) immunoglobulin chains were amplified by PCR (Polymerase Chain Reaction) using the Expand High-Fidelity PCR System (Roche Applied Science) according to the manufacturer's instructions. Heavy chain variable regions were amplified with the 5' oligonucelotide primer mix Universal Primer Mix A (mix of 5' ctaatacgactcactatagggcaagcagtggtatcaacgcagagt 3' (SEQ ID NO. 87) and 5' ctaatacgactcactatagggc 3'(SEQ ID NO. 88)) and a 3' IgG1 Constant Region specific primer, either 5' tatgcaaggcttacaaccaca 3' (SEQ ID NO. 89) or 5' gccagtggatagacagatgggggtgtcg 3' (SEQ ID NO. 90). Kappa chain variable regions were amplified with the 5' oligonucleotide primer mix Universal Primer Mix A and a 3' Kappa Constant Region specific primer, either 5' ctcattcctgttgaagctcttgacaat 3' (SEQ ID NO. 91) or 5' cgactgaggcacctccagatgtt 3' (SEQ ID NO. 92).

[0118] Individual PCR products were fractionated by agarose gel electrophoresis and purified using the Qiaquick Gel Purification kit according to the manufacturer's instructions (Qiagen). The PCR products were subsequently cloned into the pCR2.1 TOPO plasmid using the topoisomerase based cloning kit TOPO TA Cloning® Kit (with pCR®2.1-TOPO® vector) according to the manufacturer's instructions (Invitrogen, Carlsbad, CA) and transformed into DH5 bacteria using standard transformation techniques. Plasmid DNA isolated from transformed bacterial clones was sequenced using T7 (5' TAATACGACTCACTATAGGG 3') (SEQ ID NO. 93), M13 Forward (5' GTAAAACGACGGCCAGT 3') (SEQ ID NO. 94), and M13 Reverse primers (5' CAGGAAACAGCTATGACC 3') (SEQ ID NO. 95) by Agencourt

Bioscience using standard dideoxy DNA sequencing methods to identify the sequence of the variable region sequences. The sequences were analyzed using Vector NTI software (Invitrogen, Carlsbad, CA) and the IMGT/V-Quest webserver (http://imgt.cines.fr/textes/vquest) to identify and confirm variable region sequences.

c. Determination of Nucleotide Sequences Encoding Immunoglobulin Heavy and Light Chain Constant Region Sequences for 1A3, 1D3, 1F3, and 2B8 Kappa and IgG1 chains

[0119] Full Length cDNAs for the 1A3, 1D3, and 1F3 IgG1 chains were PCR amplified from the cDNA created above using the forward primer 5' ggggacaagtttgtacaaaaaagcaggctgccaccatgaactttgggctcagattgattttcc 3' (start codon underlined) (SEQ ID NO. 96) and the reverse primer 5' ggggaccactttgtacaagaaagctgggttcatttaccaggagagtgggagagg 3' (stop codon underlined) (SEQ ID NO. 97). Full Length cDNA for the 2B8 IgG1 chain was amplified from the cDNA created above using the forward primer 5' ggggacaagtttgtacaaaaaagcaggctgccaccatgggatggagctatatcatcctcttt 3' (start codon underlined) (SEQ ID NO. 98) and reverse primer 5' ggggaccactttgtacaagaaagctgggttcatttaccaggagagtgggagag 3' (stop codon underlined) (SEQ ID NO. 99).

[0120] Full Length cDNA for the 2B8 Kappa Chain was amplified using the forward primer 5' ggggacaagtttgtacaaaaaagcaggctgccaccatggaatcacagactctggtcttcata 3' (start codon underlined) (SEQ ID NO. 100) and the reverse primer 5' ggggaccactttgtacaagaaagctgggtctaacactcattcctgttgaagctc 3' (stop codon underlined) (SEQ ID NO. 101). PCR fragments were subcloned into pDONR221 (Invitrogen, Carlsbad, CA) by Gateway BP recombination reaction (Invitrogen, Carlsbad, CA) and sequenced by Agencourt Bioscience using standard dideoxy DNA sequencing methods to identify the sequence of the constant region and further confirm variable region sequences.

d. Sequence Analysis

[0121] Variable Regions (normal text) were identified using IMGT/V-QUEST webserver software (http://imgt.cines.fr/textes/vquest/). Signal Peptide sequences were predicted based on identification of the in frame start codon (ATG) that was upstream of the identified Variable Region. Signal Peptide sequences were identified and are underlined below.

[0122] The last nucleotide of each variable region is the first base of the next codon generated by the variable/constant region junction. This nucleotide is included in the variable region because it is part of that exon. Amino acid sequences of the constant regions listed below include the translation of this junction codon.

[0123] In order to create the complete heavy or kappa chain antibody sequences, the variable region sequences noted below are combined with their respective constant region sequences (the signal sequences are underlined).

[0124] (1) 1A3 Heavy Chain Variable Region (SEQ ID NO. 1)

1 atgaactttg ggctcagatt gattttcctt gtccttgttt taaaaggtgt gaagtgtgaa
61 gtgcagctgg tggagtctgg gggaggctta gtgcagcctg gagggtccct gaaactctcc
121 tgtgcagcct ctgaattcac tttcagtaac tattacatgt cttgggttcg ccagactcca
181 gagaagaggc tgcagtgggt cgcatacatt agtcctggtg gtggtagctc ctactatcca
241 gccagtgtga agggtcgatt caccatctcc agagacaatg ccaagaacac cctgtacctg
301 caaatgagca gtctgaagtc tgaggacaca gccatgtatt actgtgcaag acaaggggat
361 ggttactacg gggactatgc tatggactac tggggtcaag gaacctcagt caccgtctcc
421 tcag

[0125] (2) 1A3 Kappa Light Chain Variable Region (SEQ ID NO. 3)

1 atgagtgtgc ccactcaggt cctggggttg ctgctgctgt ggcttacaga tgccagatgt
61 gacatccaga tgactcagtc tccagcctcc ctatctgttt ctgtgggaga aactgtcacc
121 atcacatgtc gagcaagtga gaatatttat agtaatttag catggtatca gcagaaacag
181 ggaaaatctc ctcagctcct ggtctatgct gcaacaaact tagcagatgg tgtgccatca
241 aggttcagtg gcagtggatc aggcacacag ttttccctca agatcaacag cctgcagtct
301 gaagattttg ggacttatta ctgtcaacat ttttggggta ctccgtacac gttcggaggg
361 gggaccaagc tggaaataaa ac

[0126] (3) 2B8 Heavy Chain Variable Region (SEQ ID NO. 11)

1 atgggatgga gctatatcat cctcttttg gtagcaacag ctacagatgt ccactcccag
61 gtccaactgc agcagcctgg ggctgaactg gtgaagcctg ggacttcagt gaagctgtcc
121 tgcaaggctt ctggctacac cttcaccacc tactggatgc actgggtgaa tcagaggcct
181 ggacaaggcc ttgagtggat tggagagatt aatcctacca acggtcatac taactacaat
241 gagaagttca agagcaaggc cacactgact gtagacaaat cctccagcac agcctacatg

301 caactcagca gcctgacatc tgaggactct gcggtctatt actgtgcaag aaactatgtt
361 ggtagcatct ttgactactg gggccaaggc accactctca cagtctcctc ag

[0127] (4) 2B8 Kappa Light Chain Variable Region (SEQ ID NO. 13)

1 atggaatcac agactctggt cttcatatcc atactgctct ggttatatgg tgctgatggg
61 aacattgtaa tgacccaatc tcccaaatcc atgtccatgt cagtaggaga gagggtcacc
121 ttgagctgca aggccagtga gaatgtggtt tcttatgtat cctggtatca acagaaacca
181 gcgcagtctc ctaaactgct gatatacggg gcatccaacc ggaacactgg ggtccccgat
241 cgcttcacag gcagtggatc tgcaacagat ttcactctga ccatcagcag tgtgcgggct
301 gaagaccttg cagattatca ctgtgggcag agttacaact atccgtacac gttcggaggg
361 gggaccaggc tggaaataaa ac

[0128] (5) 2F8 Heavy Chain Variable Region (SEQ ID NO. 21)

1 atggaatgga gctgggtctt tctcttcctc ctgtcagtaa ctgcaggtgt ccactgccag
61 gtccagctga agcagtctgg agctgagctg gtgaggcctg ggacttcagt gaagatgtcc
121 tgcaaggctt ctggctacac cttcactacc tactatatac actgggtgaa tcagaggcct
181 ggacagggcc ttgagtggat tggaaagatt ggtcctggaa gtggtagtac ttactacaat
241 gagatgttca aagacaaggc cacattgact gtagacacat cctccagcac agcctacatg
301 cagctcagca gcctgacatc tgacgactct gcggtctatt tctgtgcaag aaggggactg
361 ggacgtggct ttgactactg gggccaaggc accactctca cagtctcctc ag

[0129] (6) 2F8 Kappa Light Chain Variable Region (SEQ ID NO. 23)

1 atggagacag acacaatcct gctatgggtg ctgctgctct gggttccagg ctccactggt
61 gacattgtgc tgacccaatc tccagcttct ttggctgtgt ctctagggca gagggccacc
121 atctcctgca aggccagcca aagtgttgat tatgatggta atagttatat caactggtac
181 caacagaaac caggacagcc acccaaagtc ctcatctatg ttgcatccaa tctagaatct
241 gggatcccag ccaggtttag tggcagtggg tctgggacag acttcaccct caacatccat
301 cctgtggagg aggaggatgc tgcaacctat tactgtcagc aaagtattga ggatcctccc
361 acgttcggtg ctgggaccaa gctggagctg aaac

[0130] (7) 3B6 Heavy Chain Variable Region (SEQ ID NO. 31)

1 atggaatggc cttgtatctt tctcttcctc ctgtcagtaa ctgaaggtgt ccactcccag
61 gttcagctgc agcagtctgg ggctgaactg gtgaggcctg ggtcctcagt gaagatttcc
121 tgcaaggctt ctggctatgt attcagtagc tactggatga actgggtgaa gcagaggcct

181 ggacagggtc ttgagtggat tggacagatt tatcctggag atggtgatag taactacaat
241 ggaaacttca agggtaaagc cacactgact gcagacaaat cctccagtac agcctacatg
301 cagctcagca gcctaacatc tgaggactct gcggtctatt tctgtgcatc ccagctcggg
361 ctacgtgaga actactttga ctactggggc caaggcacca ctctcacagt ctcctcag

[0131] (8) 3B6 Kappa Light Chain Variable Region (2 possible ATG start codons (uppercase)) (SEQ ID NO. 33)

1 ATGgacATGa ggacccctgc tcagtttctt ggaatcttgt tgctctggtt tccaggtatc
61 aaatgtgaca tcaagatgac ccagtctcca tcttccatgt atgcatctct aggagagaga
121 gtcacaatca cttgcaaggc gagtcaggac attaaaagct atttaagctg gttccagcag
181 aaaccaggga aatctcctaa gaccctgatc tatcgtgtaa acagattggt agatggggtc
241 ccatcaaggt tcagtggcag tggatctggg caagattctt ctctcaccat caccagcctg
301 gagaatgaag atatgggaat ttattattgt ctacagtatg atgagtttcc gttcacgttc
361 ggaggggggga ccaagctgga aataaagc

[0132] (9) 3D11 Heavy Chain Variable Region (SEQ ID NO. 41)

1 atggctgtcc cggtgctgtt cctctgcctg gttgcatttc caagctgtgt cctgtcccag
61 gtacagctga aggagtcagg acctggcctg gtggcgccct cacagagcct gtccatcact
121 tgcactgtct ctgggtttc attaaccagc tatagtttac actgggttcg ccagcctcca
181 ggaaagggtc tggaatggct gggagtaata tgggctggtg gaaacacaaa ttataattcg
241 tctctcatgt ccagactgac catcaggaaa gacaactcca agagccaagt tttcttaaaa
301 atgaacagtc tgcaaactga tgacacagcc atgtactact gtgccagaga gaggtttgct
361 tactggggcc aagggactct ggtcactgtc tctgcag

[0133] (10) 3D11 Kappa Light Chain Variable Region (SEQ ID NO. 43)

1 atggattttc aagtgcagat tttcagcttc ctgctaatca gtgcctcagt caaaatatcc
61 agaggacaaa ttgttctcac ccagtctcca gcaatcatgt ctgcatatcc aggggagaag
121 gtcaccatga cctgcagtgc cagctcaagt gtaagttaca tgcactggta ccagcagaag
181 tcaggcacct cccccaaaag atggatttat gacacatcca aactggcttc tggagtccct
241 gctcgcttca gtggcagtgg gtctgggacc tcttactccc tcacaatcag tagtatggag
301 gctgaagatg ctgccactta ttactgccag cagtggagta gtaacccact cacgttcggt
361 gctgggacca agctggagct gaaac

[0134] (11) 1D3 Heavy Chain Variable Region (SEQ ID NO. 51)

1 atgaactttg ggctcagatt gattttcctt gtccttgttt taaaaggtgt gaagtgtgaa
61 gtgcagctgg tggagtctgg gggaggctta gtgcagcctg gagggtccct gaaactctcc
121 tgtgcagcct ctggattcac tttcagtgac tattacatgt cttgggttcg ccagactcca
181 gagaagaggc tggagtgggt cgcatacatt agtagtggtg gtggtagcac ctactatcca
241 gacagtgtga agggtcgatt caccatctcc cgagacaatg ccaagaacac cctgtacctg
301 caaatgagca gtctgaagtc tgaggacaca gccatatatt actgtgtgag acaaggggat
361 ggttattacg gggactatgc tatggactac tggggtcaag gaacctcagt catcgtctcc
421 tcag

[0135] (12) 1D3 Kappa Light Chain Variable Region (SEQ ID NO. 53)

1 atgagtgtgc ccactcaggt cctggggttg ctgctgctgt ggcttacaga tgtcagatgt
61 gacatccaga tgactcagtc tccagcctcc ctatctgtat ctgtgggaga aactgtcacc
121 atcacatgtc gaacaagtga gaatatttac agtaatttag cgtggtatca gcagaaacag
181 ggaaaatctc ctcagctcct aatctatgct gcaacaaact tagcagatgg tgtgccatca
241 aggttcagtg gcagtggatc aggcacacag ttttccctca ggatcaacag cctgcagtct
301 gaagattttg ggaggtatta ctgtcaacat ttttgggggga ctccgtacac gttcggaggg
361 gggaccaaac tggaaataaa ac

[0136] (13) 1F3 Heavy Chain Variable Region (SEQ ID NO. 61)

1 atgaactttg ggctcagatt gattttcctt gtccttgttt taaaaggtgt gaagtgtgag
61 gtgcagctgg tggagtctgg gggaggctta gtgcagtctg gagggtccct gaaactctcc
121 tgtgcggcct ctggattcac tttcagtaac tatttcatgt cttgggttcg ccagactcca
181 gagaagaggc tggagtgggt cgcatatatt agtagtggtg gtggtagcac ctactatcca
241 gacagtgtga agggtcgatt caccatctct agagacaatg ccaagaacac cctgtacctg
301 caaatgagca gtctgaagtc tgaggacaca gccatgtatt actgtgtaag acaaggggat
361 ggttactacg gggactatgc tatggactac tggggtcaag gaacctcagt caccgtctcc
421 tcag

[0137] (14) 1F3 Kappa Light Chain Variable Region (SEQ ID NO. 63)

1 atgagtgtgc ccactcaggt cctggggttg ctgctgctgt ggcttacaga tgccagatgt
61 gacatccaga tgactcagtc tccagcctcc ctatctgtat ctgtgggaga aactgtcacc
121 atcacatgtc gagcaagtga gaatatttac agtaatttag catggtatca gcagaaacag

181 ggaaaatctc ctcagctcct ggtctatgat gcaacacact taccagatgg tgtgccatca
241 aggttcagtg gcagtggatc aggcacacag ttttccctca agatcaacag cctgcagtct
301 gaagattttg ggagttatta ctgtcaacat ttttggggta ctccgtacac gtttggaggg
361 gggaccagac tggaaattaa ac

[0138] (15) 3A12 Heavy Chain Variable Region (SEQ ID NO. 71)

1 atgaactttg ggctcagatt gattttcctt gtccttgttt taaaaggtgt gaagtgtgaa
61 gtgcagctgg tggagtctgg gggaggctta gtgcagcctg gagggtccct gaaaatctcc
121 tgtgcagcct ctggatttac tttcagtaac tatttcatgt cttgggttcg ccagactcca
181 gagaagaggc tggagtgggt cgcatacatt agtagtggtg gtggtagcac ctactatcca
241 gacagtgtga agggtcgatt caccatctcc agagacaatg ccaagaacac cctgtacctg
301 caaatgaaca gtctgaagtc tgaggacaca gccatgtatt actgtgtaag acaaggagat
361 ggttactatg gggactatgc tatggactac tggggtcaag gaacctcagt caccgtctcc
421 tcag

[0139] (16) 3A12 Kappa Light Chain Variable Region (SEQ ID NO. 73)

1 atgagtgtgc ccactcaggt cctggggttg ctgctgctgt ggcttacaga tgccagatgt
61 gacatccaga tgactcagtc gccagcctcc ctatctgtat ctgtgggaga aactgtcacc
121 atcacatgtc gagcaagtga gaatatttac attaatttag catggtatca gcagaaacag
181 ggaaaatctc ctcagctcct ggtccatgct gcaacaaagt tagcagatgg tgtgccatca
241 aggttcagtg gcagtggatc aggcacacag tattccctca agatcaacag cctgcagtct
301 gaagattttg ggagttatta ctgtcaacat ttttggggta ctccgtacac gttcggaggg
361 gggaccaaac tagaaataaa ac

[0140] (17) Reference Mouse IgG1 Heavy Chain Constant Region (J00453) (SEQ ID NO. 81)

1 ccaaaacgac acccccatct gtctatccac tggcccctgg atctgctgcc caaactaact
61 ccatggtgac cctgggatgc ctggtcaagg gctatttccc tgagccagtg acagtgacct
121 ggaactctgg atccctgtcc agcggtgtgc acaccttccc agctgtcctg gagtctgacc
181 tctacactct gagcagctca gtgactgtcc cctccagccc tcggcccagc gagaccgtca
241 cctgcaacgt tgcccacccg gccagcagca ccaaggtgga caagaaaatt gtgcccaggg
301 attgtggttg taagccttgc atatgtacag tcccagaagt atcatctgtc ttcatcttcc
361 ccccaaagcc caaggatgtg ctcaccatta ctctgactcc taaggtcacg tgtgttgtgg

421 tagacatcag caaggatgat cccgaggtcc agttcagctg gtttgtagat gatgtggagg
481 tgcacacagc tcagacgcaa ccccgggagg agcagttcaa cagcactttc cgctcagtca
541 gtgaacttcc catcatgcac caggactggc tcaatggcaa ggagttcaaa tgcagggtca
601 acagtgcagc tttccctgcc cccatcgaga aaaccatctc caaaaccaaa ggcagaccga
661 aggctccaca ggtgtacacc attccacctc ccaaggagca gatggccaag gataaagtca
721 gtctgacctg catgataaca gacttcttcc ctgaagacat tactgtggag tggcagtgga
781 atgggcagcc agcggagaac tacaagaaca ctcagcccat catgaacacg aatggctctt
841 acttcgtcta cagcaagctc aatgtgcaga agagcaactg ggaggcagga aatactttca
901 cctgctctgt gttacatgag ggcctgcaca accaccatac tgagaagagc ctctcccact
961 ctcctggtaa atga

[0141] (18) Mouse IgG1 Heavy Chain Constant Region Determined for 1A3, 1D3, 1F3, and 2B8 (derived from AJ strain mice) (SEQ ID NO. 82)

1 ccaaaacgac acccccatct gtctatccac tggcccctgg atctgctgcc caaactaact
61 ccatggtgac cctgggatgc ctggtcaagg gctatttccc tgagccagtg acagtgacct
121 ggaactctgg atccctgtcc agcggtgtgc acaccttccc agctgtcctg cagtctgacc
181 tctacactct gagcagctca gtgactgtcc cctccagcac ctggcccagc gagaccgtca
241 cctgcaacgt tgcccacccg gccagcagca ccaaggtgga caagaaaatt gtgcccaggg
301 attgtggttg taagccttgc atatgtacag tcccagaagt atcatctgtc ttcatcttcc
361 ccccaaagcc caaggatgtg ctcaccatta ctctgactcc taaggtcacg tgtgttgtgg
421 tagacatcag caaggatgat cccgaggtcc agttcagctg gtttgtagat gatgtggagg
481 tgcacacagc tcagacgcaa ccccgggagg agcagttcaa cagcactttc cgctcagtca
541 gtgaacttcc catcatgcac caggactggc tcaatggcaa ggagttcaaa tgcagggtca
601 acagtgcagc tttccctgcc cccatcgaga aaaccatctc caaaaccaaa ggcagaccga
661 aggctccaca ggtgtacacc attccacctc ccaaggagca gatggccaag gataaagtca
721 gtctgacctg catgataaca gacttcttcc ctgaagacat tactgtggag tggcagtgga
781 atgggcagcc agcggagaac tacaagaaca ctcagcccat catggacaca gatggctctt
841 acttcgtcta cagcaagctc aatgtgcaga agagcaactg ggaggcagga aatactttca
901 cctgctctgt gttacatgag ggcctgcaca accaccatac tgagaagagc ctctcccact
961 ctcctggtaa atga

[0142] (19) Reference Mouse Kappa Light Chain Constant Region (V00807) and Mouse Kappa Light Chain Constant Region Determined for 1D3, 1F3, and 2B8 (derived from AJ strain mice) (SEQ ID NO. 83)

1 gggctgatgc tgcaccaact gtatccatct tcccaccatc cagtgagcag ttaacatctg
61 gaggtgcctc agtcgtgtgc ttcttgaaca acttctaccc caaagacatc aatgtcaagt
121 ggaagattga tggcagtgaa cgacaaaatg gcgtcctgaa cagttggact gatcaggaca
181 gcaaagacag cacctacagc atgagcagca ccctcacgtt gaccaaggac gagtatgaac
241 gacataacag ctatacctgt gaggccactc acaagacatc aacttcaccc attgtcaaga
301 gcttcaacag gaatgagtgt tag

[0143] (20) Mouse Kappa Light Chain Constant Region Determined for 1A3 containing one altered nucleotide compared to 1D3, 1F3, and 2B8 (underlined) (SEQ ID NO. 84)

1 gggctgatgc tgcaccaact gtatccatct tcccaccatc cagtgagcag ttaacatctg
61 gaggtgcctc agtcgtgtgc ttcttgaaca acttctaccc caaagacatc aatgtcaagt
121 ggaagattga tggcagtgaa cgacaaaatg gcgtcctgaa cagttggact gatcaggaca
181 gcaaagacag cacctacagc atgagcagca ccctcatgtt gaccaaggac gagtatgaac
241 gacataacag ctatacctgt gaggccactc acaagacatc aacttcaccc attgtcaaga
301 gcttcaacag gaatgagtgt tag

[0144] Each of the amino acid sequences defining the immunoglobulin heavy chain variable regions for the antibodies produced in Example 1 are set forth in Figure 2. Each of the sequences are aligned with one another and the sequences defining the signal peptide, CDR_1, CDR_2 and CDR_3 are identified by boxes. Figure 3 shows an alignment of the separate CDR_1, CDR_2 and CDR_3 sequences for each of the antibodies.

[0145] Each of the amino acid sequences defining the immunoglobulin light chain variable regions for each of the antibodies produced in Example 1 are set forth in Figure 4. Each of the sequences are aligned with one another and the sequences defining the signal peptide, CDR_1, CDR_2 and CDR_3 are identified by boxes. Figure 5 shows an alignment of the separate CDR_1, CDR_2 and CDR_3 sequences for each of the antibodies.

[0146] For convenience, Table 1 provides a concordance chart showing the correspondence between the antibody sequences discussed in this Example with those presented in the Sequence Listing.

TABLE 1

SEQ ID NO:	Protein or Nucleic Acid
1	Heavy Chain Variable Region 1A3 – nucleic acid
2	Heavy Chain Variable Region 1A3 – protein
3	Light (kappa) Chain Variable Region 1A3 – nucleic acid
4	Light (kappa) Chain Variable Region 1A3 – protein
5	Heavy Chain CDR_1 1A3
6	Heavy Chain CDR_2 1A3
7	Heavy Chain CDR_3 1A3
8	Light (kappa) Chain CDR_1 1A3
9	Light (kappa) Chain CDR_2 1A3
10	Light (kappa) Chain CDR_3 1A3
11	Heavy Chain Variable Region 2B8 – nucleic acid
12	Heavy Chain Variable Region 2B8 – protein
13	Light (kappa) Chain Variable Region 2B8 – nucleic acid
14	Light (kappa) Chain Variable Region 2B8 – protein
15	Heavy Chain CDR_1 2B8
16	Heavy Chain CDR_2 2B8
17	Heavy Chain CDR_3 2B8
18	Light (kappa) Chain CDR_1 2B8
19	Light (kappa) Chain CDR_2 2B8
20	Light (kappa) Chain CDR_3 2B8
21	Heavy Chain Variable Region 2F8 – nucleic acid
22	Heavy Chain Variable Region 2F8 – protein
23	Light (kappa) Chain Variable Region 2F8 – nucleic acid
24	Light (kappa) Chain Variable Region 2F8 – protein
25	Heavy Chain CDR_1 2F8
26	Heavy Chain CDR_2 2F8
27	Heavy Chain CDR_3 2F8
28	Light (kappa) Chain CDR_1 2F8
29	Light (kappa) Chain CDR_2 2F8
30	Light (kappa) Chain CDR_3 2F8
31	Heavy Chain Variable Region 3B6 – nucleic acid
32	Heavy Chain Variable Region 3B6 – protein
33	Light (kappa) Chain Variable Region 3B6 – nucleic acid
34	Light (kappa) Chain Variable Region 3B6 – protein
35	Heavy Chain CDR_1 3B6
36	Heavy Chain CDR_2 3B6
37	Heavy Chain CDR_3 3B6
38	Light (kappa) Chain CDR_1 3B6
39	Light (kappa) Chain CDR_2 3B6
40	Light (kappa) Chain CDR_3 3B6
41	Heavy Chain Variable Region 3D11 – nucleic acid
42	Heavy Chain Variable Region 3D11 – protein
43	Light (kappa) Chain Variable Region 3D11 – nucleic acid
44	Light (kappa) Chain Variable Region 3D11 – protein
45	Heavy Chain CDR_1 3D11
46	Heavy Chain CDR_2 3D11
47	Heavy Chain CDR_3 3D11

SEQ ID NO:	Protein or Nucleic Acid
48	Light (kappa) Chain CDR_1 3D11
49	Light (kappa) Chain CDR_2 3D11
50	Light (kappa) Chain CDR_3 3D11
51	Heavy Chain Variable Region 1D3 – nucleic acid
52	Heavy Chain Variable Region 1D3 – protein
53	Light (kappa) Chain Variable Region 1D3 – nucleic acid
54	Light (kappa) Chain Variable Region 1D3 – protein
55	Heavy Chain CDR_1 1D3
56	Heavy Chain CDR_2 1D3
57	Heavy Chain CDR_3 1D3
58	Light (kappa) Chain CDR_1 1D3
59	Light (kappa) Chain CDR_2 1D3
60	Light (kappa) Chain CDR_3 1D3
61	Heavy Chain Variable Region 1F3 – nucleic acid
62	Heavy Chain Variable Region 1F3 – protein
63	Light (kappa) Chain Variable Region 1F3 – nucleic acid
64	Light (kappa) Chain Variable Region 1F3 – protein
65	Heavy Chain CDR_1 1F3
66	Heavy Chain CDR_2 1F3
67	Heavy Chain CDR_3 1F3
68	Light (kappa) Chain CDR_1 1F3
69	Light (kappa) Chain CDR_2 1F3
70	Light (kappa) Chain CDR_3 1F3
71	Heavy Chain Variable Region 3A12 – nucleic acid
72	Heavy Chain Variable Region 3A12 – protein
73	Light (kappa) Chain Variable Region 3A12 – nucleic acid
74	Light (kappa) Chain Variable Region 3A12 – protein
75	Heavy Chain CDR_1 3A12
76	Heavy Chain CDR_2 3A12
77	Heavy Chain CDR_3 3A12
78	Light (kappa) Chain CDR_1 3A12
79	Light (kappa) Chain CDR_2 3A12
80	Light (kappa) Chain CDR_3 3A12

[0147] Also, for convenience, the following sequences represent the actual or contemplated full length heavy and light chain sequences (i.e., containing both the variable and constant region sequences) for each of the antibodies described in this Example. It is noted that the constant regions of the murine antibodies 2F8, 3A12, 3B6, and 3D11 were not sequenced but are presumed to have the same constant region sequences as the 1D3, 1F3, and 2B8 antibodies, which were sequenced, as they were all derived from AJ strain mice. It is appreciated, however, that the variable region sequences described herein can be ligated to each of a number of other constant region sequences known to those skilled in the art to produce active full length immunoglobulin heavy and light chains.

[0148] (1) Nucleic Acid Sequence Encoding the Full Length 1A3 Heavy Chain Sequence (1A3 Heavy Chain Variable Region and IgG1 Constant Region) (signal sequence underlined) (SEQ ID NO. 122)

```
   1 atgaactttg ggctcagatt gattttcctt gtccttgttt taaaaggtgt gaagtgtgaa
  61 gtgcagctgg tggagtctgg gggaggctta gtgcagcctg gagggtccct gaaactctcc
 121 tgtgcagcct ctgaattcac tttcagtaac tattacatgt cttgggttcg ccagactcca
 181 gagaagaggc tgcagtgggt cgcatacatt agtcctggtg gtggtagctc ctactatcca
 241 gccagtgtga agggtcgatt caccatctcc agagacaatg ccaagaacac cctgtacctg
 301 caaatgagca gtctgaagtc tgaggacaca gccatgtatt actgtgcaag acaaggggat
 361 ggttactacg gggactatgc tatggactac tggggtcaag gaacctcagt caccgtctcc
 421 tcagccaaaa cgacaccccc atctgtctat ccactggccc ctggatctgc tgcccaaact
 481 aactccatgg tgaccctggg atgcctggtc aagggctatt tccctgagcc agtgacagtg
 541 acctggaact ctggatccct gtccagcggt gtgcacacct tcccagctgt cctgcagtct
 601 gacctctaca ctctgagcag ctcagtgact gtcccctcca gcacctggcc cagcgagacc
 661 gtcacctgca acgttgccca cccggccagc agcaccaagg tggacaagaa aattgtgccc
 721 agggattgtg gttgtaagcc ttgcatatgt acagtcccag aagtatcatc tgtcttcatc
 781 ttcccccaa agcccaagga tgtgctcacc attactctga ctcctaaggt cacgtgtgtt
 841 gtggtagaca tcagcaagga tgatcccgag gtccagttca gctggtttgt agatgatgtg
 901 gaggtgcaca cagctcagac gcaaccccgg gaggagcagt tcaacagcac tttccgctca
 961 gtcagtgaac ttcccatcat gcaccaggac tggctcaatg gcaaggagtt caaatgcagg
1021 gtcaacagtg cagctttccc tgcccccatc gagaaaacca tctccaaaac caaaggcaga
1081 ccgaaggctc cacaggtgta caccattcca cctcccaagg agcagatggc caaggataaa
1141 gtcagtctga cctgcatgat aacagacttc ttccctgaag acattactgt ggagtggcag
1201 tggaatgggc agccagcgga gaactacaag aacactcagc ccatcatgga cacagatggc
1261 tcttacttcg tctacagcaa gctcaatgtg cagaagagca actgggaggc aggaaatact
1321 ttcacctgct ctgtgttaca tgagggcctg cacaaccacc atactgagaa gagcctctcc
1381 cactctcctg gtaaatga
```

[0149] (2) Protein Sequence Defining the Full Length 1A3 Heavy Chain Sequence (1A3 Heavy Chain Variable Region and IgG1 Constant Region) (without signal sequence) (SEQ ID NO. 123)

```
   1 evqlvesggg lvqpggslkl scaaseftfs nyymswvrqt pekrlqwvay ispgggssyy
  61 pasvkgrfti srdnakntly lqmsslksed tamyycarqg dgyygdyamd ywgqgtsvtv
 121 ssakttppsv yplapgsaaq tnsmvtlgcl vkgyfpepvt vtwnsgslss gvhtfpavlq
 181 sdlytlsssv tvpsstwpse tvtcnvahpa sstkvdkkiv prdcgckpci ctvpevssvf
 241 ifppkpkdvl titltpkvtc vvvdiskddp evqfswfvdd vevhtaqtqp reeqfnstfr
 301 svselpimhq dwlngkefkc rvnsaafpap iektisktkg rpkapqvyti pppkeqmakd
 361 kvsltcmitd ffpeditvew qwngqpaeny kntqpimdtd gsyfvyskln vqksnweagn
 421 tftcsvlheg lhnhhteksl shspgk
```

[0150] (3) Nucleic Acid Sequence Encoding the Full Length 1A3 Light Chain Sequence (1A3 Kappa Variable Region and Constant Region) (signal sequence underlined) (SEQ ID NO. 124)

```
   1 atgagtgtgc ccactcaggt cctggggttg ctgctgctgt ggcttacaga tgccagatgt
  61 gacatccaga tgactcagtc tccagcctcc ctatctgttt ctgtgggaga aactgtcacc
 121 atcacatgtc gagcaagtga gaatatttat agtaatttag catggtatca gcagaaacag
 181 ggaaaatctc ctcagctcct ggtctatgct gcaacaaact tagcagatgg tgtgccatca
 241 aggttcagtg gcagtggatc aggcacacag ttttccctca agatcaacag cctgcagtct
 301 gaagattttg ggacttatta ctgtcaacat ttttggggta ctccgtacac gttcggaggg
```

```
361 gggaccaagc tggaaataaa acgggctgat gctgcaccaa ctgtatccat cttcccacca
421 tccagtgagc agttaacatc tggaggtgcc tcagtcgtgt gcttcttgaa caacttctac
481 cccaaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa tggcgtcctg
541 aacagttgga ctgatcagga cagcaaagac agcacctaca gcatgagcag caccctcatg
601 ttgaccaagg acgagtatga acgacataac agctatacct gtgaggccac tcacaagaca
661 tcaacttcac ccattgtcaa gagcttcaac aggaatgagt gttag
```

[0151] (4) Protein Sequence Defining the Full Length 1A3 Light Chain Sequence (1A3 Kappa Variable Region and Constant Region) (without signal sequence) (SEQ ID NO. 125)

```
  1 diqmtqspas lsvsvgetvt itcraseniy snlawyqqkq gkspqllvya atnladgvps
 61 rfsgsgsgtq fslkinslqs edfgtyycqh fwgtpytfgg gtkleikrad aaptvsifpp
121 sseqltsgga svvcflnnfy pkdinvkwki dgserqngvl nswtdqdskd stysmsstlm
181 ltkdeyerhn sytceathkt stspivksfn rnec
```

[0152] (5) Nucleic Acid Sequence Encoding the Full Length 2B8 Heavy Chain Sequence (2B8 Heavy Chain Variable Region and IgG1 Constant Region) (signal sequence underlined) (SEQ ID NO. 126)

```
   1 atgggatgga gctatatcat cctctttttg gtagcaacag ctacagatgt ccactcccag
  61 gtccaactgc agcagcctgg ggctgaactg gtgaagcctg ggacttcagt gaagctgtcc
 121 tgcaaggctt ctggctacac cttcaccacc tactggatgc actgggtgaa tcagaggcct
 181 ggacaaggcc ttgagtggat tggagagatt aatcctacca acggtcatac taactacaat
 241 gagaagttca agagcaaggc cacactgact gtagacaaat cctccagcac agcctacatg
 301 caactcagca gcctgacatc tgaggactct gcggtctatt actgtgcaag aaactatgtt
 361 ggtagcatct ttgactactg gggccaaggc accactctca cagtctcctc agccaaaacg
 421 acaccccccat ctgtctatcc actggccct ggatctgctg cccaaactaa ctccatggtg
 481 accctgggat gcctggtcaa gggctatttc cctgagccag tgacagtgac ctggaactct
 541 ggatccctgt ccagcggtgt gcacaccttc ccagctgtcc tgcagtctga cctctacact
 601 ctgagcagct cagtgactgt cccctccagc acctggccca gcgagaccgt cacctgcaac
 661 gttgcccacc cggccagcag caccaaggtg gacaagaaaa ttgtgcccag ggattgtggt
 721 tgtaagcctt gcatatgtac agtcccagaa gtatcatctg tcttcatctt cccccaaag
 781 cccaaggatg tgctcaccat tactctgact cctaaggtca cgtgtgttgt ggtagacatc
 841 agcaaggatg atcccgaggt ccagttcagc tggtttgtag atgatgtgga ggtgcacaca
 901 gctcagacgc aaccccggga ggagcagttc aacagcactt tccgctcagt cagtgaactt
 961 cccatcatgc accaggactg gctcaatggc aaggagttca aatgcagggt caacagtgca
1021 gctttccctg cccccatcga gaaaaccatc tccaaaacca aaggcagacc gaaggctcca
1081 caggtgtaca ccattccacc tcccaaggag cagatggcca aggataaagt cagtctgacc
1141 tgcatgataa cagacttctt ccctgaagac attactgtgg agtggcagtg gaatgggcag
1201 ccagcggaga actacaagaa cactcagccc atcatggaca cagatggctc ttacttcgtc
1261 tacagcaagc tcaatgtgca gaagagcaac tgggaggcag gaaatacttt cacctgctct
1321 gtgttacatg agggcctgca caaccaccat actgagaaga gcctctccca ctctcctggt
1381 aaatga
```

[0153] (6) Protein Sequence Defining the Full Length 2B8 Heavy Chain Sequence (2B8 Heavy Chain Variable Region and IgG1 Constant Region) (without signal sequence) (SEQ ID NO. 127)

```
  1 qvqlqqpgae lvkpgtsvkl sckasgytft tywmhwvnqr pgqglewige inptnghtny
 61 nekfkskatl tvdkssstay mqlssltsed savyycarny vgsifdywgq gttltvssak
121 ttppsvypla pgsaaqtnsm vtlgclvkgy fpepvtvtwn sgslssgvht fpavlqsdly
181 tlsssvtvps stwpsetvtc nvahpasstk vdkkivprdc gckpcictvp evssvfifpp
241 kpkdvltitl tpkvtcvvvd iskddpevqf swfvddvevh taqtqpreeq fnstfrsvse
```

```
301 lpimhqdwln gkefkcrvns aafpapiekt isktkgrpka pqvytipppk eqmakdkvsl
361 tcmitdffpe ditvewqwng qpaenykntq pimdtdgsyf vysklnvqks nweagntftc
421 svlheglhnh htekslshsp gk
```

[0154] (7) Nucleic Acid Sequence Encoding the Full Length 2B8 Light Chain Sequence (2B8 Kappa Variable Region and Constant Region) (signal sequence underlined) (SEQ ID NO. 128)

```
   1 atggaatcac agactctggt cttcatatcc atactgctct ggttatatgg tgctgatggg
  61 aacattgtaa tgacccaatc tcccaaatcc atgtccatgt cagtaggaga gagggtcacc
 121 ttgagctgca aggccagtga gaatgtggtt tcttatgtat cctggtatca acagaaacca
 181 gcgcagtctc ctaaactgct gatatacggg gcatccaacc ggaacactgg ggtccccgat
 241 cgcttcacag gcagtggatc tgcaacagat ttcactctga ccatcagcag tgtgcgggct
 301 gaagaccttg cagattatca ctgtgggcag agttacaact atccgtacac gttcggaggg
 361 gggaccaggc tggaaataaa acgggctgat gctgcaccaa ctgtatccat cttcccacca
 421 tccagtgagc agttaacatc tggaggtgcc tcagtcgtgt gcttcttgaa caacttctac
 481 cccaaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa tggcgtcctg
 541 aacagttgga ctgatcagga cagcaaagac agcacctaca gcatgagcag caccctcacg
 601 ttgaccaagg acgagtatga acgacataac agctatacct gtgaggccac tcacaagaca
 661 tcaacttcac ccattgtcaa gagcttcaac aggaatgagt gttag
```

[0155] (8) Protein Sequence Defining the Full Length 2B8 Light Chain Sequence (2B8 Kappa Variable Region and Constant Region) (without signal sequence) (SEQ ID NO. 129)

```
   1 nivmtqspks msmsvgervt lsckasenvv syvswyqqkp aqspklliyg asnrntgvpd
  61 rftgsgsatd ftltissvra edladyhcgq synypytfgg gtrleikrad aaptvsifpp
 121 sseqltsgga svvcflnnfy pkdinvkwki dgserqngvl nswtdqdskd stysmsstlt
 181 ltkdeyerhn sytceathkt stspivksfn rnec
```

[0156] (9) Nucleic Acid Sequence Encoding the Full Length 2F8 Heavy Chain Sequence (2F8 Heavy Chain Variable Region and IgG1 Constant Region) (signal sequence underlined) (SEQ ID NO. 130)

```
   1 atggaatgga gctgggtctt tctcttcctc ctgtcagtaa ctgcaggtgt ccactgccag
  61 gtccagctga agcagtctgg agctgagctg gtgaggcctg ggacttcagt gaagatgtcc
 121 tgcaaggctt ctggctacac cttcactacc tactatatac actgggtgaa tcagaggcct
 181 ggacagggcc ttgagtggat tggaaagatt ggtcctggaa gtggtagtac ttactacaat
 241 gagatgttca aagacaaggc cacattgact gtagacacat cctccagcac agcctacatg
 301 cagctcagca gcctgacatc tgacgactct gcggtctatt tctgtgcaag aaggggactg
 361 ggacgtggct ttgactactg gggccaaggc accactctca cagtctcctc agccaaaacg
 421 acaccccccat ctgtctatcc actggccccct ggatctgctg cccaaactaa ctccatggtg
 481 accctgggat gcctggtcaa gggctatttc cctgagccag tgacagtgac ctggaactct
 541 ggatccctgt ccagcggtgt gcacaccttc ccagctgtcc tgcagtctga cctctacact
 601 ctgagcagct cagtgactgt cccctccagc acctggccca gcgagaccgt cacctgcaac
 661 gttgcccacc cggccagcag caccaaggtg gacaagaaaa ttgtgcccag ggattgtggt
 721 tgtaagcctt gcatatgtac agtcccagaa gtatcatctg tcttcatctt cccccaaag
 781 cccaaggatg tgctcaccat tactctgact cctaaggtca cgtgtgttgt ggtagacatc
 841 agcaaggatg atcccgaggt ccagttcagc tggtttgtag atgatgtgga ggtgcacaca
 901 gctcagacgc aaccccggga ggagcagttc aacagcactt tccgctcagt cagtgaactt
 961 cccatcatgc accaggactg gctcaatggc aaggagttca aatgcagggt caacagtgca
1021 gctttccctg cccccatcga gaaaaccatc tccaaaacca aaggcagacc gaaggctcca
1081 caggtgtaca ccattccacc tcccaaggag cagatggcca aggataaagt cagtctgacc
1141 tgcatgataa cagacttctt ccctgaagac attactgtgg agtggcagtg gaatgggcag
```

```
1201 ccagcggaga actacaagaa cactcagccc atcatggaca cagatggctc ttacttcgtc
1261 tacagcaagc tcaatgtgca gaagagcaac tgggaggcag gaaatacttt cacctgctct
1321 gtgttacatg agggcctgca caaccaccat actgagaaga gcctctccca ctctcctggt
1381 aaatga
```

[0157] (10) Protein Sequence Defining the Full Length 2F8 Heavy Chain Sequence (2F8 Heavy Chain Variable Region and IgG1 Constant Region) (without signal sequence) (SEQ ID NO. 131)

```
   1 qvqlkqsgae lvrpgtsvkm sckasgytft tyyihwvnqr pgqglewigk igpgsgstyy
  61 nemfkdkatl tvdtssstay mqlssltsdd savyfcarrg lgrgfdywgq gttltvssak
 121 ttppsvypla pgsaaqtnsm vtlgclvkgy fpepvtvtwn sgslssgvht fpavlqsdly
 181 tlsssvtvps stwpsetvtc nvahpasstk vdkkivprdc gckpcictvp evssvfifpp
 241 kpkdvltitl tpkvtcvvvd iskddpevqf swfvddvevh taqtqpreeq fnstfrsvse
 301 lpimhqdwln gkefkcrvns aafpapiekt isktkgrpka pqvytipppk eqmakdkvsl
 361 tcmitdffpe ditvewqwng qpaenykntq pimdtdgsyf vysklnvqks nweagntftc
 421 svlheglhnh hteklshsp gk
```

[0158] (11) Nucleic Acid Sequence Encoding the Full Length 2F8 Light Chain Sequence (2F8 Kappa Variable Region and Constant Region) (signal sequence underlined) (SEQ ID NO. 132)

```
   1 atggagacag acacaatcct gctatgggtg ctgctgctct gggttccagg ctccactggt
  61 gacattgtgc tgacccaatc tccagcttct ttggctgtgt ctctagggca gagggccacc
 121 atctcctgca aggccagcca aagtgttgat tatgatggta atagttatat caactggtac
 181 caacagaaac caggacagcc acccaaagtc ctcatctatg ttgcatccaa tctagaatct
 241 gggatcccag ccaggtttag tggcagtggg tctgggacag acttcaccct caacatccat
 301 cctgtggagg aggaggatgc tgcaacctat tactgtcagc aaagtattga ggatcctccc
 361 acgttcggtg ctgggaccaa gctggagctg aaacgggctg atgctgcacc aactgtatcc
 421 atcttcccac catccagtga gcagttaaca tctggaggtg cctcagtcgt gtgcttcttg
 481 aacaacttct accccaaaga catcaatgtc aagtggaaga ttgatggcag tgaacgacaa
 541 aatggcgtcc tgaacagttg gactgatcag gacagcaaag acagcaccta cagcatgagc
 601 agcaccctca cgttgaccaa ggacgagtat gaacgacata acagctatac ctgtgaggcc
 661 actcacaaga catcaacttc acccattgtc aagagcttca acaggaatga gtgttag
```

[0159] (12) Protein Sequence Defining the Full Length 2F8 Light Chain Sequence (2F8 Kappa Variable Region and Constant Region) (without signal sequence) (SEQ ID NO. 133)

```
   1 divltqspas lavslgqrat isckasqsvd ydgnsyinwy qqkpgqppkv liyvasnles
  61 giparfsgsg sgtdftlnih pveeedaaty ycqqsiedpp tfgagtklel kradaaptvs
 121 ifppsseqlt sggasvvcfl nnfypkdinv kwkidgserq ngvlnswtdq dskdstysms
 181 stltltkdey erhnsytcea thktstspiv ksfnrnec
```

[0160] (13) Nucleic Acid Sequence Encoding the Full Length 3B6 Heavy Chain Sequence (3B6 Heavy Chain Variable Region and IgG1 Constant Region) (signal sequence underlined) (SEQ ID NO. 134)

```
   1 atggaatggc cttgtatctt tctcttcctc ctgtcagtaa ctgaaggtgt ccactcccag
  61 gttcagctgc agcagtctgg ggctgaactg gtgaggcctg ggtcctcagt gaagatttcc
 121 tgcaaggctt ctggctatgt attcagtagc tactggatga actgggtgaa gcagaggcct
 181 ggacagggtc ttgagtggat tggacagatt tatcctggag atggtgatag taactacaat
```

```
 241 ggaaacttca agggtaaagc cacactgact gcagacaaat cctccagtac agcctacatg
 301 cagctcagca gcctaacatc tgaggactct gcggtctatt tctgtgcatc ccagctcggg
 361 ctacgtgaga actactttga ctactggggc caaggcacca ctctcacagt ctcctcagcc
 421 aaaacgacac ccccatctgt ctatccactg gcccctggat ctgctgccca aactaactcc
 481 atggtgaccc tgggatgcct ggtcaagggc tatttccctg agccagtgac agtgacctgg
 541 aactctggat ccctgtccag cggtgtgcac accttcccag ctgtcctgca gtctgacctc
 601 tacactctga gcagctcagt gactgtcccc tccagcacct ggcccagcga gaccgtcacc
 661 tgcaacgttg cccacccggc cagcagcacc aaggtggaca agaaaattgt gcccagggat
 721 tgtggttgta agccttgcat atgtacagtc ccagaagtat catctgtctt catcttcccc
 781 ccaaagccca aggatgtgct caccattact ctgactccta aggtcacgtg tgttgtggta
 841 gacatcagca aggatgatcc cgaggtccag ttcagctggt ttgtagatga tgtggaggtg
 901 cacacagctc agacgcaacc ccgggaggag cagttcaaca gcactttccg ctcagtcagt
 961 gaacttccca tcatgcacca ggactggctc aatggcaagg agttcaaatg cagggtcaac
1021 agtgcagctt tccctgcccc catcgagaaa accatctcca aaaccaaagg cagaccgaag
1081 gctccacagg tgtacaccat tccacctccc aaggagcaga tggccaagga taaagtcagt
1141 ctgacctgca tgataacaga cttcttccct gaagacatta ctgtggagtg gcagtggaat
1201 gggcagccag cggagaacta caagaacact cagcccatca tggacacaga tggctcttac
1261 ttcgtctaca gcaagctcaa tgtgcagaag agcaactggg aggcaggaaa tactttcacc
1321 tgctctgtgt tacatgaggg cctgcacaac caccatactg agaagagcct ctcccactct
1381 cctggtaaat ga
```

[0161] (14) Protein Sequence Defining the Full Length 3B6 Heavy Chain Sequence (3B6 Heavy Chain Variable Region and IgG1 Constant Region) (without signal sequence) (SEQ ID NO. 135)

```
   1 qvqlqqsgae lvrpgssvki sckasgyvfs sywmnwvkqr pgqglewigq iypgdgdsny
  61 ngnfkgkatl tadkssstay mqlssltsed savyfcasql glrenyfdyw gqgttltvss
 121 akttppsvyp lapgsaaqtn smvtlgclvk gyfpepvtvt wnsgslssgv htfpavlqsd
 181 lytlsssvtv psstwpsetv tcnvahpass tkvdkkivpr dcgckpcict vpevssvfif
 241 ppkpkdvlti tltpkvtcvv vdiskddpev qfswfvddve vhtaqtqpre eqfnstfrsv
 301 selpimhqdw lngkefkcrv nsaafpapie ktisktkgrp kapqvytipp pkeqmakdkv
 361 sltcmitdff peditvewqw ngqpaenykn tqpimdtdgs yfvysklnvq ksnweagntf
 421 tcsvlheglh nhhtekslsh spgk
```

[0162] (15) Nucleic Acid Sequence Encoding the Full Length 3B6 Light Chain Sequence (3B6 Kappa Variable Region and Constant Region) (signal sequence underlined) (SEQ ID NO. 136)

```
   1 ATGgacATGa ggaccctgc tcagtttctt ggaatcttgt tgctctggtt tccaggtatc
  61 aaatgtgaca tcaagatgac ccagtctcca tcttccatgt atgcatctct aggagagaga
 121 gtcacaatca cttgcaaggc gagtcaggac attaaaagct atttaagctg gttccagcag
 181 aaaccaggga aatctcctaa gaccctgatc tatcgtgtaa acagattggt agatggggtc
 241 ccatcaaggt tcagtggcag tggatctggg caagattctt ctctcaccat caccagcctg
 301 gagaatgaag atatgggaat ttattattgt ctacagtatg atgagtttcc gttcacgttc
 361 ggaggggga ccaagctgga aataaagcgg gctgatgctg caccaactgt atccatcttc
 421 ccaccatcca gtgagcagtt aacatctgga ggtgcctcag tcgtgtgctt cttgaacaac
 481 ttctacccca aagacatcaa tgtcaagtgg aagattgatg gcagtgaacg acaaaatggc
 541 gtcctgaaca gttggactga tcaggacagc aaagacagca cctacagcat gagcagcacc
 601 ctcacgttga ccaaggacga gtatgaacga cataacagct atacctgtga ggccactcac
 661 aagacatcaa cttcacccat tgtcaagagc ttcaacagga atgagtgtta g
```

[0163] (16) Protein Sequence Defining the Full Length 3B6 Light Chain Sequence (3B6 Kappa Variable Region and Constant Region) (without signal sequence) (SEQ ID NO. 137)

```
  1 dikmtqspss myaslgervt itckasqdik sylswfqqkp gkspktliyr vnrlvdgvps
 61 rfsgsgsgqd ssltitslen edmgiyyclq ydefpftfgg gtkleikrad aaptvsifpp
121 sseqltsgga svvcflnnfy pkdinvkwki dgserqngvl nswtdqdskd stysmsstlt
181 ltkdeyerhn sytceathkt stspivksfn rnec
```

[0164] (17) Nucleic Acid Sequence Encoding the Full Length 3D11 Heavy Chain Sequence (3D11 Heavy Chain Variable Region and IgG1 Constant Region) (signal sequence underlined) (SEQ ID NO. 138)

```
   1 atggctgtcc cggtgctgtt cctctgcctg gttgcatttc caagctgtgt cctgtcccag
  61 gtacagctga aggagtcagg acctggcctg gtggcgccct cacagagcct gtccatcact
 121 tgcactgtct ctgggttttc attaaccagc tatagtttac actgggttcg ccagcctcca
 181 ggaaagggtc tggaatggct gggagtaata tgggctggtg gaaacacaaa ttataattcg
 241 tctctcatgt ccagactgac catcaggaaa gacaactcca agagccaagt tttcttaaaa
 301 atgaacagtc tgcaaactga tgacacagcc atgtactact gtgccagaga gaggtttgct
 361 tactggggcc aagggactct ggtcactgtc tctgcagcca aaacgacacc cccatctgtc
 421 tatccactgg cccctggatc tgctgcccaa actaactcca tggtgaccct gggatgcctg
 481 gtcaagggct atttccctga gccagtgaca gtgacctgga actctggatc cctgtccagc
 541 ggtgtgcaca ccttcccagc tgtcctgcag tctgacctct acactctgag cagctcagtg
 601 actgtccccc ccagcacctg gcccagcgag accgtcacct gcaacgttgc ccacccggcc
 661 agcagcacca aggtggacaa gaaaattgtg cccagggatt gtggttgtaa gccttgcata
 721 tgtacagtcc cagaagtatc atctgtcttc atcttccccc caaagcccaa ggatgtgctc
 781 accattactc tgactcctaa ggtcacgtgt gttgtggtag acatcagcaa ggatgatccc
 841 gaggtccagt tcagctggtt tgtagatgat gtggaggtgc acacagctca gacgcaaccc
 901 cgggaggagc agttcaacag cactttccgc tcagtcagtg aacttcccat catgcaccag
 961 gactggctca atggcaagga gttcaaatgc agggtcaaca gtgcagcttt ccctgccccc
1021 atcgagaaaa ccatctccaa aaccaaaggc agaccgaagg ctccacaggt gtacaccatt
1081 ccacctccca aggagcagat ggccaaggat aaagtcagtc tgacctgcat gataacagac
1141 ttcttccctg aagacattac tgtggagtgg cagtggaatg ggcagccagc ggagaactac
1201 aagaacactc agcccatcat ggacacagat ggctcttact tcgtctacag caagctcaat
1261 gtgcagaaga gcaactggga ggcaggaaat actttcacct gctctgtgtt acatgagggc
1321 ctgcacaacc accatactga gaagagcctc tcccactctc ctggtaaatg a
```

[0165] (18) Protein Sequence Defining the Full Length 3D11 Heavy Chain Sequence (3D11 Heavy Chain Variable Region and IgG1 Constant Region) (without signal sequence) (SEQ ID NO. 139)

```
  1 qvqlkesgpg lvapsqslsi tctvsgfslt syslhwvrqp pgkglewlgv iwaggntnyn
 61 sslmsrltir kdnsksqvfl kmnslqtddt amyycarerf aywgqgtlvt vsaakttpps
121 vyplapgsaa qtnsmvtlgc lvkgyfpepv tvtwnsgsls sgvhtfpavl qsdlytlsss
181 vtvpsstwps etvtcnvahp asstkvdkki vprdcgckpc ictvpevssv fifppkpkdv
241 ltitltpkvt cvvvdiskdd pevqfswfvd dvevhtaqtq preeqfnstf rsvselpimh
301 qdwlngkefk crvnsaafpa piektisktk grpkapqvyt ipppkeqmak dkvsltcmit
361 dffpeditve wqwngqpaen ykntqpimdt dgsyfvyskl nvqksnweag ntftcsvlhe
421 glhnhhteks lshspgk
```

[0166] (19) Nucleic Acid Sequence Encoding the Full Length 3D11 Light Chain Sequence (3D11 Kappa Variable Region and Constant Region) (signal sequence underlined) (SEQ ID NO. 140)

```
   1 atggattttc aagtgcagat tttcagcttc ctgctaatca gtgcctcagt caaaatatcc
  61 agaggacaaa ttgttctcac ccagtctcca gcaatcatgt ctgcatatcc aggggagaag
 121 gtcaccatga cctgcagtgc cagctcaagt gtaagttaca tgcactggta ccagcagaag
 181 tcaggcacct cccccaaaag atggatttat gacacatcca aactggcttc tggagtccct
 241 gctcgcttca gtggcagtgg gtctgggacc tcttactccc tcacaatcag tagtatggag
 301 gctgaagatg ctgccactta ttactgccag cagtggagta gtaacccact cacgttcggt
 361 gctgggacca agctggagct gaaacgggct gatgctgcac caactgtatc catcttccca
 421 ccatccagtg agcagttaac atctggaggt gcctcagtcg tgtgcttctt gaacaacttc
 481 taccccaaag acatcaatgt caagtggaag attgatggca gtgaacgaca aaatggcgtc
 541 ctgaacagtt ggactgatca ggacagcaaa gacagcacct acagcatgag cagcaccctc
 601 acgttgacca aggacgagta tgaacgacat aacagctata cctgtgaggc cactcacaag
 661 acatcaactt cacccattgt caagagcttc aacaggaatg agtgttag
```

[0167] (20) Protein Sequence Defining the Full Length 3D11 Light Chain Sequence (3D11 Kappa Variable Region and Constant Region) (without signal sequence) (SEQ ID NO. 141)

```
   1 qivltqspai msaypgekvt mtcsasssvs ymhwyqqksg tspkrwiydt sklasgvpar
  61 fsgsgsgtsy sltissmeae daatyycqqw ssnpltfgag tklelkrada aptvsifpps
 121 seqltsggas vvcflnnfyp kdinvkwkid gserqngvln swtdqdskds tysmsstltl
 181 tkdeyerhns ytceathkts tspivksfnr nec
```

[0168] (21) Nucleic Acid Sequence Encoding the Full Length 1D3 Heavy Chain Sequence (1D3 Heavy Chain Variable Region and IgG1 Constant Region) (signal sequence underlined) (SEQ ID NO. 142)

```
   1 atgaactttg ggctcagatt gattttcctt gtccttgttt taaaaggtgt gaagtgtgaa
  61 gtgcagctgg tggagtctgg gggaggctta gtgcagcctg gagggtccct gaaactctcc
 121 tgtgcagcct ctggattcac tttcagtgac tattacatgt cttgggttcg ccagactcca
 181 gagaagaggc tggagtgggt cgcatacatt agtagtggtg gtggtagcac ctactatcca
 241 gacagtgtga agggtcgatt caccatctcc cgagacaatg ccaagaacac cctgtacctg
 301 caaatgagca gtctgaagtc tgaggacaca gccatatatt actgtgtgag acaaggggat
 361 ggttattacg gggactatgc tatggactac tggggtcaag gaacctcagt catcgtctcc
 421 tcagccaaaa cgacacccca atctgtctat ccactggccc ctggatctgc tgcccaaact
 481 aactccatgg tgaccctggg atgcctggtc aagggctatt tccctgagcc agtgacagtg
 541 acctggaact ctggatccct gtccagcggt gtgcacacct tcccagctgt cctgcagtct
 601 gacctctaca ctctgagcag ctcagtgact gtccctcca gcacctggcc cagcgagacc
 661 gtcacctgca acgttgccca cccggccagc agcaccaagg tggacaagaa aattgtgccc
 721 agggattgtg gttgtaagcc ttgcatatgt acagtcccag aagtatcatc tgtcttcatc
 781 ttccccccaa agcccaagga tgtgctcacc attactctga ctcctaaggt cacgtgtgtt
 841 gtggtagaca tcagcaagga tgatcccgag gtccagttca gctggtttgt agatgatgtg
 901 gaggtgcaca cagctcagac gcaaccccgg gaggagcagt tcaacagcac tttccgctca
 961 gtcagtgaac ttcccatcat gcaccaggac tggctcaatg gcaaggagtt caaatgcagg
1021 gtcaacagtg cagctttccc tgcccccatc gagaaaacca tctccaaaac caaaggcaga
1081 ccgaaggctc cacaggtgta caccattcca cctcccaagg agcagatggc caaggataaa
1141 gtcagtctga cctgcatgat aacagacttc ttccctgaag acattactgt ggagtggcag
1201 tggaatgggc agccagcgga gaactacaag aacactcagc ccatcatgga cacagatggc
1261 tcttacttcg tctacagcaa gctcaatgtg cagaagagca actgggaggc aggaaatact
1321 ttcacctgct ctgtgttaca tgagggcctg cacaaccacc atactgagaa gagcctctcc
1381 cactctcctg gtaaatga
```

[0169] (22) Protein Sequence Defining the Full Length 1D3 Heavy chain sequence (1D3 Heavy Chain Variable Region and IgG1 Constant Region) (without signal sequence) (SEQ ID NO. 143)

```
  1 evqlvesggg lvqpggslkl scaasgftfs dyymswvrqt pekrlewvay issgggstyy
 61 pdsvkgrfti srdnakntly lqmsslksed taiyycvrqg dgyygdyamd ywgqgtsviv
121 ssakttppsv yplapgsaaq tnsmvtlgcl vkgyfpepvt vtwnsgslss gvhtfpavlq
181 sdlytlsssv tvpsstwpse tvtcnvahpa sstkvdkkiv prdcgckpci ctvpevssvf
241 ifppkpkdvl titltpkvtc vvvdiskddp evqfswfvdd vevhtaqtqp reeqfnstfr
301 svselpimhq dwlngkefkc rvnsaafpap iektisktkg rpkapqvyti pppkeqmakd
361 kvsltcmitd ffpeditvew qwngqpaeny kntqpimdtd gsyfvyskln vqksnweagn
421 tftcsvlheg lhnhhteksl shspgk
```

[0170] (23) Nucleic Acid Sequence Encoding the Full Length 1D3 Light Chain Sequence (1D3 Kappa Variable Region and Constant Region) (signal sequence underlined) (SEQ ID NO. 144)

```
  1 atgagtgtgc ccactcaggt cctggggttg ctgctgctgt ggcttacaga tgtcagatgt
 61 gacatccaga tgactcagtc tccagcctcc ctatctgtat ctgtgggaga aactgtcacc
121 atcacatgtc gaacaagtga gaatatttac agtaatttag cgtggtatca gcagaaacag
181 ggaaaatctc ctcagctcct aatctatgct gcaacaaact tagcagatgg tgtgccatca
241 aggttcagtg gcagtggatc aggcacacag ttttccctca ggatcaacag cctgcagtct
301 gaagattttg ggaggtatta ctgtcaacat ttttgggggga ctccgtacac gttcggaggg
361 gggaccaaac tggaaataaa acgggctgat gctgcaccaa ctgtatccat cttcccacca
421 tccagtgagc agttaacatc tggaggtgcc tcagtcgtgt gcttcttgaa caacttctac
481 cccaaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa tggcgtcctg
541 aacagttgga ctgatcagga cagcaaagac agcacctaca gcatgagcag caccctcacg
601 ttgaccaagg acgagtatga acgacataac agctatacct gtgaggccac tcacaagaca
661 tcaacttcac ccattgtcaa gagcttcaac aggaatgagt gttag
```

[0171] (24) Protein Sequence Defining the Full Length 1D3 Light Chain Sequence (1D3 Kappa Variable Region and Constant Region) (without signal sequence) (SEQ ID NO. 145)

```
  1 diqmtqspas lsvsvgetvt itcrtseniy snlawyqqkq gkspqlliya atnladgvps
 61 rfsgsgsgtq fslrinslqs edfgryycqh fwgtpytfgg gtkleikrad aaptvsifpp
121 sseqltsgga svvcflnnfy pkdinvkwki dgserqngvl nswtdqdskd stysmsstlt
181 ltkdeyerhn sytceathkt stspivksfn rnec
```

[0172] (25) Nucleic Acid Sequence Encoding the Full Length 1F3 Heavy Chain Sequence (1F3 Heavy Chain Variable Region and IgG1 Constant Region) (signal sequence underlined) (SEQ ID NO. 146)

```
  1 atgaactttg ggctcagatt gattttcctt gtccttgttt taaaaggtgt gaagtgtgag
 61 gtgcagctgg tggagtctgg gggaggctta gtgcagtctg gagggtccct gaaactctcc
121 tgtgcggcct ctggattcac tttcagtaac tatttcatgt cttgggttcg ccagactcca
181 gagaagaggc tggagtgggt cgcatatatt agtagtggtg gtggtagcac ctactatcca
241 gacagtgtga agggtcgatt caccatctct agagacaatg ccaagaacac cctgtacctg
301 caaatgagca gtctgaagtc tgaggacaca gccatgtatt actgtgtaag acaaggggat
361 ggttactacg gggactatgc tatggactac tggggtcaag gaacctcagt caccgtctcc
```

```
 421 tcagccaaaa cgacaccccc atctgtctat ccactggccc ctggatctgc tgcccaaact
 481 aactccatgg tgaccctggg atgcctggtc aagggctatt tccctgagcc agtgacagtg
 541 acctggaact ctggatccct gtccagcggt gtgcacacct tcccagctgt cctgcagtct
 601 gacctctaca ctctgagcag ctcagtgact gtcccctcca gcacctggcc cagcgagacc
 661 gtcacctgca acgttgccca cccggccagc agcaccaagg tggacaagaa aattgtgccc
 721 agggattgtg gttgtaagcc ttgcatatgt acagtcccag aagtatcatc tgtcttcatc
 781 ttcccccaa agcccaagga tgtgctcacc attactctga ctcctaaggt cacgtgtgtt
 841 gtggtagaca tcagcaagga tgatcccgag gtccagttca gctggtttgt agatgatgtg
 901 gaggtgcaca cagctcagac gcaacccegg gaggagcagt tcaacagcac tttccgctca
 961 gtcagtgaac ttcccatcat gcaccaggac tggctcaatg gcaaggagtt caaatgcagg
1021 gtcaacagtg cagctttccc tgcccccatc gagaaaacca tctccaaaac caaaggcaga
1081 ccgaaggctc cacaggtgta caccattcca cctcccaagg agcagatggc caaggataaa
1141 gtcagtctga cctgcatgat aacagacttc ttccctgaag acattactgt ggagtggcag
1201 tggaatgggc agccagcgga gaactacaag aacactcagc ccatcatgga cacagatggc
1261 tcttacttcg tctacagcaa gctcaatgtg cagaagagca actgggaggc aggaaatact
1321 ttcacctgct ctgtgttaca tgagggcctg cacaaccacc atactgagaa gagcctctcc
1381 cactctcctg gtaaatga
```

[0173] (26) Protein Sequence Defining the Full Length 1F3 Heavy Chain Sequence (1F3 Heavy Chain Variable Region and IgG1 Constant Region) (without signal sequence) (SEQ ID NO. 147)

```
   1 evqlvesggg lvqsggslkl scaasgftfs nyfmswvrqt pekrlewvay issgggstyy
  61 pdsvkgrfti srdnakntly lqmsslksed tamyycvrqg dgyygdyamd ywgqgtsvtv
 121 ssakttppsv yplapgsaaq tnsmvtlgcl vkgyfpepvt vtwnsgslss gvhtfpavlq
 181 sdlytlsssv tvpsstwpse tvtcnvahpa sstkvdkkiv prdcgckpci ctvpevssvf
 241 ifppkpkdvl titltpkvtc vvvdiskddp evqfswfvdd vevhtaqtqp reeqfnstfr
 301 svselpimhq dwlngkefkc rvnsaafpap iektisktkg rpkapqvyti pppkeqmakd
 361 kvsltcmitd ffpeditvew qwngqpaeny kntqpimdtd gsyfvyskln vqksnweagn
 421 tftcsvlheg lhnhhteksl shspgk
```

[0174] (27) Nucleic Acid Sequence Encoding the Full Length 1F3 Light Chain Sequence (1F3 Kappa Variable Region and Constant Region) (signal sequence underlined) (SEQ ID NO. 148)

```
   1 atgagtgtgc ccactcaggt cctggggttg ctgctgctgt ggcttacaga tgccagatgt
  61 gacatccaga tgactcagtc tccagcctcc ctatctgtat ctgtgggaga aactgtcacc
 121 atcacatgtc gagcaagtga gaatatttac agtaatttag catggtatca gcagaaacag
 181 ggaaaatctc ctcagctcct ggtctatgat gcaacacact taccagatgg tgtgccatca
 241 aggttcagtg gcagtggatc aggcacacag ttttccctca agatcaacag cctgcagtct
 301 gaagattttg ggagttatta ctgtcaacat ttttggggta ctccgtacac gtttggaggg
 361 gggaccagac tggaaattaa acgggctgat gctgcaccaa ctgtatccat cttcccacca
 421 tccagtgagc agttaacatc tggaggtgcc tcagtcgtgt gcttcttgaa caacttctac
 481 cccaaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa tggcgtcctg
 541 aacagttgga ctgatcagga cagcaaagac agcacctaca gcatgagcag caccctcacg
 601 ttgaccaagg acgagtatga acgacataac agctatacct gtgaggccac tcacaagaca
 661 tcaacttcac ccattgtcaa gagcttcaac aggaatgagt gttag
```

[0175] (28) Protein Sequence Defining the Full Length 1F3 Light Chain Sequence (1F3 Kappa Variable Region and Constant Region) (without signal sequence) (SEQ ID NO. 149)

```
  1 diqmtqspas lsvsvgetvt itcraseniy snlawyqqkq gkspqllvyd athlpdgvps
 61 rfsgsgsgtq fslkinslqs edfgsyycqh fwgtpytfgg gtrleikrad aaptvsifpp
121 sseqltsgga svvcflnnfy pkdinvkwki dgserqngvl nswtdqdskd stysmsstlt
181 ltkdeyerhn sytceathkt stspivksfn rnec
```

[0176] (29) Nucleic Acid Sequence Encoding the Full Length 3A12 Heavy Chain Sequence (3A12 Heavy Chain Variable Region and IgG1 Constant Region) (signal sequence underlined) (SEQ ID NO. 150)

```
   1 atgaactttg ggctcagatt gattttcctt gtccttgttt taaaaggtgt gaagtgtgaa
  61 gtgcagctgg tggagtctgg gggaggctta gtgcagcctg gagggtccct gaaaatctcc
 121 tgtgcagcct ctggatttac tttcagtaac tatttcatgt cttgggttcg ccagactcca
 181 gagaagaggc tggagtgggt cgcatacatt agtagtggtg gtggtagcac ctactatcca
 241 gacagtgtga agggtcgatt caccatctcc agagacaatg ccaagaacac cctgtacctg
 301 caaatgaaca gtctgaagtc tgaggacaca gccatgtatt actgtgtaag acaaggagat
 361 ggttactatg gggactatgc tatggactac tggggtcaag gaacctcagt caccgtctcc
 421 tcagccaaaa cgacacccca atctgtctat ccactggccc ctggatctgc tgcccaaact
 481 aactccatgg tgaccctggg atgcctggtc aagggctatt tccctgagcc agtgacagtg
 541 acctggaact ctggatccct gtccagcggt gtgcacacct tcccagctgt cctgcagtct
 601 gacctctaca ctctgagcag ctcagtgact gtccctccca gcacctggcc cagcgagacc
 661 gtcacctgca acgttgccca cccggccagc agcaccaagg tggacaagaa aattgtgccc
 721 agggattgtg gttgtaagcc ttgcatatgt acagtcccag aagtatcatc tgtcttcatc
 781 ttccccccaa agcccaagga tgtgctcacc attactctga ctcctaaggt cacgtgtgtt
 841 gtggtagaca tcagcaagga tgatcccgag gtccagttca gctggtttgt agatgatgtg
 901 gaggtgcaca cagctcagac gcaaccccgg gaggagcagt tcaacagcac tttccgctca
 961 gtcagtgaac ttcccatcat gcaccaggac tggctcaatg gcaaggagtt caaatgcagg
1021 gtcaacagtg cagctttccc tgcccccatc gagaaaacca tctccaaaac caaaggcaga
1081 ccgaaggctc cacaggtgta caccattcca cctcccaagg agcagatggc caaggataaa
1141 gtcagtctga cctgcatgat aacagacttc ttccctgaag acattactgt ggagtggcag
1201 tggaatgggc agccagcgga gaactacaag aacactcagc ccatcatgga cacagatggc
1261 tcttacttcg tctacagcaa gctcaatgtg cagaagagca actgggaggc aggaaatact
1321 ttcacctgct ctgtgttaca tgagggcctg cacaaccacc atactgagaa gagcctctcc
1381 cactctcctg gtaaatga
```

[0177] (30) Protein Sequence Defining the Full Length 3A12 Heavy Chain Sequence (3A12 Heavy Chain Variable Region and IgG1 Constant Region) (without signal sequence) (SEQ ID NO. 151)

```
  1 evqlvesggg lvqpggslki scaasgftfs nyfmswvrqt pekrlewvay issgggstyy
 61 pdsvkgrfti srdnakntly lqmnslksed tamyycvrqg dgyygdyamd ywgqgtsvtv
121 ssakttppsv yplapgsaaq tnsmvtlgcl vkgyfpepvt vtwnsgslss gvhtfpavlq
181 sdlytlsssv tvpsstwpse tvtcnvahpa sstkvdkkiv prdcgckpci ctvpevssvf
241 ifppkpkdvl titltpkvtc vvvdiskddp evqfswfvdd vevhtaqtqp reeqfnstfr
301 svselpimhq dwlngkefkc rvnsaafpap iektisktkg rpkapqvyti pppkeqmakd
361 kvsltcmitd ffpeditvew qwngqpaeny kntqpimdtd gsyfvyskln vqksnweagn
421 tftcsvlheg lhnhhteksl shspgk
```

[0178] (31) Nucleic Acid Sequence Encoding the Full Length 3A12 Light Chain Sequence (3A12 Kappa Variable Region and Constant Region) (signal sequence underlined) (SEQ ID NO. 152)

```
   1 atgagtgtgc ccactcaggt cctggggttg ctgctgctgt ggcttacaga tgccagatgt
  61 gacatccaga tgactcagtc gccagcctcc ctatctgtat ctgtgggaga aactgtcacc
 121 atcacatgtc gagcaagtga gaatatttac attaatttag catggtatca gcagaaacag
 181 ggaaaatctc ctcagctcct ggtccatgct gcaacaaagt tagcagatgg tgtgccatca
 241 aggttcagtg gcagtggatc aggcacacag tattccctca agatcaacag cctgcagtct
 301 gaagattttg ggagttatta ctgtcaacat ttttggggta ctccgtacac gttcggaggg
 361 gggaccaaac tagaaataaa acgggctgat gctgcaccaa ctgtatccat cttcccacca
 421 tccagtgagc agttaacatc tggaggtgcc tcagtcgtgt gcttcttgaa caacttctac
 481 cccaaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa tggcgtcctg
 541 aacagttgga ctgatcagga cagcaaagac agcacctaca gcatgagcag caccctcacg
 601 ttgaccaagg acgagtatga acgacataac agctatacct gtgaggccac tcacaagaca
 661 tcaacttcac ccattgtcaa gagcttcaac aggaatgagt gttag
```

[0179] (32) Protein Sequence Defining the Full Length 3A12 Light Chain Sequence (3A12 Kappa Variable Region and Constant Region) (without signal sequence) (SEQ ID NO. 153)

```
   1 diqmtqspas lsvsvgetvt itcraseniy inlawyqqkq gkspqllvha atkladgvps
  61 rfsgsgsgtq yslkinslqs edfgsyycqh fwgtpytfgg gtkleikrad aaptvsifpp
 121 sseqltsgga svvcflnnfy pkdinvkwki dgserqngvl nswtdqdskd stysmsstlt
 181 ltkdeyerhn sytceathkt stspivksfn rnec
```

[0180] For convenience, Table 2 provides a concordance chart showing the correspondence between the full length sequences of the antibodies discussed in this Example with those presented in the Sequence Listing.

TABLE 2

SEQ. ID NO.	Protein or Nucleic Acid
122	1A3 Heavy Variable + IgG1 constant – nucleic acid
123	1A3 Heavy Variable + IgG1 constant – protein
124	1A3 Light Variable + constant – nucleic acid
125	1A3 Light Variable + constant – protein
126	2B8 Heavy Variable + IgG1 constant – nucleic acid
127	2B8 Heavy Variable + IgG1 constant – protein
128	2B8 Light Variable + constant – nucleic acid
129	2B8 Light Variable + constant – protein
130	2F8 Heavy Variable + IgG1 constant – nucleic acid
131	2F8 Heavy Variable + IgG1 constant – protein
132	2F8 Light Variable + constant – nucleic acid
133	2F8 Light Variable + constant – protein
134	3B6 Heavy Variable + IgG1 constant – nucleic acid
135	3B6 Heavy Variable + IgG1 constant – protein
136	3B6 Light Variable + constant – nucleic acid
137	3B6 Light Variable + constant – protein
138	3D11 Heavy Variable + IgG1 constant – nucleic acid
139	3D11 Heavy Variable + IgG1 constant – protein
140	3D11 Light Variable + constant – nucleic acid

SEQ ID NO	Protein or Nucleic Acid
141	3D11 Light Variable + constant – protein
142	1D3 Heavy Variable + IgG1 constant – nucleic acid
143	1D3 Heavy Variable + IgG1 constant – protein
144	1D3 Light Variable + constant – nucleic acid
145	1D3 Light Variable + constant – protein
146	1F3 Heavy Variable + IgG1 constant – nucleic acid
147	1F3 Heavy Variable + IgG1 constant – protein
148	1F3 Light Variable + constant – nucleic acid
149	1F3 Light Variable + constant – protein
150	3A12 Heavy Variable + IgG1 constant – nucleic acid
151	3A12 Heavy Variable + IgG1 constant – protein
152	3A12 Light Variable + constant – nucleic acid
153	3A12 Light Variable + constant – protein

Example 3 – Production of Various Recombinant hHGF Proteins

[0181] This Example describes the cloning and expression of a number of recombinant proteins used to characterize the antibodies created in Example 1 and in Example 14. In particular, this Example describes the cloning and expression of recombinant hHGF protein, a recombinant hHGF protein containing a glycine to glutamate substitution at position 555 (G555E), a recombinant hHGF protein containing a cysteine to arginine substitution at position 561 (C561R), a recombinant mouse-human-mouse (mhm) chimeric HGF protein containing the human V495-L585 HGF sequence disposed within mouse HGF sequence, a recombinant mhm chimeric HGF protein containing the human I499-R566 HGF sequence disposed within mouse HGF sequence, and a recombinant mhm chimeric HGF protein containing human W507-L585 HGF sequence disposed within mouse HGF sequence.

[0182] The following expression constructs were generated using standard molecular techniques and the resulting cDNA sequences were confirmed by DNA sequencing:

a. hHGF-Fc

[0183] In a first round of PCR, two overlapping PCR fragments were generated introducing a Not I site and encoding a 6xHis tag between hHGF and hIgFc. The overlapping PCR fragments served as template in a second round to amplify hHGF-his-IgFc. The resulting fragment was digested by NheI and BamHI and cloned into pcDNA5/FRT (Invitrogen, #35-3014). Then, hHGF was amplified from Invitrogen clone ID: IOH29794 (human HGF cDNA).

The sequence was found to correspond to the sequence deposited at the NCBI under accession number NM_000601.4.

(1) 5'hHGF NheI Primer

[0184] ACTGGCTAGCATGTGGGTGACCAAACTCCT (SEQ ID NO. 102)

(2) 3' hHGF NotI His Tag Primer

[0185] GTGATGGTGATGGTGATGGCGGCCGCATGACTGTGGTACCTTATATG (SEQ ID NO. 103)

(3) 5' HisIgFc Primer

[0186] ACTGGCGGCCGCCATCACCATCACCATCAC (SEQ ID NO. 104)

(4) 3' IgFc BamHI Primer

[0187] ACTGGGATCCTCACTATTTACCCGGGGACAG (SEQ ID NO. 105)

b. hHGF-Fc G555E and hHGF-Fc C561R

[0188] hHGF-Fc mutants G555E and C561R were generated by site directed mutagenesis using the QuikChange II XL site-directed mutagenesis kit (Stratagene) according to manufacturer's instructions.

(1) hHGF-Fc (G555E) Sense Primer

[0189] CATGATGTCCACGAAAGAGGAGATGAG (SEQ ID NO. 106)

(2) hHGF-Fc (G555E) Anti-sense Primer

[0190] CTCATCTCCTCTTTCGTGGACATCATG (SEQ ID NO. 107)

(3) hHGF-Fc (C561R) Sense Primer

[0191] GGAAGAGGAGATGAGAAACGCAAACAGGTTCTCAATG (SEQ ID NO. 108)

(4) hHGF-Fc (C561R) Anti-sense Primer

[0192] CATTGAGAACCTGTTTGCGTTTCTCATCTCCTCTTCC (SEQ ID NO. 109)

c. Mouse-human-mouse chimera Fc

[0193] The mouse-human-mouse chimera IgFc construct contains mHGF alpha chain-hHGF, β-chain amino acids Val 495-Leu 585 of human HGF, and mHGF C-terminal beta chain followed by 6xHis tag and IgG-Fc.

[0194] Human HGF cDNA encoding amino acids V495-L585 was amplified from Invitrogen clone ID: IOH29794 (human HGF cDNA). The sequence corresponds to the sequence deposited at the NCBI under accession number NM_000601.4. Mouse HGF sequences were amplified by RT-PCR from mouse liver total RNA (Clontech, # 636603) using the Super Script One Step RT-PCR kit from Invitrogen (#10928-034) according to manufacturer's instructions. The mHGF cDNA sequence corresponds to the sequence deposited at the NCBI under accession number D10213.1.

[0195] Three fragments, referred to as Fragments 1, 2, and 3, were generated using overlapping PCR primers and annealed in consecutive rounds of PCR amplification. The final product was cleaved with NheI and NotI and cloned into pcDNA5/FRT IgGFc.

(1) Fragment 1 Primers for mHGF alpha chain 5'NheI

[0196] 5'ATCGGCTAGCATGATGTGGGGGACCAAAC (SEQ ID NO. 110)

[0197] 3' GAATCCCATTTACAACCCGCAGTTGTTTTGTTTTGG (SEQ ID NO. 111)

(2) Fragment 2 Primers for hHGF beta chain aa V495-L585

[0198] 5' CCAAAACAAAACAACTGCGGGTTGTAAATGGGATTC (SEQ ID NO. 112)

[0199] 3' CAGGATTGCAGGTCGAGCAAGCTTCATTAAAACCAGATCT (SEQ ID NO. 113)

(3) Fragment 3 Primer for mHGF beta chain C-terminus 3'NotI

[0200] 5' AGATCTGGTTTTAATGAAGCTTGCTCGACCTGCAATCCTG (SEQ ID NO. 114)

[0201] 3' GTAATTTTGACATACAAGTTGTGCGGCCGCCATCACCATCACCATCAC (SEQ ID NO. 115)

d. Construction of hHGF and mhm chimera

[0202] The vectors encoding hHGF and mhm chimera (V495-L585), pcDNA5/FRT hHGF and pcDNA5/FRT-mhm chimera (V495-L585), without Fc-tag were generated by site directed mutagenesis. A stop codon was introduced 3' of the 6xHis tag using the QuikChange II XL site-directed mutagenesis kit (Stratagene) according to manufacturer's instructions. The mutagenesis primer included Primer 1: CATCACCATCACCATCACTAAGCGGGTCTGGTGCCACG (SEQ ID NO. 116), and Primer 2: CGTGGCACCAGACCCGCTTAGTGATGGTGATGGTGATG (SEQ ID NO. 117).

[0203] In addition, two additional mhm chimeras were created from the pcDNA5/FRT-mhm (V495-L585) construct by site directed mutagenesis using the QuikChange II XL site-directed mutagenesis kit (Stratagene) according to manufacturer's instructions. One mhm construct contained the region of I499-R556 of hHGF disposed between murine sequences. The other mhm construct contained the region of W507-L585 of hHGF disposed between murine sequences.

[0204] For the mhm chimera (I499-R556), the following point mutations were made in order in the template pcDNA5/FRT-mhm chimera (V495-L585) construct: D558E, C561R, V564I, V567I and M583L, using the appropriate oligonucleotide sequences. For the mhm chimera (W507-L585), the following point mutations were introduced in one step in the template pcDNA5/FRT-mhm chimera (V495-L585) construct: Q502R, N504T and I505V, using the appropriate oligonucleotide sequences.

[0205] The resulting nucleotide sequence of the hHGF-Fc protein is set forth as SEQ ID NO. 118, including signal sequence (nucleotides 1-93) and prodomain (nucleotides 94-162). The amino acid sequence of the hHGF-Fc protein is set forth as SEQ ID NO. 119.

[0206] The resulting nucleotide sequence encoding the mhm (V495-L585)-Fc chimeric protein is set forth in SEQ ID NO. 120, including signal sequence (nucleotides 1-96) and prodomain (nucleotides 97-165). The amino acid sequence of the mhm (V495-L585)-Fc chimeric protein is set forth in SEQ ID NO. 121.

[0207] The resulting nucleotide sequence encoding, and the protein sequence defining, the mhm (V495-L585) construct are set forth in SEQ ID NOS. 211 and 212, respectively. The nucleic acid sequence set forth in SEQ ID NO. 211 includes the signal sequence (nucleotides 1-

96) and the prodomain (nucleotides 97-165), and the protein sequence set forth in SEQ ID NO. 212 includes the active protein sequence (without the signal sequence or the prodomain). The resulting nucleotide sequence encoding, and the protein sequence defining, the mhm (I499-R556) construct are set forth in SEQ ID NOS. 213 and 214, respectively. The nucleic acid sequence set forth in SEQ ID NO. 213 includes the signal sequence (nucleotides 1-96) and the prodomain (nucleotides 97-165), and the protein sequence set forth in SEQ ID NO. 214 includes the active protein sequence (without the signal sequence or the prodomain). The resulting nucleotide sequence encoding, and the protein sequence defining, the mhm (W507-L585) are set forth in SEQ ID NOS. 215 and 216, respectively. The nucleic acid sequence set forth in SEQ ID NO. 215 includes the signal sequence (nucleotides 1-96) and the prodomain (nucleotides 97-165), and the protein sequence set forth in SEQ ID NO. 216 includes the active protein sequence (without the signal sequence or the prodomain).

e. Protein Expression

(1) Cell culture

[0208] CHO FlpIn cells (Invitrogen, Catalog No. R758-07)) were grown in F12K media (ATCC, Catalog No. 30-2004), 10% FCS (Invitrogen, Catalog No. 10438026), 1% Penicillin (10000 units/mL) /Streptomycin (10,000 µg/mL) (Invitrogen, Catalog No. 15140-122) at 37°C, 5% CO_2, 100 µg/mL Zeocin (Invitrogen, Catalog No. R250-01).

(2) Generation of Stable CHO FlpIn Cell Lines

[0209] CHO FlpIn host cells were transfected with a 9:1 ratio of pOG44:pcDNA5/FRT expression plasmid DNA using lipofectamine 2000 according to the manufacturer's instructions (Invitrogen, Catalog No. 11668-027). As controls, cells were transfected with empty pcDNA5/FRT vector/pOG44 and pOG44 plasmid (Invitrogen, Catalog No. 35-3018) alone. Twenty four hours after transfection, the cells were split, and after forty eight hours 0.5 mg/mL Hygromycin B (Sigma, Catalog No. H0654-SPEC) was added to the cells. Polyclonal selection of stable cells was performed in F12K, 10% FCS, 1% Penicillin/Streptomycin, 0.5 mg/mL Hygromycin B.

(3) Protein expression in stable CHO FlpIn cell lines

[0210] Approximately $2x10^6$ cells were seeded in 15 cm plates and grown in F12K (ATCC, Catalog No. 30-2004)/DMEM high glucose (Invitrogen, Catalog No. 11995065) 1:1, 5% ultra low IgG FCS (Invitrogen, #16250-78) at 37°C, 5% CO_2 for 5-6 days. Supernatants were harvested and resulting proteins analyzed by ELISA and by surface plasmon resonance.

Example 4 – Binding Characteristics of Anti-hHGF Monoclonal Antibodies

[0211] The monoclonal antibodies produced in Example 1 were characterized by their ability to bind hHGF, and certain of the recombinant HGF proteins produced in Example 3.

[0212] The antibodies were analyzed by surface-plasmon resonance using a BIAcore T100 instrument to assess their ability to bind HGF and certain of the fusion proteins discussed in Example 3. Each antibody was immobilized on a carboxymethylated dextran CM5 sensor chip (BIAcore, Catalog No. BR-1006-68) by amine coupling (BIAcore, Catalog No. BR-1000-50) using a standard coupling protocol according to manufacturer's instructions.

[0213] Analyses were performed at 25°C using PBS (GIBCO, Catalog No. 14040-133) containing 0.05% surfactant P20 (BIAcore, Catalog No. R-1000-54), 2 mg/mL BSA (EMD, Catalog No. 2930) and 10 mg/mL CM-Dextran Sodium salt (Fluka, Catalog No. 86524) as running buffer. Supernatant containing different HGF fusion proteins or supernatant from cells transfected with empty vector were injected over each antibody at a flow rate of 30 μL/min for 3 minutes. The resulting binding was determined as resonance units (RU) over baseline 30 seconds after the end of injection. Binding was compared to human HGF (R&D Systems, Catalog No. 294-HGN-025) diluted in running buffer. Non-specific binding was monitored by comparing binding to a control surface where mouse IgG (Rockland, Catalog No. 010-0102) was immobilized using the same amine coupling procedure.

[0214] The results are summarized in the Table 3.

TABLE 3

Antibody	rhHGF (R&D Systems)	rmHGF (R&D Systems)	mhm chimera (V495-L585)	human HGF	G555E	C561R
1A3	Yes	No	No	Yes	Yes	Yes
1D3	Yes	No	Yes	Yes	Yes	Yes
1F3	Yes	Yes	Yes	Yes	Yes	Yes
2B8	Yes	No	Yes	Yes	Yes	Yes
2F8	Yes	Yes	No	Yes	Yes	Yes
3A12	Yes	No	No	Yes	Yes	Yes
3B6	Yes	No	No	Yes	Yes	Yes
3D11	Yes	No	No	Yes	Yes	Yes

[0215] The results in Table 3 demonstrate that each of the antibodies bind rHGF and purified human HGF. Furthermore, all of the antibodies bind hHGF containing point mutations G555E and C561R. In general, all of the antibodies except for 1F3 and 2F8 did not bind murine HGF demonstrating that the antibodies 1A3, 1D3, 2B8, 3A12, 3B6, and 3D11 specifically bind human HGF. Antibodies 1D3, 1F3, and 2B8 bind the mouse-human-mouse chimera whereas the remaining antibodies did not. The results suggest that the antibodies 1D3 and 2B8 at least in part bind to residues 495-585 of human HGF. The antibodies 1A3, 3A12, 3B6, and 3D11 appear to bind portions of human hHGF other than residues 495-585. At present, it is uncertain why 2F8 does not bind the mhm chimera as it appears to bind both hHGF and mHGF.

Example 5 – Ability of Anti-hHGF Monoclonal Antibodies to Bind Reduced and Non-reduced HGF

[0216] In this Example, the anti-hHGF monoclonal antibodies produced in Example 1 were analyzed for their ability to bind reduced and non-reduced HGF.

[0217] The reactivity of the anti-HGF sera with the recombinant hHGF was assessed by immunoblotting. Eight μg of recombinant hHGF protein in NuPAGE MOPS SDS running buffer (Invitrogen) with or without NuPAGE sample reducing buffer (Invitrogen) was fractionated on a 4-12% Bis-Tris 1.0mmX2D well gel (Invitrogen, Carlsbad, CA). The fractionated proteins then were transferred onto a nitrocellulose membrane using standard procedures. The nitrocellulose membranes were blocked with 5% nonfat milk powder solution

in Tris buffered Saline with 0.1% Tween-20® (TBST), and then mounted onto a Mini Protean II Multi-Screen apparatus (BioRad) for further blocking.

[0218] The resulting membranes were probed with the purified antibodies on a Multi-Screen apparatus. The purified antibodies were diluted to 5μg/mL in blocking buffer. The nitrocellulose membrane then was removed from the apparatus, and incubated with horseradish peroxidase-labeled anti-mouse IgG antibodies. The results are summarized in Table 4, where the numbers reflect the extent of binding with - representing the least (little or no binding) and 3+ representing the most binding.

TABLE 4

Antibody	Reduced (exposure: 3-5min)	Non-Reduced (exposure: 20sec)
1A3	2+	2+
1D3	2+	2+
1F3	2+	2+
2B8	-	1+
2F8	2+	2+
3A12	-	2+
3B6	3+	2+
3D11	-	3+

[0219] The data in Table 4 demonstrate that all the antibodies bind non-reduced rhHGF. In contrast, monoclonal antibodies 1A3, 1D3, 1F3, 2F8, 3B6 bound reduced rhHGF but antibodies 2B8, 3A12, and 3D11 did not bind to reduced rhHGF.

Example 6 – Binding Affinities

[0220] The binding affinities and kinetics of interaction of each of the antibodies produced in Example 1 against hHGF were measured by surface plasmon resonance.

[0221] Rabbit anti-mouse immunoglobulins (BIAcore, Catalog No. BR-1005-14) were immobilized on carboxymethylated dextran CM5 sensor chips (BIAcore, Catalog No. BR-1006-68) by amine coupling (BIAcore, Catalog No. BR-1000-50) using a standard coupling protocol according to manufacturer's instructions. The analyses were performed at 25°C using PBS (GIBCO, Catalog No. 14040-133) containing 0.05% surfactant P20 (BIAcore, Catalog No.

BR-1000-54), 2 mg/mL BSA (EMD, Catalog No. 2930), and 10 mg/mL CM-Dextran Sodium salt (Fluka, Catalog No. 86524) as running buffer.

[0222] The antibodies were captured in an individual flow cell at a flow rate of 10 μL/min. Injection time was variable for each antibody to yield approximately 20 RU of antibody captured for each cycle. Buffer or HGF (R&D Systems, Catalog No. 294-HGN-025) diluted in running buffer was injected sequentially over a reference surface (no antibody captured) and the active surface (antibody to be tested) for 2 minutes at 60 μL/min. The dissociation phase was monitored for 15 or 90 minutes, depending on concentration. The surface then was regenerated with 10mM Glycine-HCl, pH 1.7 (BIAcore, Catalog No. BR-1003-54) injected for 3 minutes at a flow rate of 60 μL/min before another cycle was initiated. HGF concentrations tested were 0.46 nM to 7.5 nM.

[0223] Kinetic parameters were determined using the kinetic function of the BIAevalutation software with reference subtraction. Kinetic parameters for each antibody, k_a (association rate constant), k_d (dissociation rate constant) and K_D (equilibrium dissociation constant) are summarized in Table 5.

TABLE 5

Antibody	ka (1/Ms)	SE (ka)	kd (1/s)	SE (kd)	K_D (pM)	SD
1A3	$1.7x10^6$	$7.3x10^4$	$5.2x10^{-5}$	$8.4x10^{-7}$	30.1	5.6
1D3	$1.7x10^6$	$3.1x10^4$	$8.2x10^{-5}$	$1.7x10^{-6}$	54.2	27.4
1F3	$1.5x10^6$	$5.0x10^4$	$2.6x10^{-5}$	$6.6x10^{-7}$	18.1	8.2
2B8	$1.6x10^6$	$2.9x10^4$	$2.1x10^{-5}$	$1.4x10^{-7}$	13.5	4.4
3A12	$1.6x10^6$	$3.7x10^4$	$1.6x10^{-4}$	$1.6x10^{-6}$	103.0	10.4
3B6	$2.0x10^6$	$6.5x10^4$	$3.9x10^{-5}$	$3.2x10^{-7}$	17.0	3.4

[0224] The data in Table 5 demonstrate that the antibodies bind hHGF with a K_D of about 100 pM or less, about 50 pM or less, or 20 pM or less.

Example 7 – Neutralization Activity of Anti-hHGF Antibodies

[0225] In this Example, the antibodies produced in Example 1 were characterized for their ability to (a) inhibit the binding of hHGF to c-Met, and (b) inhibit HGF stimulated BrdU incorporation in 4MBr-5 cells.

a. HGF-Met Binding Inhibition Assay (Neutralization Assay)

[0226] The antibodies were tested by ELISA for their ability to inhibit hHGF binding to c-Met.

[0227] Specifically, Wallac 96-well DELFIA assay plates (Wallac Inc., Catalog No. AAAND-0001) were coated with 100 μL of 6.25 μg/mL HGF (R&D Systems, Catalog No. 294-HGN-025) in carbonate coating buffer (15 mM Na_2CO_3 and 34 mM $NaHCO_3$, pH 9.0) for 16 hours at 4°C. The plates then were blocked with 200 μL of 5% non-fat dry milk in PBS for 1 hour at room temperature. The antibodies were prepared in a separate plate by adding increasing concentrations of the antibodies under investigation (0.033-667nM, 3-fold-serial dilution) to 2nM c-Met (R&D Systems, Catalog No. 358-MT/CF) in 5% non-fat dry milk in PBS. 100 μL of sample per well was transferred to the assay plate and incubated overnight at 4°C. The assay plates then were washed 3 times with PBS-0.1% Tween 20, and incubated for 2 hours at room temperature with 100 μL/well of 2 μg/mL biotinylated anti-human c-Met antibody (R&D Systems, Catalog No. BAF358) prepared in 5% non-fat dry milk in PBS.

[0228] The resulting plates then were washed three times with PBS-0.1% Tween 20, and incubated for 1 hour at room temperature with Eu-labeled Streptavidin (Wallac, Catalog No. 1244-360) diluted 1:1000 in DELFIA assay buffer (Wallac, Catalog No. 4002-0010). The resulting plates were washed 3 times with DELFIA wash solution (Wallac, Catalog No. 4010-0010) and incubated with 100 μL/well DELFIA enhancement solution (Wallac #4001-0010) for 15 minutes at room temperature with agitation.

[0229] The plates were read on Victor^{3}V instrument (Perkin Elmer) using the Europium method. The IC_{50} values were calculated and are summarized in Table 6.

TABLE 6

Antibody	IC_{50} (nM)	SD
1A3	5.65	0.91
1D3	4.43	2.27
1F3	6.57	0.28
2B8	5.57	1.19
2F8	5.36	0.88
3A12	5.26	2.11
3B6	-	-
3D11	5.66	2.75

[0230] The results demonstrate that all the antibodies (i.e., 1D3, 1A3, 2B8, 3A12, 1F3, 3D11, and 2F8) other than 3B6 efficiently neutralize HGF binding to c-Met.

b. Neutralization of HGF Stimulated BrdU Incorporation in 4MBr-5 cells

[0231] Ten µL of 12.5 nM of hHGF was dispensed into individual wells of a 96-well tissue culture microtiter plate (Costar Catalog No. 3903). Ten µL of serially diluted antibodies at concentrations of 6667, 2222, 740, 247, 82, 27, 9.1, 3.0, 1.0, 0.33 nM were added to each well. The HGF antibody mixture then was incubated at room temperature for 30 minutes. Monkey bronchial epithelial cells 4MBr-5 (ATCC, CCL208) cultured in F-12K (ATCC, 30-2004), 15% FBS (Gibco 10438-026), 30 ng/mL EGF (Sigma E9644), 1% penicillin/streptomycin (PS, Gibco Catalog No. 15140-122) were dissociated with Trypsin (Gibco Catalog No. 25200-056), resuspended in assay media (F-12K, 2.5% FBS, 1% PS) at 75,000 cells/mL, and 80 µL of the cell suspension was dispensed to the HGF antibody mixture.

[0232] The resulting cells were incubated at 37°C, 5% CO_2. Forty eight hours later, 10 µL of 100 µM BrdU (Roche Catalog No. 1669915) was added. Seventy two hours later, the media was removed, the plates were dried with a hair dryer and were processed with the BrdU ELISA in accordance with manufacturer's instructions (Roche Catalog No. 1669915).

[0233] The luminescent signal was quantified by a Synergy HT plate reader (Bio-Tek). The data were fit to a sigmoidal dose response with variable slope with the equation y = bottom + (top-bottom)/(1+10^(log(EC50-x)*hill slope)) in GraphPad Prism (GraphPad Software). Each experiment was repeated at least 3 times in duplicates, and average EC_{50} values are presented in Table 7.

TABLE 7

Antibody	IC_{50} (nM)
1A3	4.69
1D3	4.99
1F3	1.94
2B8	1.41
2F8	19.24
3A12	30.30
3B6	36.08
3D11	51.12

[0234] The results in Table 7 demonstrate that all of the antibodies, 1A3, 1D3, 1F3, 2B8, 2F8, 3A12, 3B6, and 3D11 inhibit HGF induced proliferation in 4MBr-5 cells.

Example 8 – Anti-Scatter Activity of Anti-hHGF Antibodies

[0235] This Example describes a characterization of the antibodies produced in Example 1 for their ability to inhibit HGF induced scatter activity. HGF induces "scattering" (motility) of clusters in MDCK cells (ATCC, Manassas, VA, Catalog No. CCL-34).

[0236] MDCK cells were seeded in 96-well Costar tissue culture plates (Corning Incorporated, Corning, NY, Catalog No. 3595) at a density of $4x10^3$ cells per well in 80 µL MEM (ATCC, Manassas, VA, Catalog No. 30-2003) containing 10% Fetal Bovine Serum (Invitrogen Catalog No. 10438026), and 1% penicillin-streptomycin (Invitrogen Catalog No. 15140122). Each of the antibodies to be investigated was diluted to 6,667 nM in MEM containing 10% Fetal Bovine Serum and 1% penicillin-streptomycin. Each of the different antibody dilutions, as well as MEM containing 10% Fetal Bovine Serum and 1% penicillin-streptomycin without antibody, then was separately combined with an equal volume of MEM containing 10% Fetal Bovine Serum and 1% penicillin-streptomycin, and 100 ng/ml HGF (R&D Systems Catalog No. 294-HGN-025). The antibody/HGF dilutions were incubated for 30 minutes at 25°C. Twenty µL of each antibody/HGF dilution was added separately to individual wells, yielding a final antibody concentration of 666.7 nM, and a final HGF concentration of 10 ng/ml. The MDCK cells then were incubated for 24 hours at 37°C with 5% CO_2.

[0237] After 24 hours incubation, the MDCK cells were carefully washed once with 100 µL per well of ice-cold PBS (Invitrogen Catalog No. 14190144), and fixed with 100 µL per well of ice-cold methanol while rocking for 10 minutes at 25°C. The plates then were washed

carefully once with distilled water. A volume of 100 µL crystal violet solution, consisting of 0.5% crystal violet (Sigma, St. Louis, MO, Catalog No. C3886) and 50% ethanol in distilled water, was added to each well, and the cells were incubated for 20 minutes at 25°C while rocking.

[0238] Following staining with crystal violet solution, the cells were washed carefully three times with distilled water. Then, PBS was added to each well to prevent drying of samples. The cells were imaged using the Leica DMIRB microscope (Leica Microsystems GmbH, Wetzler, Germany), DC500 camera (Leica Microsystems GmbH, Wetzler, Germany), and MagnaFire 2.1C software (Optronics, Goleta, CA), and samples were rated for level of scattering. The results are summarized in Table 8.

TABLE 8

Inhibition of HGF-induced MDCK Cell Scattering		
Antibody	Trial 1	Trial 2
1A3	++	+
1D3	++	++
1F3	+	+
2B8	+++	+++
2F8	+	+
3A12	-	-/+
3B6	++	++
3D11	-	-

- No Inhibition
+++ Very strong, nearly complete inhibition
++ Strong inhibition
+ Detectable inhibition

[0239] The results in Table 8 demonstrate that antibody 2B8 inhibited HGF-induced scattering more than the other antibodies. Antibodies 1D3 and 3B6 displayed an intermediate level of inhibition; antibody 1A3 displayed a low to intermediate level of inhibition: antibodies 1F3 and 2F8 displayed a low level of inhibition; and antibodies 3A12 and 3D11 gave little or no detectable inhibition.

Example 9 – Inhibition of HGF-stimulated c-Met Phosphorylation

[0240] This Example describes a characterization of the antibodies produced in Example 1 for their ability to inhibit the HGF-stimulated c-Met phosphorylation in PC-3 cells. HGF induces phosphorylation of Met in PC-3 cells (ATCC No. CRL-1435).

[0241] PC-3 cells were seeded into individual wells of 96-well Costar tissue culture plates (Corning Catalog No. 3595) at a density of $4.5x10^4$ cells per well in 100 µL F-12K (ATCC, Manassas, VA, Catalog No. 30-2004) containing 10% Fetal Bovine Serum (Invitrogen Catalog No. 10438026) and 1% penicillin-streptomycin (Invitrogen Catalog No. 15140122). After 24 hours at 37°C with 5% CO_2, the media was removed, and cells were rinsed once with serum-free F-12K containing 1% penicillin-streptomycin. Cells then were incubated for 24 hours in 100 µL serum-free F-12K containing 1% penicillin-streptomycin.

[0242] The following 10 different dilutions of each of the antibodies being investigated were prepared in serum-free F-12K containing 1% penicillin-streptomycin: 6667 nM, 2222 nM, 741 nM, 247 nM, 82.3 nM, 27.4 nM, 9.1 nM, 3.0 nM, 1.0 nM, and 0.3 nM. Each antibody dilution, and, serum-free F-12K containing 1% penicillin-streptomycin without antibody, were separately combined with an equal volume of serum-free F-12K containing 1% penicillin-streptomycin and 500 ng/mL HGF (R&D Systems Catalog No. 294-HGN-025). These antibody/HGF dilutions were incubated for 30 minutes at 25°C. This resulted in a final concentration of 1.25 nM HGF.

[0243] The PC-3 cells then were rinsed once with serum-free F-12K containing 1% penicillin-streptomycin. Next, 70 µL of serum-free F-12K containing 1% penicillin-streptomycin was added to the cells, followed by 10 µL of 10 mM Na_3VO_4 (Sigma Catalog No. S6508) in serum-free F-12K containing 1% penicillin-streptomycin. The cells then were incubated for 60 minutes at 37°C with 5% CO_2. Following this incubation, 20 µL of each antibody/HGF dilution was added separately to separate wells, yielding a final HGF concentration of 50 ng/mL, and the following final concentrations of each antibody: 666.7 nM, 222.2 nM, 74.1 nM, 24.7 nM, 8.23 nM, 2.74 nM, 0.91 nM, 0.30 nM, 0.10 nM, 0.03 nM. The cells then were incubated for 10 minutes at 37°C with 5% CO_2, after which point the media/antibody/HGF mixture was removed, the plates were placed on ice. The cells then were rinsed once with 100 µL per well of ice-cold PBS (Invitrogen Catalog No. 14190144) containing 1 mM Na_3VO_4. The cells then were incubated for 30 minutes at 4°C in 100 µL per

well ice-cold lysis buffer consisting of 1% OmniPur Triton X-100 (MERCK KGaA, Darmstadt, Germany, Catalog No. 9410), 50 mM Tris-HCl pH 8.0, 100 mM NaCl, 0.3 mM Na_3VO_4, 1x protease inhibitor cocktail (Sigma Catalog No. P8340), and 1x phosphatase inhibitor cocktail 2 (Sigma Catalog No. 5726).

[0244] Biotinylated anti-human HGF-R (c-met) antibody (R&D Systems Catalog No. BAF358) was diluted to a concentration of 2 μg/mL in DELFIA Assay Buffer (PerkinElmer, Turku, Finland, Catalog No. 4002-0010) containing 1% bovine serum albumin (Sigma Catalog No. A2153), and 50 μL of this dilution was added per well of yellow streptavidin microtitration plates (PerkinElmer Catalog No. AAAND-0005). The plates then were incubated with antibody for 30 minutes at 25°C with rocking. Following incubation, the plates were washed with DELFIA wash solution (PerkinElmer Catalog No. 4010-0010), and 80 μL of each of the different PC-3 cell lysates was added separately to individual wells of the washed streptavidin microtitration plates.

[0245] The streptavidin microtitration plates containing PC-3 cell lysates were incubated for 60 minutes at 25°C with shaking, and then washed with DELFIA wash solution. 100 μL of 600 ng/mL DELFIA Eu-N1 P-Tyr-100 antibody (PerkinElmer Catalog No. AD0159) diluted in DELFIA Assay Buffer containing 1% bovine serum albumin was added to each well of the washed streptavidin microtitration plates previously incubated with PC-3 cell lysates. The plates were incubated for 60 minutes at 25°C, with rocking. The plates were washed a final time with DELFIA wash solution. Then 200 μL of DELFIA Enhancement Solution (PerkinElmer Catalog No. 4001-0010) was added to each well of the washed streptavidin microtitration plates, and the plates were incubated in the dark for 5 minutes at 25°C, with shaking.

[0246] Signal then was measured using the Europium protocol on the Victor3V reader (PerkinElmer). EC_{50} values were calculated using Prism 4 for Windows (GraphPad Software, Inc., San Diego, CA) and the sigmoidal dose-response equation.

[0247] The results summarized as EC50s in nM are tabulated in Table 9.

TABLE 9

Antibody	Average of Two Trials	Standard Deviation
1A3	0.684	0.242
1D3	0.984	0.129
1F3	1.19	1.01
2B8	0.287	0.104
2F8	1.39	2.12
3A12	2.00	0.553
3B6	1.01	1.11
3D11	2.28	N/A

[0248] The data in Table 9 demonstrate that all eight antibodies are potent inhibitors of HGF-induced c-Met phosphorylation in PC-3 cells.

Example 10 – Tumor Inhibition in U87MG Xenograft Model

[0249] The ability of murine monoclonal antibodies of the invention to inhibit tumor growth was tested in an U87MG xenograft model. U87MG cells (ATCC) were expanded in culture at 37°C in an atmosphere containing 5% CO2 and 95% air, using a medium comprising Dulbecco's Modified Eagle medium (DMEM) with 10% fetal bovine serum, 100 units/mL penicillin and 100 μg/mL streptomycin. The cells were subcultured and maintained by detaching the cells from the wall of the culture dish using trypsin-EDTA.

[0250] Near-confluent cells were collected by trypsinization and then $5x10^6$ cells in 50% Matrigel (BD Biosciences; catalog no. 356237) were injected subcutaneously into the upper dorsal area between the shoulder blades of 7-week old female ICR SCID mice (Taconic Labs). The long (L) and short (W) diameters (mm) of tumors were measured with a caliper. Tumor volume (vol.) was calculated as: volume (mm^3) = $L \times W^2 / 2$. When the tumors grew to approximately 200 mm^3, the tumor-bearing mice were randomized into 5 groups of 10 mice each. One group received PBS. Each of the other 4 groups received one of the antibody 1A3, 1D3, 1F3 or 2B8. All antibodies were dosed at 1 mg/kg body weight, twice per week, by intraperitoneal injections of 5 doses. Tumor volumes and mouse body weights were recorded twice per week. Tumor growth inhibition was analyzed using Student's t-test. The results are summarized in Figure 6 and Table 10.

Table 10

	Percent Inhibition	
2B8 vs PBS	93%	p=0.001
1A3 vs PBS	73%	p=0.0075
1D3 vs PBS	51%	p=0.075
1F3 vs PBS	60%	p=0.027

[0251] Partial regression was achieved in 2B8 treated group (Figure 6). Statistically significant growth inhibition was observed in the 1A3-treated and 1F3-treated groups (Table 10). There was 51% tumor growth inhibition for 1D3 with a p value of 0.075. No significant body weight loss was observed.

Example 11 – Tumor Inhibition in U118 Xenograft Model

[0252] The ability of the antibodies 1A3, 1D3, 1F3 and 2B8 to inhibit tumor growth was tested in an U118 xenograft model. U118 cells (ATCC) were expanded as described in Example 10 (above) with respect to the U87MG cells.

[0253] Subcutaneous tumors were established as described in Example 10 above, except that the mice used were 7 weeks old female NCr nude mice (Taconic), and treatment was started when the tumors grew to approximately 80 mm^3. As in the U87MG model, all the antibodies were dosed at 1 mg/kg body weight twice a week by intra-peritoneal injections for 4 doses. Tumor volumes and body weights of the mice were recorded twice per week. Tumor growth inhibition was analyzed using Student's t-test. The results are summarized in Figure 7 and Table 11.

Table 11

	Percent Inhibition	
2B8 vs IgG	75%	p=0.007
1A3 vs IgG	57%	p=0.01
1D3 vs IgG	47%	p=0.12
1F3 vs IgG	30%	p=0.39

[0254] Statistically significant tumor growth inhibition was observed in 2B8 and 1A3 treated groups (Figure 7). There was modest tumor growth inhibition in 1F3 and 1D3 groups with p values less than 0.05, which was defined as statistical significance in this study (Table 11). No significant body weight loss was observed.

Example 12 – Humanization of Murine Monoclonal Antibodies

[0255] This Example describes the humanization of the murine 2B8 antibody, together with a characterization of the resulting humanized antibodies. The murine 2B8 Heavy and Light Variable Regions were "humanized" by two methods.

A. Humanization Procedure 1

[0256] In the first method, three humanized heavy chain variable regions and two humanized kappa light chain variable regions were designed based on the "superhumanization" method described in Hwang *et al.* (2005) METHODS 36:35-42; Tan *et al.* (2002) J. IMMUNOL. 169:1119-1125; U.S. Patent No. 6,881,557.

[0257] The Chothia canonical structural class was determined for each mouse 2B8 CDR based on CDR length and amino acid composition. Human germline variable regions consisting of the same Chothia canonical structural class light and heavy variable regions were identified based on known human germline variable region reference alleles described at the International Immunogentics Information System (IMGT) website (available on the world wide web at imgt.cines.fr and biochem.unizh.ch/antibody/Sequences/index.html). These human germline variable regions of the same structural class were compared to murine 2B8 variable regions by calculating the percent identity or similarity between CDR amino acid residues. Those human germline variable regions with the highest identity and/or similarity with mouse 2B8 CDR residues were chosen for CDR grafting. The framework residues of the human germline variable regions were preserved while the mouse 2B8 CDR residues were used to replace the corresponding human germline variable region residues that were different between mouse 2B8 CDR and human germline CDRs. The human J region that was most similar to the 2B8 mouse J region was then added to the carboxyl terminus of the "superhumanized" variable region. A signal sequence was then added to the amino terminus of the "superhumanized" variable regions and these amino acid sequences were converted into nucleic acid sequences.

[0258] The complete variable region nucleic acid sequence was constructed using gene synthesis PCR methods (Young *et al.* (2004) NUCL. ACIDS RES. 32:e59) and cloned into a mammalian expression vector (based on pcDNA3.2 DEST (Invitrogen)) containing human constant IgG1 (G1m(17,1) allotype) or Kappa (Km(3) allotype (allele 2)) regions (downstream of the variable regions) using standard molecular biology techniques. All four heavy chain IgG1 antibodies (chimeric 2B8 and 3 humanized heavy chains (Hu2B8 Hv1-f.1, Hu2B8 Hv5-

a.1, Hu2B8 Hv5-51.1) were expressed in the possible combinations with all 3 kappa chain antibodies (chimera 2B8 and 2 humanized light chains (Hu2B8 Kv1-39.1 and Hu2B8 Kv3-15.1) creating 12 different antibody proteins. Binding of the chimeric, chimeric/humanized, and humanized antibodies to human HGF was then measured as described below and the results are summarized in Figure 8. Each of the possible combinations of immunoglobulin heavy chain and immunoglobulin light chain variable regions are set forth below in Table 12A.

Table 12A

Heavy Chain Variable Region	Light Chain Variable Region
Chimeric 2B8 (SEQ ID NO: 12)	Chimeric 2B8 (SEQ ID NO: 14)
Chimeric 2B8 (SEQ ID NO: 12)	Hu2B8 Kv1-39.1 (SEQ ID NO: 173)
Chimeric 2B8 (SEQ ID NO: 12)	Hu2B8 Kv3-15.1 (SEQ ID NO: 179)
Hu2B8 Hv1-f.1 (SEQ ID NO: 159)	Chimeric 2B8 (SEQ ID NO: 14)
Hu2B8 Hv1-f.1 (SEQ ID NO: 159)	Hu2B8 Kv1-39.1 (SEQ ID NO: 173)
Hu2B8 Hv1-f.1 (SEQ ID NO: 159)	Hu2B8 Kv3-15.1 (SEQ ID NO: 179)
Hu2B8 Hv5-a.1 (SEQ ID NO: 165)	Chimeric 2B8 (SEQ ID NO: 14)
Hu2B8 Hv5-a.1 (SEQ ID NO: 165)	Hu2B8 Kv1-39.1 (SEQ ID NO: 173)
Hu2B8 Hv5-a.1 (SEQ ID NO: 165)	Hu2B8 Kv3-15.1 (SEQ ID NO: 179)
Hu2B8 Hv5-51.1 (SEQ ID NO: 169)	Chimeric 2B8 (SEQ ID NO: 14)
Hu2B8 Hv5-51.1 (SEQ ID NO: 169)	Hu2B8 Kv1-39.1 (SEQ ID NO: 173)
Hu2B8 Hv5-51.1 (SEQ ID NO: 169)	Hu2B8 Kv3-15.1 (SEQ ID NO: 179)

[0259] Each of the possible combinations of immunoglobulin heavy chains and immunoglobulin light chains are set forth below in Table 12B.

Table 12B

Immunoglobulin Heavy Chain	Immunoglobulin Light Chain
Chimeric 2B8 IgG1 (SEQ ID NO: 155)	Chimeric 2B8 Kappa (Km(3)) (SEQ ID NO: 157)
Chimeric 2B8 IgG1 (SEQ ID NO: 155)	Hu2B8 Kv1-39.1 + Kappa Constant (Km(3) allotype) (allele 2) (SEQ ID NO: 177)
Chimeric 2B8 IgG1 (SEQ ID NO: 155)	Hu2B8 Kv3-15.1 + Kappa Constant (Km(3) allotype) (allele 2) (SEQ ID NO: 181)
Hu2B8 Hv1-f.1 + IgG1 Constant (G1M(17,1)) allotype (SEQ ID NO: 163)	Chimeric 2B8 Kappa (Km(3)) (SEQ ID NO: 157)
Hu2B8 Hv1-f.1 + IgG1 Constant (G1M(17,1)) allotype (SEQ ID NO: 163)	Hu2B8 Kv1-39.1 + Kappa Constant (Km(3) allotype) (allele 2) (SEQ ID NO: 177)
Hu2B8 Hv1-f.1 + IgG1 Constant (G1M(17,1)) allotype (SEQ ID NO: 163)	Hu2B8 Kv3-15.1 + Kappa Constant (Km(3) allotype) (allele 2) (SEQ ID NO: 181)
Hu2B8 Hv5-a.1 + IgG1 Constant (G1M(17,1)) allotype (SEQ ID NO: 167)	Chimeric 2B8 Kappa (Km(3)) (SEQ ID NO: 157)
Hu2B8 Hv5-a.1 + IgG1 Constant (G1M(17,1)) allotype (SEQ ID NO: 167)	Hu2B8 Kv1-39.1 + Kappa Constant (Km(3) allotype) (allele 2) (SEQ ID NO: 177)
Hu2B8 Hv5-a.1 + IgG1 Constant (G1M(17,1)) allotype (SEQ ID NO: 167)	Hu2B8 Kv3-15.1 + Kappa Constant (Km(3) allotype) (allele 2) (SEQ ID NO: 181)
Hu2B8 Hv5-51.1 + IgG1 Constant (G1M(17,1)) allotype (SEQ ID NO: 171)	Chimeric 2B8 Kappa (Km(3)) (SEQ ID NO: 157)
Hu2B8 Hv5-51.1 + IgG1 Constant (G1M(17,1)) allotype (SEQ ID NO: 171)	Hu2B8 Kv1-39.1 + Kappa Constant (Km(3) allotype) (allele 2) (SEQ ID NO: 177)
Hu2B8 Hv5-51.1 + IgG1 Constant (G1M(17,1)) allotype (SEQ ID NO: 171)	Hu2B8 Kv3-15.1 + Kappa Constant (Km(3) allotype) (allele 2) (SEQ ID NO: 181)

[0260] Two of the possible antibody constructs containing the full length immunoglobulin heavy and light chains containing humanized variable regions are designated below:

sh2B8-9 (G1m(17,1)) = hu2B8 Hv5-51.1 (+ IgG1 constant region (G1m(17,1) allotype) (SEQ ID NO. 171) plus hu2B8 Kv 1-39.1 (+ Kappa constant region (Km(3) allotype (allele 2))) (SEQ ID NO. 177)

sh2B8-12 (G1m(17,1)) = hu2B8 Hv5-51.1 (+ IgG1 constant region (G1m(17,1) allotype)) (SEQ ID NO. 171) plus hu2B8 Kv 3-15.1 (+ Kappa constant region (Km(3) allotype (allele 2))) (SEQ ID NO. 181).

[0261] The nucleic acid sequences encoding and the protein sequences defining each of the humanized antibodies are summarized below. In this section, the last nucleotide of each variable region is the first base of the next codon generated by the variable/constant region

junction. This nucleotide is included in the Variable Region because it is part of that exon. Amino acid sequences of Constant Regions listed below include the translation of this junction codon.

[0262] (1) Nucleic Acid Sequence Encoding the Full Length Chimeric 2B8 Heavy Chain (Mouse Variable Region and Human IgG1 Constant Region) (allotype G1m(17,1)) (signal sequence underlined) (SEQ ID NO. 154)

```
   1 atgggatgga gctatatcat cctcttttg gtagcaacag ctacagatgt ccactcccag
  61 gtccaactgc agcagcctgg ggctgaactg gtgaagcctg ggacttcagt gaagctgtcc
 121 tgcaaggctt ctggctacac cttcaccacc tactggatgc actgggtgaa tcagaggcct
 181 ggacaaggcc ttgagtggat tggagagatt aatcctacca acggtcatac taactacaat
 241 gagaagttca agagcaaggc cacactgact gtagacaaat cctccagcac agcctacatg
 301 caactcagca gcctgacatc tgaggactct gcggtctatt actgtgcaag aaactatgtt
 361 ggtagcatct ttgactactg gggccaaggc accactctca ccgtctcctc agcctccacc
 421 aagggcccat cggtcttccc cctggcaccc tcctccaaga gcacctctgg gggcacagcg
 481 gccctgggct gcctggtcaa ggactacttc cccgaaccgg tgacggtgtc gtggaactca
 541 ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcctc aggactctac
 601 tccctcagca gcgtggtgac cgtgccctcc agcagcttgg gcacccagac ctacatctgc
 661 aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttgagcc caaatcttgt
 721 gacaaaactc acacatgccc accgtgccca gcacctgaac tcctgggggg accgtcagtc
 781 ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca
 841 tgcgtggtgg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac
 901 ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac
 961 cgtgtggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag
1021 tgcaaggtct ccaacaaagc cctcccagcc cccatcgaga aaaccatctc caaagccaaa
1081 gggcagcccc gagaaccaca ggtgtacacc ctgccccccat cccgggatga gctgaccaag
1141 aaccaggtca gcctgacctg cctggtcaaa ggcttctatc ccagcgacat cgccgtggag
1201 tgggagagca atgggcagcc ggagaacaac tacaagacca cgcctcccgt gctggactcc
1261 gacggctcct tcttcctcta cagcaagctc accgtggaca agagcaggtg gcagcagggg
1321 aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc
1381 ctctccctgt ctccgggtaa atga
```

[0263] (2) Protein Sequence Defining the Full Length Chimeric 2B8 Heavy Chain (Chimeric 2B8 IgG1 (G1m(17,1) allotype) (without signal sequence) (SEQ ID NO. 155)

```
   1 qvqlqqpgae lvkpgtsvkl sckasgytft tywmhwvnqr pgqglewige inptnghtny
  61 nekfkskatl tvdkssstay mqlssltsed savyycarny vgsifdywgq gttltvssas
 121 tkgpsvfpla psskstsggt aalgclvkdy fpepvtvswn sgaltsgvht fpavlqssgl
 181 yslssvvtvp ssslgtqtyi cnvnhkpsnt kvdkkvepks cdkthtcppc papellggps
 241 vflfppkpkd tlmisrtpev tcvvvdvshe dpevkfnwyv dgvevhnakt kpreeqynst
 301 yrvvsvltvl hqdwlngkey kckvsnkalp apiektiska kgqprepqvy tlppsrdelt
 361 knqvsltclv kgfypsdiav ewesngqpen nykttppvld sdgsfflysk ltvdksrwqq
 421 gnvfscsvmh ealhnhytqk slslspgk
```

[0264] (3) Nucleic Acid Sequence Encoding the Full Length Chimeric 2B8 Light Chain (Mouse Variable Region and Human Constant Region) (Chimeric 2B8 Kappa (Km(3))) (signal sequence underlined) (SEQ ID NO. 156)

```
   1 atggaatcac agactctggt cttcatatcc atactgctct ggttatatgg tgctgatggg
  61 aacattgtaa tgacccaatc tcccaaatcc atgtccatgt cagtaggaga gagggtcacc
```

```
121 ttgagctgca aggccagtga gaatgtggtt tcttatgtat cctggtatca acagaaacca
181 gcgcagtctc ctaaactgct gatatacggg gcatccaacc ggaacactgg ggtccccgat
241 cgcttcacag gcagtggatc tgcaacagat ttcactctga ccatcagcag tgtgcgggct
301 gaagaccttg cagattatca ctgtgggcag agttacaact atccgtacac gttcggaggg
361 gggaccaggc tggaaataaa acgaactgtg gctgcaccat ctgtcttcat cttcccgcca
421 tctgatgagc agttgaaatc tggaactgcc tctgttgtgt gcctgctgaa taacttctat
481 cccagagagg ccaaagtaca gtggaaggtg gataacgccc tccaatcggg taactcccag
541 gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg
601 ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc
661 ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gttga
```

[0265] (4) Protein Sequence Defining the Full Length Chimeric 2B8 Light Chain (Chimeric 2B8 Kappa (Km(3))) (without signal sequence) (SEQ ID NO. 157)

```
  1 nivmtqspks msmsvgervt lsckasenvv syvswyqqkp aqspklliyg asnrntgvpd
 61 rftgsgsatd ftltissvra edladyhcgq synypytfgg gtrleikrtv aapsvfifpp
121 sdeqlksgta svvcllnnfy preakvqwkv dnalqsgnsq esvteqdskd styslsstlt
181 lskadyekhk vyacevthqg lsspvtksfn rgec
```

[0266] (5) Nucleic Acid Sequence Encoding Humanized Hu2B8 Hv1-f.1 Heavy Chain Variable Region (signal sequence underlined) (SEQ ID NO. 158)

```
  1 atggactgca cctggaggat cctcctcttg gtggcagcag ctacaggcac ccacgccgag
 61 gtccagctgg tacagtctgg ggctgaggtg aagaagcctg gggctacagt gaaaatctcc
121 tgcaaggttt ctggatacac cttcaccacc tactggatgc actgggtgca acaggccccc
181 ggaaaagggc ttgagtggat gggagagatt aatcctacca acggtcatac taactacaat
241 gagaagttcc agggcagagt caccataacc gcggacacgt ctacagacac agcctacatg
301 gagctgagca gcctgagatc tgaggacacg gccgtgtatt actgtgcaac aaactatgtt
361 ggtagcatct ttgactactg gggccaagga accctggtca ccgtctcctc ag
```

[0267] (6) Protein Sequence Defining Humanized Hu2B8 Hv1-f.1 Heavy Chain Variable Region (without signal sequence) (SEQ ID NO. 159)

```
 1 evqlvqsgae vkkpgatvki sckvsgytft tywmhwvqqa pgkglewmge inptnghtny
61 nekfqgrvti tadtstdtay melsslrsed tavyycatny vgsifdywgq gtlvtvss
```

[0268] (7) Nucleic Acid Sequence Encoding Human IgG1 Heavy Chain Constant Region (G1m(17,1) allotype) (SEQ ID NO. 160)

1 cctccaccaa gggcccatcg gtcttccccc tggcaccctc ctccaagagc acctctgggg
61 gcacagcggc cctgggctgc ctggtcaagg actacttccc cgaaccggtg acggtgtcgt
121 ggaactcagg cgccctgacc agcggcgtgc acaccttccc ggctgtccta cagtcctcag
181 gactctactc cctcagcagc gtggtgaccg tgccctccag cagcttgggc acccagacct
241 acatctgcaa cgtgaatcac aagcccagca acaccaaggt ggacaagaaa gttgagccca
301 aatcttgtga caaaactcac acatgcccac cgtgcccagc acctgaactc ctggggggac
361 cgtcagtctt cctcttcccc ccaaaaccca aggacaccct catgatctcc cggacccctg
421 aggtcacatg cgtggtggtg gacgtgagcc acgaagaccc tgaggtcaag ttcaactggt
481 acgtggacgg cgtggaggtg cataatgcca agacaaagcc gcgggaggag cagtacaaca
541 gcacgtaccg tgtggtcagc gtcctcaccg tcctgcacca ggactggctg aatggcaagg
601 agtacaagtg caaggtctcc aacaaagccc tcccagcccc catcgagaaa accatctcca
661 aagccaaagg gcagccccga gaaccacagg tgtacaccct gcccccatcc cgggatgagc
721 tgaccaagaa ccaggtcagc ctgacctgcc tggtcaaagg cttctatccc agcgacatcg
781 ccgtggagtg ggagagcaat gggcagccgg agaacaacta caagaccacg cctcccgtgc
841 tggactccga cggctccttc ttcctctaca gcaagctcac cgtggacaag agcaggtggc
901 agcaggggaa cgtcttctca tgctccgtga tgcatgaggc tctgcacaac cactacacgc
961 agaagagcct ctccctgtct ccgggtaaat ga

[0269] (8) Protein Sequence Defining Human IgG1 Heavy Chain Constant Region (G1m(17,1) allotype) (SEQ ID NO. 161). The first amino acid is derived from translation of the last nucleotide of variable region and beginning two nucleotides of the IgG1 Heavy Chain sequence.

1 astkgpsvfp lapsskstsg gtaalgclvk dyfpepvtvs wnsgaltsgv htfpavlqss
61 glyslssvvt vpssslgtqt yicnvnhkps ntkvdkkvep kscdkthtcp pcpapellgg
121 psvflfppkp kdtlmisrtp evtcvvvdvs hedpevkfnw yvdgvevhna ktkpreeqyn
181 styrvvsvlt vlhqdwlngk eykckvsnka lpapiektis kakgqprepq vytlppsrde
241 ltknqvsltc lvkgfypsdi avewesngqp ennykttppv ldsdgsffly skltvdksrw
301 qqgnvfscsv mhealhnhyt qkslslspgk

[0270] (9) Nucleic Acid Sequence Encoding the Full Length Heavy Chain Humanized Hu2B8 Hv1f.1 Variable Region and Human IgG1 (G1m(17,1) allotype) Heavy Chain Constant Region (signal sequence underlined) (SEQ ID NO. 162)

```
   1 atggactgca cctggaggat cctcctcttg gtggcagcag ctacaggcac ccacgccgag
  61 gtccagctgg tacagtctgg ggctgaggtg aagaagcctg gggctacagt gaaaatctcc
 121 tgcaaggttt ctggatacac cttcaccacc tactggatgc actgggtgca acaggccgct
 181 ggaaaagggc ttgagtggat gggagagatt aatcctacca acggtcatac taactacaat
 241 gagaagttcc agggcagagt caccataacc gcggacacgt ctacagacac agcctacatg
 301 gagctgagca gcctgagatc tgaggacacg gccgtgtatt actgtgcaac aaactatgtt
 361 ggtagcatct ttgactactg gggccaagga accctggtca ccgtctcctc agcctccacc
 421 aagggcccat cggtcttccc cctggcaccc tcctccaaga gcacctctgg gggcacagcg
 481 gccctgggct gcctggtcaa ggactacttc cccgaaccgg tgacggtgtc gtggaactca
 541 ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcctc aggactctac
 601 tccctcagca gcgtggtgac cgtgccctcc agcagcttgg gcacccagac ctacatctgc
 661 aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttgagcc caaatcttgt
 721 gacaaaactc acacatgccc accgtgccca gcacctgaac tcctgggggg accgtcagtc
 781 ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca
 841 tgcgtggtgg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac
 901 ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac
 961 cgtgtggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag
1021 tgcaaggtct ccaacaaagc cctcccagcc cccatcgaga aaaccatctc caaagccaaa
1081 gggcagcccc gagaaccaca ggtgtacacc ctgccccat cccgggatga gctgaccaag
1141 aaccaggtca gcctgacctg cctggtcaaa ggcttctatc ccagcgacat cgccgtggag
1201 tgggagagca atgggcagcc ggagaacaac tacaagacca cgcctcccgt gctggactcc
1261 gacggctcct tcttcctcta cagcaagctc accgtggaca agagcaggtg gcagcagggg
1321 aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc
1381 ctctccctgt ctccgggtaa atga
```

[0271] (10) Protein Sequence Defining the Full Length Heavy Chain Humanized Hu2B8 Hv1f.1 Variable Region and Human IgG1 Heavy Chain Constant Region (G1m(17,1) allotype) (without signal sequence) (SEQ ID NO. 163)

```
   1 evqlvqsgae vkkpgatvki sckvsgytft tywmhwvqqa pgkglewmge inptnghtny
  61 nekfqgrvti tadtstdtay melsslrsed tavyycatny vgsifdywgq gtlvtvssas
 121 tkgpsvfpla psskstsggt aalgclvkdy fpepvtvswn sgaltsgvht fpavlqssgl
 181 yslssvvtvp ssslgtqtyi cnvnhkpsnt kvdkkvepks cdkthtcppc papellggps
 241 vflfppkpkd tlmisrtpev tcvvvdvshe dpevkfnwyv dgvevhnakt kpreeqynst
 301 yrvvsvltvl hqdwlngkey kckvsnkalp apiektiska kgqprepqvy tlppsrdelt
 361 knqvsltclv kgfypsdiav ewesngqpen nykttppvld sdgsfflysk ltvdksrwqq
 421 gnvfscsvmh ealhnhytqk slslspgk
```

[0272] (11) Nucleic Acid Sequence Encoding Humanized Hu2B8 Hv5a.1 Heavy Chain Variable Region (signal sequence underlined) (SEQ ID NO. 164)

```
   1 atggggtcaa ccgccatcct cgccctcctc ctggctgttc tccaaggagt ctgtgccgaa
  61 gtgcagctgg tgcagtctgg agcagaggtg aaaaagcccg gggagtctct gaggatctcc
 121 tgtaagggtt ctggatacag ctttaccacc tactggatgc actgggtgcg ccagatgccc
 181 gggaaaggcc tggagtggat gggggagatt aatcctacca acggtcatac taactacaat
```

241 ccgtccttcc aaggccacgt caccatctca gctgacaagt ccatcagcac tgcctacctg

301 cagtggagca gcctgaaggc ctcggacacc gccatgtatt actgtgcgag aaactatgtt

361 ggtagcatct ttgactactg gggccaagga accctggtca ccgtctcctc ag

[0273] (12) Protein Sequence Defining Humanized Hu2B8 Hv5a.1 Heavy Chain Variable Region (without signal sequence) (SEQ ID NO. 165)

1 evqlvqsgae vkkpgeslri sckgsgysft tywmhwvrqm pgkglewmge inptnghtny

61 npsfqghvti sadksistay lqwsslkasd tamyycarny vgsifdywgq gtlvtvss

[0274] (13) Nucleic Acid Sequence Encoding the Full Length Humanized Hu2B8 Hv5a.1 Heavy Chain Variable Region and Human IgG1 (G1m(17,1) allotype) Heavy Chain Constant Region (signal sequence underlined) (SEQ ID NO. 166)

```
   1 atggggtcaa ccgccatcct cgccctcctc ctggctgttc tccaaggagt ctgtgccgaa
  61 gtgcagctgg tgcagtctgg agcagaggtg aaaaagcccg gggagtctct gaggatctcc
 121 tgtaagggtt ctggatacag ctttaccacc tactggatgc actgggtgcg ccagatgccc
 181 gggaaaggcc tggagtggat gggggagatt aatcctacca acggtcatac taactacaat
 241 ccgtccttcc aaggccacgt caccatctca gctgacaagt ccatcagcac tgcctacctg
 301 cagtggagca gcctgaaggc ctcggacacc gccatgtatt actgtgcgag aaactatgtt
 361 ggtagcatct ttgactactg gggccaagga accctggtca ccgtctcctc agcctccacc
 421 aagggcccat cggtcttccc cctggcaccc tcctccaaga gcacctctgg gggcacagcg
 481 gccctgggct gcctggtcaa ggactacttc cccgaaccgg tgacggtgtc gtggaactca
 541 ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcctc aggactctac
 601 tccctcagca gcgtggtgac cgtgccctcc agcagcttgg gcacccagac ctacatctgc
 661 aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttgagcc caaatcttgt
 721 gacaaaactc acacatgccc accgtgccca gcacctgaac tcctgggggg accgtcagtc
 781 ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca
 841 tgcgtggtgg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac
 901 ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac
 961 cgtgtggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag
1021 tgcaaggtct ccaacaaagc cctcccagcc cccatcgaga aaaccatctc caaagccaaa
1081 gggcagcccc gagaaccaca ggtgtacacc ctgccccat cccgggatga gctgaccaag
1141 aaccaggtca gcctgacctg cctggtcaaa ggcttctatc ccagcgacat cgccgtggag
1201 tgggagagca atgggcagcc ggagaacaac tacaagacca cgcctcccgt gctggactcc
1261 gacggctcct tcttcctcta cagcaagctc accgtggaca agagcaggtg gcagcagggg
1321 aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc
1381 ctctccctgt ctccgggtaa atga
```

[0275] (14) Protein Sequence Defining the Full Length Humanized Hu2B8 Hv5a.1 Heavy Chain Variable Region and Human IgG1 (G1m(17,1) allotype) Heavy Chain Constant Region (without signal sequence) (SEQ ID NO. 167)

```
   1 evqlvqsgae vkkpgeslri sckgsgysft tywmhwvrqm pgkglewmge inptnghtny
  61 npsfqghvti sadksistay lqwsslkasd tamyycarny vgsifdywgq gtlvtvssas
 121 tkgpsvfpla psskstsggt aalgclvkdy fpepvtvswn sgaltsgvht fpavlqssgl
 181 yslssvvtvp ssslgtqtyi cnvnhkpsnt kvdkkvepks cdkthtcppc papellggps
 241 vflfppkpkd tlmisrtpev tcvvvdvshe dpevkfnwyv dgvevhnakt kpreeqynst
 301 yrvvsvltvl hqdwlngkey kckvsnkalp apiektiska kgqprepqvy tlppsrdelt
```

361 knqvsltclv kgfypsdiav ewesngqpen nykttppvld sdgsfflysk ltvdksrwqq
421 gnvfscsvmh ealhnhytqk slslspgk

[0276] (15) Nucleic Acid Sequence Encoding Humanized Hu2B8 Hv5-51.1 Heavy Chain Variable Region (signal sequence underlined) (SEQ ID NO. 168)

1 atggggtcaa ccgccatcct cgccctcctc ctggctgttc tccaaggagt ctgtgccgaa
61 gtgcagctgg tgcagtctgg agcagaggtg aaaaagcccg gggagtctct gaagatctcc
121 tgtaagggtt ctggatacag ctttaccacc tactggatgc actgggtgcg ccagatgccc
181 gggaaaggcc tggagtggat gggggagatt aatcctacca acggtcatac taactacaat
241 ccgtccttcc aaggccaggt caccatctca gctgacaagt ccatcagcac tgcctacctg
301 cagtggagca gcctgaaggc ctcggacacc gccatgtatt actgtgcgag aaactatgtt
361 ggtagcatct ttgactactg gggccaagga accctggtca ccgtctcctc ag

[0277] (16) Protein Sequence Defining Humanized Hu2B8 Hv5-51.1 Heavy Chain Variable Sequence (without signal sequence) (SEQ ID NO. 169)

1 evqlvqsgae vkkpgeslki sckgsgysft tywmhwvrqm pgkglewmge inptnghtny
61 npsfqgqvti sadksistay lqwsslkasd tamyycarny vgsifdywgq gtlvtvss

[0278] (17) Nucleic Acid Sequence Encoding the Full Length Humanized Hu2B8 Hv5-51.1 Heavy Chain Variable Region and Human IgG1 (G1m(17,1) allotype) Heavy Chain Constant Region (signal sequence underlined) (SEQ ID NO. 170)

1 atggggtcaa ccgccatcct cgccctcctc ctggctgttc tccaaggagt ctgtgccgaa
61 gtgcagctgg tgcagtctgg agcagaggtg aaaaagcccg gggagtctct gaagatctcc
121 tgtaagggtt ctggatacag ctttaccacc tactggatgc actgggtgcg ccagatgccc
181 gggaaaggcc tggagtggat gggggagatt aatcctacca acggtcatac taactacaat
241 ccgtccttcc aaggccaggt caccatctca gctgacaagt ccatcagcac tgcctacctg
301 cagtggagca gcctgaaggc ctcggacacc gccatgtatt actgtgcgag aaactatgtt
361 ggtagcatct ttgactactg gggccaagga accctggtca ccgtctcctc agcctccacc
421 aagggcccat cggtcttccc cctggcaccc tcctccaaga gcacctctgg gggcacagcg
481 gccctgggct gcctggtcaa ggactacttc cccgaaccgg tgacggtgtc gtggaactca
541 ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcctc aggactctac
601 tccctcagca gcgtggtgac cgtgccctcc agcagcttgg gcacccagac ctacatctgc
661 aacgtgaatc acaagcccag caacaccaag gtggacaaga aagttgagcc caaatcttgt
721 gacaaaactc acacatgccc accgtgccca gcacctgaac tcctgggggg accgtcagtc
781 ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca
841 tgcgtggtgg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac
901 ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac
961 cgtgtggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag
1021 tgcaaggtct ccaacaaagc cctcccagcc cccatcgaga aaaccatctc caaagccaaa
1081 gggcagcccc gagaaccaca ggtgtacacc ctgccccat cccgggatga gctgaccaag
1141 aaccaggtca gcctgacctg cctggtcaaa ggcttctatc ccagcgacat cgccgtggag
1201 tgggagagca atgggcagcc ggagaacaac tacaagacca cgcctcccgt gctggactcc
1261 gacggctcct tcttcctcta cagcaagctc accgtggaca agagcaggtg gcagcagggg

```
1321 aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc
1381 ctctccctgt ctccgggtaa atga
```

[0279] (18) Protein Sequence Defining the Full Length Humanized Hu2B8 Hv5-51.1 Heavy Chain Variable Region and Human IgG1 (G1m(17,1) allotype) Heavy Chain Constant Region (without signal sequence) (SEQ ID NO. 171)

```
   1 evqlvqsgae vkkpgeslki sckgsgysft tywmhwvrqm pgkglewmge inptnghtny
  61 npsfqgqvti sadksistay lqwsslkasd tamyycarny vgsifdywgq gtlvtvssas
 121 tkgpsvfpla psskstsggt aalgclvkdy fpepvtvswn sgaltsgvht fpavlqssgl
 181 yslssvvtvp ssslgtqtyi cnvnhkpsnt kvdkkvepks cdkthtcppc papellggps
 241 vflfppkpkd tlmisrtpev tcvvvdvshe dpevkfnwyv dgvevhnakt kpreeqynst
 301 yrvvsvltvl hqdwlngkey kckvsnkalp apiektiska kgqprepqvy tlppsrdelt
 361 knqvsltclv kgfypsdiav ewesngqpen nykttppvld sdgsfflysk ltvdksrwqq
 421 gnvfscsvmh ealhnhytqk slslspgk
```

[0280] (19) Nucleic Acid Sequence Encoding Humanized Hu2B8 Kv1-39.1 Kappa Chain Variable Region (signal sequence underlined) (SEQ ID NO. 172). Two possible start ATGs are shown in uppercase.

```
   1 ATGgacATGa gggtccccgc tcagctcctg gggctcctgc tactctggct ccgaggtgcc
  61 agatgtgaca tccagatgac ccagtctcca tcctccctgt ctgcatctgt aggagacaga
 121 gtcaccatca cttgcaaggc cagtgagaat gtggtttctt atgtatcctg gtatcagcag
 181 aaaccaggga aagcccctaa gctcctgatc tatggggcat ccaaccggaa cactggggtc
 241 ccatcaaggt tcagtggcag tggatctggg acagatttca ctctcaccat cagcagtctg
 301 caacctgaag attttgcaac ttactactgt gggcagagtt acaactatcc gtacacgttt
 361 ggccagggga ccaagctgga gatcaaac
```

[0281] (20) Protein Sequence Defining Humanized Hu2B8 Kv1-39.1 Kappa Chain Variable Region (without signal sequence) (SEQ ID NO. 173)

```
   1 diqmtqspss lsasvgdrvt itckasenvv syvswyqqkp gkapklliyg asnrntgvps
  61 rfsgsgsgtd ftltisslqp edfatyycgq synypytfgq gtkleik
```

[0282] (21) Nucleic Acid Sequence Encoding Human Kappa Chain Constant Region (Km(3) allotype) (allele 2) (SEQ ID NO. 174)

```
   1 gaactgtggc tgcaccatct gtcttcatct tcccgccatc tgatgagcag ttgaaatctg
  61 gaactgcctc tgttgtgtgc ctgctgaata acttctatcc cagagaggcc aaagtacagt
 121 ggaaggtgga taacgccctc caatcgggta actcccagga gagtgtcaca gagcaggaca
 181 gcaaggacag cacctacagc ctcagcagca ccctgacgct gagcaaagca gactacgaga
```

241 aacacaaagt ctacgcctgc gaagtcaccc atcagggcct gagctcgccc gtcacaaaga

301 gcttcaacag gggagagtgt tga

[0283] (22) Protein Sequence Defining Human Kappa Chain Constant Region (Km(3) allotype) (allele 2) (SEQ ID NO. 175). The first amino acid is derived from translation of the last nucleotide of variable region and beginning two nucleotides of the Kappa Light Chain sequence.

1 rtvaapsvfi fppsdeqlks gtasvvclln nfypreakvq wkvdnalqsg nsqesvteqd

61 skdstyslss tltlskadye khkvyacevt hqglsspvtk sfnrgec

[0284] (23) Nucleic Acid Sequence Encoding the Full Length Humanized Hu2B8 Kv1-39.1 Light Chain Variable Region and Human Kappa Chain Constant Region (Km(3) allotype) (allele 2) (signal sequence underlined) (SEQ ID NO. 176)

```
  1 atggacatga gggtccccgc tcagctcctg gggctcctgc tactctggct ccgaggtgcc
 61 agatgtgaca tccagatgac ccagtctcca tcctccctgt ctgcatctgt aggagacaga
121 gtcaccatca cttgcaaggc cagtgagaat gtggtttctt atgtatcctg gtatcagcag
181 aaaccaggga aagcccctaa gctcctgatc tatggggcat ccaaccggaa cactggggtc
241 ccatcaaggt tcagtggcag tggatctggg acagatttca ctctcaccat cagcagtctg
301 caacctgaag attttgcaac ttactactgt gggcagagtt acaactatcc gtacacgttt
361 ggccagggga ccaagctgga gatcaaacga actgtggctg caccatctgt cttcatcttc
421 ccgccatctg atgagcagtt gaaatctgga actgcctctg ttgtgtgcct gctgaataac
481 ttctatccca gagaggccaa agtacagtgg aaggtggata acgccctcca atcgggtaac
541 tcccaggaga gtgtcacaga gcaggacagc aaggacagca cctacagcct cagcagcacc
601 ctgacgctga gcaaagcaga ctacgagaaa cacaaagtct acgcctgcga agtcacccat
661 cagggcctga gctcgcccgt cacaaagagc ttcaacaggg gagagtgttg a
```

[0285] (24) Protein Sequence Defining the Full Length Humanized Hu2B8 Kv1-39.1 Light Chain Variable Region and Human Kappa Chain Constant Region (Km(3) allotype) (allele 1) (SEQ ID NO. 177)

```
  1 diqmtqspss lsasvgdrvt itckasenvv syvswyqqkp gkapklliyg asnrntgvps
 61 rfsgsgsgtd ftltisslqp edfatyycgq synypytfgq gtkleikrtv aapsvfifpp
121 sdeqlksgta svvcllnnfy preakvqwkv dnalqsgnsq esvteqdskd styslsstlt
181 lskadyekhk vyacevthqg lsspvtksfn rgec
```

[0286] (25) Nucleic Acid Sequence Encoding Humanized Hu2B8 Kv3-15.1 Light Chain Variable Region (signal sequence underlined) (SEQ ID NO. 178)

1 atggaagccc cagcgcagct tctcttcctc ctgctactct ggctcccaga taccactgga

61 gaaatagtga tgacgcagtc tccagccacc ctgtctgtgt ctccagggga aagagccacc

121 ctctcctgca aggccagtga gaatgtggtt tcttatgtat cctggtacca gcagaaacct

181 ggccaggctc ccaggctcct catctatggg gcatccaacc ggaacactgg tatcccagcc

241 aggttcagtg gcagtgggtc tgggacagag ttcactctca ccatcagcag cctgcagtct

301 gaagattttg cagtttatta ctgtgggcag agttacaact atccgtacac gtttggccag

361 gggaccaagc tggagatcaa ac

[0287] (26) Protein Sequence Defining Humanized Hu2B8 Kv3-15.1 Light Chain Variable Region (without signal sequence) (SEQ ID NO. 179)

1 eivmtqspat lsvspgerat lsckasenvv syvswyqqkp gqaprlliyg asnrntgipa

61 rfsgsgsgte ftltisslqs edfavyycgq synypytfgq gtkleik

[0288] (27) Nucleic Acid Encoding the Full Length Humanized Hu2B8 Kv3-15.1 Light Chain Variable Region and Human Kappa Chain Constant Region (Km(3) allotype) (allele 2) (signal sequence underlined) (SEQ ID NO. 180)

```
  1 atggaagccc cagcgcagct tctcttcctc ctgctactct ggctcccaga taccactgga
 61 gaaatagtga tgacgcagtc tccagccacc ctgtctgtgt ctccagggga aagagccacc
121 ctctcctgca aggccagtga gaatgtggtt tcttatgtat cctggtacca gcagaaacct
181 ggccaggctc ccaggctcct catctatggg gcatccaacc ggaacactgg tatcccagcc
241 aggttcagtg gcagtgggtc tgggacagag ttcactctca ccatcagcag cctgcagtct
301 gaagattttg cagtttatta ctgtgggcag agttacaact atccgtacac gtttggccag
361 gggaccaagc tggagatcaa acgaactgtg gctgcaccat ctgtcttcat cttcccgcca
421 tctgatgagc agttgaaatc tggaactgcc tctgttgtgt gcctgctgaa taacttctat
481 cccagagagg ccaaagtaca gtggaaggtg gataacgccc tccaatcggg taactcccag
541 gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg
601 ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc
661 ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gttga
```

[0289] (28) Protein Sequence Defining Humanized Hu2B8 Kv3-15.1 Light Chain Variable Region and Human Kappa Chain Constant Region (Km(3) allotype) (allele 2) (without signal sequence) (SEQ ID NO. 181)

```
  1 eivmtqspat lsvspgerat lsckasenvv syvswyqqkp gqaprlliyg asnrntgipa
 61 rfsgsgsgte ftltisslqs edfavyycgq synypytfgq gtkleikrtv aapsvfifpp
121 sdeqlksgta svvcllnnfy preakvqwkv dnalqsgnsq esvteqdskd styslsstlt
181 lskadyekhk vyacevthqg lsspvtksfn rgec
```

[0290] For convenience, Table 13 provides a concordance chart showing the correspondence between the full length sequences and of the antibodies discussed in this section with those presented in the Sequence Listing.

TABLE 13

SEQ. ID NO.	Protein or Nucleic Acid
154	Chimeric 2B8 IgG1 (G1m(17,1)) – nucleic acid
155	Chimeric 2B8 IgG1 (G1m(17,1)) – protein
156	Chimeric 2B8 Kappa (Km(3)) – nucleic acid
157	Chimeric 2B8 Kappa (Km(3)) – protein
158	Hu2B8 Hv1f.1 Heavy Chain Variable Region – nucleic acid
159	Hu2B8 Hv1f.1 Heavy Chain Variable Region – protein
160	Human IgG1 Heavy Chain Constant Region (G1m(17,1)) allotype – nucleic acid
161	Human IgG1 Heavy Chain Constant Region (G1m(17,1)) allotype – protein
162	Hu2B8 Hv1f.1 + IgG1 Constant (G1m(17,1) allotype) – nucleic acid
163	Hu2B8 Hv1f.1 + IgG1 Constant (G1m(17,1) allotype) – protein
164	Hu2B8 Hv5a.1 Heavy Chain Variable Region – nucleic acid
165	Hu2B8 Hv5a.1 Heavy Chain Variable Region – protein
166	Hu2B8 Hv5a.1 + IgG1 Constant (G1m(17,1) allotype) – nucleic acid
167	Hu2B8 Hv5a.1 + IgG1 Constant (G1m(17,1) allotype) – protein
168	Hu2B8 Hv5-51.1 Heavy Chain Variable Region – nucleic acid
169	Hu2B8 Hv5-51.1 Heavy Chain Variable Region – protein
170	Hu2B8 Hv5-51.1 + IgG1 Constant (G1m(17,1 allotype) – nucleic acid
171	Hu2B8 Hv5-51.1 + IgG1 Constant (G1m(17,1 allotype) – protein
172	Hu2B8 Kv1-39.1 Kappa Chain Variable Region – nucleic acid
173	Hu2B8 Kv1-39.1 Kappa Chain Variable Region – protein
174	Human Kappa Chain Constant Region (Km(3) allotype) (allele 2) – nucleic acid
175	Human Kappa Chain Constant Region (Km(3) allotype) (allele 2) – protein
176	Hu2B8 Kv1-39.1 + Kappa Constant (Km(3) allotype) (allele 2) – nucleic acid
177	Hu2B8 Kv1-39.1 + Kappa Constant (Km(3) allotype) (allele 2) – protein
178	Hu2B8 Kv3-15.1 Kappa Chain Variable Region – nucleic acid
179	Hu2B8 Kv3-15.1 Kappa Chain Variable Region – protein
180	Hu2B8 Kv3-15.1 + Kappa Constant (Km(3) allotype) (allele 2) – nucleic acid
181	Hu2B8 Kv3-15.1 + Kappa Constant (Km(3) allotype) (allele 2) – protein

B. Humanization Procedure 2

[0291] The second humanization method employed for reducing immunogenicity of the mouse 2B8 antibody is based on the method described in Studnicka *et al.* (1994) PROTEIN ENG. 7:805-814. The heavy and kappa human germline variable regions most identical (at the amino acid level) to those of mouse 2B8 were identified. Residues that differed between mouse and human were converted into the human sequence depending on the likely risk that such a change would affect binding or immunogenicity. Low risk residues (i.e., residues that when changed would likely not affect antigen binding and would also reduce potential immunogenicity) were changed to the human amino acid in the heavy variable region (creating LR2B8HC) and the kappa variable region (creating LR2B8LC). Additionally, low risk and medium risk (i.e., residues that when changed are somewhat likely to have an effect on antigen

binding residues and would also reduce potential immunogenicity) were changed to the human amino acid in the heavy variable region (creating LRMR2B8HC) and the kappa variable region (creating LRMR2B8LC). The human IgG1 heavy chain constant region (G1m(3) allotype (allele 1)) was added to the carboxyl terminus of the two human engineered heavy variable regions and the human Kappa constant region (Km(3) allotype (allele 1)) was added to the carboxyl terminus of two human engineered light variable regions, thus creating four human engineered antibody chains. Variable region nucleic acid sequences were first synthesized by gene synthesis methods and then added to human constant region sequences. These human engineered antibodies were cloned into mammalian protein expression vectors, and protein was expressed in the four possible combinations of heavy chain plus light chain. Binding of the chimeric, chimeric/humanized, or humanized antibodies to human HGF was measured using conventional techniques, as described below.

[0292] The nucleic acid sequences encoding and the protein sequences defining each of the humanized antibodies are summarized below. In this section, the last nucleotide of each variable region is the first base of the next codon generated by the variable/constant region junction. This nucleotide is included in the Variable Region because it is part of that exon. Amino acid sequences of Constant Regions listed below include the translation of this junction codon.

[0293] (1) Nucleic Acid Sequence Encoding the Humanized LR2B8HC Heavy Chain Variable Region (signal sequence underlined) (SEQ ID NO. 182)

1 atgggctggt catatattat tctctttctt gttgctaccg ctaccgatgt gcactctcaa
61 gtccaactcg tacaaccagg cgctgaagtc gtaaaacccg gaacatctgt taaactctca
121 tgcaaagcct caggatacac tttcacaact tactggatgc attgggtcaa tcaagccccc
181 ggacaaggcc tcgaatggat tggcgaaatt aacccaacta acggacatac taattataat
241 gaaaaattta agggcaaagc tacactcacc gtcgataaat caacctctac agcttatatg
301 gaactttcat ccctgagatc agaagataca gccgtctact attgcgccag aaactacgta
361 ggatcaatat tcgattactg gggtcaaggc actctcctca cagtcagctc ag

[0294] (2) Protein Sequence Defining Humanized LR2B8HC Heavy Chain Variable Region (without signal sequence) (SEQ ID NO. 183)

1 qvqlvqpgae vvkpgtsvkl sckasgytft tywmhwvnqa pgqglewige inptnghtny
61 nekfkgkatl tvdkststay melsslrsed tavyycarny vgsifdywgq gtlltvss

[0295] (3) Nucleic Acid Sequence Encoding the Human IgG1 Heavy Chain Constant Region (G1m(3) allotype) (allele 1) (SEQ ID NO. 184)

1 ccagcacaaa gggcccatcg gtcttccccc tggcaccctc ctccaagagc acctctgggg
61 gcacagcggc cctgggctgc ctggtcaagg actacttccc cgaaccggtg acggtgtcgt
121 ggaactcagg cgccctgacc agcggcgtgc acaccttccc ggctgtccta cagtcctcag
181 gactctactc cctcagcagc gtggtgaccg tgccctccag cagcttgggc acccagacct
241 acatctgcaa cgtgaatcac aagcccagca acaccaaggt ggacaagaga gttgagccca
301 aatcttgtga caaaactcac acatgtccac cgtgcccagc acctgaactc ctggggggac
361 cgtcagtctt cctcttcccc ccaaaaccca aggacaccct catgatctcc cggacccctg
421 aggtcacatg cgtggtggtg gacgtgagcc acgaagaccc tgaggtcaag ttcaactggt
481 acgtggacgg cgtggaggtg cataatgcca agacaaagcc gcgggaggag cagtacaaca
541 gcacgtaccg tgtggtcagc gtcctcaccg tcctgcacca ggactggctg aatggcaagg
601 agtacaagtg caaggtctcc aacaaagccc tcccagcccc catcgagaaa accatctcca
661 aagccaaagg gcagccccga gaaccacagg tgtacaccct gcccccatcc cgggaggaga
721 tgaccaagaa ccaggtcagc ctgacctgcc tggtcaaagg cttctatccc agcgacatcg
781 ccgtggagtg ggagagcaat gggcagccgg agaacaacta caagaccacg cctcccgtgc
841 tggactccga cggctccttc ttcctctata gcaagctcac cgtggacaag agcaggtggc
901 agcaggggaa cgtcttctca tgctccgtga tgcatgaggc tctgcacaac cactacacgc
961 agaagagcct ctccctgtcc ccgggtaaat ga

[0296] (4) Protein Sequence Defining Human IgG1 Heavy Chain Constant Region (G1m(3) allotype) (allele 1 or 2) (SEQ ID NO. 185). The first amino acid is derived from translation of the last nucleotide of variable region and the beginning two nucleotides of the IgG1 Heavy Chain sequence.

1 astkgpsvfp lapsskstsg gtaalgclvk dyfpepvtvs wnsgaltsgv htfpavlqss
61 glyslssvvt vpssslgtqt yicnvnhkps ntkvdkrvep kscdkthtcp pcpapellgg
121 psvflfppkp kdtlmisrtp evtcvvvdvs hedpevkfnw yvdgvevhna ktkpreeqyn

181 styrvvsvlt vlhqdwlngk eykckvsnka lpapiektis kakgqprepq vytlppsree

241 mtknqvsltc lvkgfypsdi avewesngqp ennykttppv ldsdgsffly skltvdksrw

301 qqgnvfscsv mhealhnhyt qkslslspgk

[0297] (5) Nucleic Acid Sequence Encoding the Full Length Heavy Chain Humanized LR2B8HC Heavy Chain Variable Region and Human IgG1 Heavy Chain Constant Region (G1m(3) allotype) (allele 1) (signal sequence underlined) (SEQ ID NO. 186)

```
   1 atgggctggt catatattat tctctttctt gttgctaccg ctaccgatgt gcactctcaa
  61 gtccaactcg tacaaccagg cgctgaagtc gtaaaacccg gaacatctgt taaactctca
 121 tgcaaagcct caggatacac tttcacaact tactggatgc attgggtcaa tcaagccccc
 181 ggacaaggcc tcgaatggat tggcgaaatt aacccaacta acggacatac taattataat
 241 gaaaaattta agggcaaagc tacactcacc gtcgataaat caacctctac agcttatatg
 301 gaactttcat ccctgagatc agaagataca gccgtctact attgcgccag aaactacgta
 361 ggatcaatat tcgattactg gggtcaaggc actctcctca cagtcagctc agccagcaca
 421 aagggcccat cggtcttccc cctggcaccc tcctccaaga gcacctctgg gggcacagcg
 481 gccctgggct gcctggtcaa ggactacttc cccgaaccgg tgacggtgtc gtggaactca
 541 ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcctc aggactctac
 601 tccctcagca gcgtggtgac cgtgccctcc agcagcttgg gcacccagac ctacatctgc
 661 aacgtgaatc acaagcccag caacaccaag gtggacaaga gagttgagcc caaatcttgt
 721 gacaaaactc acacatgtcc accgtgccca gcacctgaac tcctgggggg accgtcagtc
 781 ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca
 841 tgcgtggtgg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac
 901 ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac
 961 cgtgtggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag
1021 tgcaaggtct ccaacaaagc cctcccagcc cccatcgaga aaaccatctc caaagccaaa
1081 gggcagcccc gagaaccaca ggtgtacacc ctgccccccat cccgggagga gatgaccaag
1141 aaccaggtca gcctgacctg cctggtcaaa ggcttctatc ccagcgacat cgccgtggag
1201 tgggagagca atgggcagcc ggagaacaac tacaagacca cgcctcccgt gctggactcc
1261 gacggctcct tcttcctcta tagcaagctc accgtggaca agagcaggtg gcagcagggg
1321 aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc
1381 ctctccctgt ccccgggtaa atga
```

[0298] (6) Protein Sequence Defining the Full Length Heavy Chain Humanized LR2B8HC Heavy Chain Variable Region and Human IgG1 Heavy Chain Constant Region (G1m(3) allotype) (allele 1) (without signal sequence) (SEQ ID NO. 187)

```
   1 qvqlvqpgae vvkpgtsvkl sckasgytft tywmhwvnqa pgqglewige inptnghtny
  61 nekfkgkatl tvdkststay melsslrsed tavyycarny vgsifdywgq gtlltvssas
 121 tkgpsvfpla psskstsggt aalgclvkdy fpepvtvswn sgaltsgvht fpavlqssgl
 181 yslssvvtvp ssslgtqtyi cnvnhkpsnt kvdkrvepks cdkthtcppc papellggps
 241 vflfppkpkd tlmisrtpev tcvvvdvshe dpevkfnwyv dgvevhnakt kpreeqynst
 301 yrvvsvltvl hqdwlngkey kckvsnkalp apiektiska kgqprepqvy tlppsreemt
 361 knqvsltclv kgfypsdiav ewesngqpen nykttppvld sdgsfflysk ltvdksrwqq
 421 gnvfscsvmh ealhnhytqk slslspgk
```

[0299] (7) Nucleic Acid Sequence Encoding the Humanized LRMR2B8HC Heavy Chain Variable Region (signal sequence underlined) (SEQ ID NO. 188)

1 atgggttggt catatattat actctttctc gtagccaccg ccaccgacgt acactctcag

61 gttcaactcg tacaacccgg cgccgaagtc aagaaaccag gaacatcagt caaactctca

121 tgtaaagcaa gcggatacac ctttactact tattggatgc attgggtaag acaagccccc

181 ggacaaggac tcgaatggat aggcgaaata aatcccacta atggacatac aaattataat

241 caaaaatttc aaggacgcgc tacactcacc gtcgataaat caacctcaac cgcatacatg

301 gaactcagct ccctccgatc cgaagacact gccgtttatt attgtgccag aaactatgta

361 ggatctattt tcgattactg gggacaagga acacttctca ccgtaagctc ag

[0300] (8) Protein Sequence Defining Humanized LRMR2B8HC Heavy Chain Variable Region (without signal sequence) (SEQ ID NO. 189)

1 qvqlvqpgae vkkpgtsvkl sckasgytft tywmhwvrqa pgqglewige inptnghtny

61 nqkfqgratl tvdkststay melsslrsed tavyycarny vgsifdywgq gtlltvss

[0301] (9) Nucleic Acid Sequence Encoding the Full Length Heavy Chain Humanized LRMR2B8HC Heavy Chain Variable Region and Human IgG1 Heavy Chain Constant Region (G1m(3) allotype) (allele 1) (signal sequence underlined) (SEQ ID NO. 190)

```
   1 atgggttggt catatattat actctttctc gtagccaccg ccaccgacgt acactctcag
  61 gttcaactcg tacaacccgg cgccgaagtc aagaaaccag gaacatcagt caaactctca
 121 tgtaaagcaa gcggatacac ctttactact tattggatgc attgggtaag acaagccccc
 181 ggacaaggac tcgaatggat aggcgaaata aatcccacta atggacatac aaattataat
 241 caaaaatttc aaggacgcgc tacactcacc gtcgataaat caacctcaac cgcatacatg
 301 gaactcagct ccctccgatc cgaagacact gccgtttatt attgtgccag aaactatgta
 361 ggatctattt tcgattactg gggacaagga acacttctca ccgtaagctc agccagcaca
 421 aagggcccat cggtcttccc cctggcaccc tcctccaaga gcacctctgg gggcacagcg
 481 gccctgggct gcctggtcaa ggactacttc cccgaaccgg tgacggtgtc gtggaactca
 541 ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcctc aggactctac
 601 tccctcagca gcgtggtgac cgtgccctcc agcagcttgg gcacccagac ctacatctgc
 661 aacgtgaatc acaagcccag caacaccaag gtggacaaga gagttgagcc caaatcttgt
 721 gacaaaactc acacatgtcc accgtgccca gcacctgaac tcctgggggg accgtcagtc
 781 ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca
 841 tgcgtggtgg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac
 901 ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac
 961 cgtgtggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag
1021 tgcaaggtct ccaacaaagc cctcccagcc cccatcgaga aaaccatctc caaagccaaa
1081 gggcagcccc gagaaccaca ggtgtacacc ctgccccat cccgggagga gatgaccaag
1141 aaccaggtca gcctgacctg cctggtcaaa ggcttctatc ccagcgacat cgccgtggag
1201 tgggagagca atgggcagcc ggagaacaac tacaagacca cgcctcccgt gctggactcc
1261 gacggctcct tcttcctcta tagcaagctc accgtggaca agagcaggtg gcagcagggg
1321 aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc
1381 ctctccctgt ccccgggtaa atga
```

[0302] (10) Protein Sequence Defining the Full Length Heavy Chain Humanized LRMR2B8HC Heavy Chain Variable Region and Human IgG1 Heavy Chain Constant Region (G1m(3) allotype) (allele 1) (without signal sequence) (SEQ ID NO. 191)

```
  1 qvqlvqpgae vkkpgtsvkl sckasgytft tywmhwvrqa pgqglewige inptnghtny
 61 nqkfqgratl tvdkststay melsslrsed tavyycarny vgsifdywgq gtlltvssas
121 tkgpsvfpla psskstsggt aalgclvkdy fpepvtvswn sgaltsgvht fpavlqssgl
181 yslssvvtvp ssslgtqtyi cnvnhkpsnt kvdkrvepks cdkthtcppc papellggps
241 vflfppkpkd tlmisrtpev tcvvvdvshe dpevkfnwyv dgvevhnakt kpreeqynst
301 yrvvsvltvl hqdwlngkey kckvsnkalp apiektiska kgqprepqvy tlppsreemt
361 knqvsltclv kgfypsdiav ewesngqpen nykttppvld sdgsfflysk ltvdksrwqq
421 gnvfscsvmh ealhnhytqk slslspgk
```

[0303] (11) Nucleic Acid Sequence Encoding the Humanized LR2B8LC Light Chain Variable Region (signal sequence underlined) (SEQ ID NO. 192)

1 atggaaagtc agacccttgt attcatctct attcttcttt ggttgtatgg agcagacggc

61 gacattgtga tgacccaatc ccccgatagt atggccatga gtgtaggaga aagagtcacc

121 cttaattgca aagcctccga aaatgtcgtt tcatatgtgt cttggtatca acaaaaaccc

181 ggccaatcac ccaaacttct catatacggc gcttcaaaca gaaacacagg cgttcccgac

241 agatttagtg gatccggatc agctacagat ttcaccctta ccatcagttc agttcaagca

301 gaagacgttg cagactatca ttgcggacaa tcttataact acccttacac attcggacaa

[0304] (12) Protein Sequence Defining Humanized LR2B8LC Light Chain Variable Region (without signal sequence) (SEQ ID NO. 193)

1 divmtqspds mamsvgervt lnckasenvv syvswyqqkp gqspkllivg asnrntgvpd

61 rfsgsgsatd ftltissvqa edvadyhcgq synypytfgq gtkleik

[0305] (13) Nucleic Acid Sequence Encoding the Human Kappa Chain Constant Region (Km(3) allotype) (allele 1) (SEQ ID NO. 194)

1 gtacggtggc tgcaccatct gtcttcatct tcccgccatc tgatgagcag ttgaaatctg

61 gaactgcctc tgttgtgtgc ctgctgaata acttctatcc cagagaggcc aaagtacagt

121 ggaaggtgga taacgccctc caatcgggta actcccagga gagtgtcaca gagcaggaca

181 gcaaggacag cacctacagc ctcagcagca ccctgacgct gagcaaagca gactacgaga

241 aacacaaagt ctacgcctgc gaagtcaccc atcagggcct gagctcgccc gtcacaaaga

301 gcttcaacag gggagagtgt tag

[0306] (14) Protein Sequence Defining the Human Kappa Chain Constant Region (Km(3) allotype) (allele 1) (SEQ ID NO. 195). The first amino acid derived from translation of the last nucleotide of variable region and beginning two nucleotides of the Kappa Light Chain sequence.

1 rtvaapsvfi fppsdeqlks gtasvvclln nfypreakvq wkvdnalqsg nsqesvteqd

61 skdstyslss tltlskadye khkvyacevt hqglsspvtk sfnrgec

[0307] (15) Nucleic Acid Sequence Encoding the Full Length Humanized LR2B8LC Light Chain Variable Region and the Human Kappa Chain Constant Region (Km(3) allotype) (allele 1) (SEQ ID NO. 196)

```
   1 atggaaagtc agacccttgt attcatctct attcttcttt ggttgtatgg agcagacggc
  61 gacattgtga tgacccaatc ccccgatagt atggccatga gtgtaggaga aagagtcacc
 121 cttaattgca aagcctccga aaatgtcgtt tcatatgtgt cttggtatca acaaaaaccc
 181 ggccaatcac ccaaacttct catatacggc gcttcaaaca gaaacacagg cgttcccgac
 241 agatttagtg gatccggatc agctacagat ttcaccctta ccatcagttc agttcaagca
 301 gaagacgttg cagactatca ttgcggacaa tcttataact acccttacac attcggacaa
 361 ggaaccaaac tcgaaattaa acgtacggtg gctgcaccat ctgtcttcat cttcccgcca
 421 tctgatgagc agttgaaatc tggaactgcc tctgttgtgt gcctgctgaa taacttctat
 481 cccagagagg ccaaagtaca gtggaaggtg gataacgccc tccaatcggg taactcccag
 541 gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg
 601 ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc
 661 ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gttag
```

[0308] (16) Protein Sequence Encoding the Full Length Humanized LR2B8LC Light Chain Variable Region and the Human Kappa Chain Constant Region (Km(3) allotype) (allele 1) (SEQ ID NO. 197)

```
   1 divmtqspds mamsvgervt lnckasenvv syvswyqqkp gqspklliyg asnrntgvpd
  61 rfsgsgsatd ftltissvqa edvadyhcgq synypytfgq gtkleikrtv aapsvfifpp
 121 sdeqlksgta svvcllnnfy preakvqwkv dnalqsgnsq esvteqdskd styslsstlt
 181 lskadyekhk vyacevthqg lsspvtksfn rgec
```

[0309] (17) Nucleic Acid Sequence Encoding the Humanized LRMR2B8LC Light Chain Variable Region (signal sequence underlined) (SEQ ID NO. 198)

1 atggaatccc aaacccttgt tttcatctct atccttctct ggctttatgg cgccgacgga

61 gacatcgtaa tgacacaatc ccctgactct cttgctatga gcttgggcga acgagtaaca

121 cttaactgca aagcatccga aaatgtcgta tcttacgtat cctggtatca gcaaaaacct

181 ggtcaaagtc ctaaacttct tatatatggt gcaagtaatc gtgaaagtgg cgtcccagac

241 agatttagcg gttcaggttc agcaactgac tttacactta caatttctag cgttcaggcc

301 gaagacgttg cagactatca ttgtggacaa tcttataact atccttatac tttcggacaa

361 ggcactaaac ttgaaattaa ac

[0310] (18) Protein Sequence Defining the Humanized LRMR2B8LC Light Chain Variable Region (without signal sequence) (SEQ ID NO. 199)

1 divmtqspds lamslgervt lnckasenvv syvswyqqkp gqspklliyg asnresgvpd

61 rfsgsgsatd ftltissvqa edvadyhcgq synypytfgq gtkleik

[0311] (19) Nucleic Acid Sequence Encoding the Full Length Humanized LRMR2B8LC Light Chain Variable Region and the Human Kappa Chain Constant Region (Km(3) allotype) (allele 1) (signal sequence underlined) (SEQ ID NO. 200)

```
   1 atggaatccc aaacccttgt tttcatctct atccttctct ggctttatgg cgccgacgga
  61 gacatcgtaa tgacacaatc ccctgactct cttgctatga gcttgggcga acgagtaaca
 121 cttaactgca aagcatccga aaatgtcgta tcttacgtat cctggtatca gcaaaaacct
 181 ggtcaaagtc ctaaacttct tatatatggt gcaagtaatc gtgaaagtgg cgtcccagac
 241 agatttagcg gttcaggttc agcaactgac tttacactta caatttctag cgttcaggcc
 301 gaagacgttg cagactatca ttgtggacaa tcttataact atccttatac tttcggacaa
 361 ggcactaaac ttgaaattaa acgtacggtg gctgcaccat ctgtcttcat cttcccgcca
 421 tctgatgagc agttgaaatc tggaactgcc tctgttgtgt gcctgctgaa taacttctat
 481 cccagagagg ccaaagtaca gtggaaggtg gataacgccc tccaatcggg taactcccag
 541 gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg
 601 ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc
 661 ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gttag
```

[0312] (20) Protein Sequence Defining the Full Length Humanized LRMR2B8LC Light Chain Variable Region and the Human Kappa Chain Constant Region (Km(3) allotype) (allele 1) (SEQ ID NO. 201)

```
   1 divmtqspds lamslgervt lnckasenvv syvswyqqkp gqspklliyg asnresgvpd
  61 rfsgsgsatd ftltissvqa edvadyhcgq synypytfgq gtkleikrtv aapsvfifpp
 121 sdeqlksgta svvcllnnfy preakvqwkv dnalqsgnsq esvteqdskd styslsstlt
 181 lskadyekhk vyacevthqg lsspvtksfn rgec
```

[0313] For convenience, Table 14 provides a concordance chart showing the correspondence between the full length sequences and of the antibodies discussed in this section with those presented in the Sequence Listing.

TABLE 14

SEQ. ID NO:	Protein or Nucleic Acid
182	LR2B8HC Heavy Chain Variable Region – nucleic acid
183	LR2B8HC Heavy Chain Variable Region – protein
184	Human IgG1 Heavy Chain Constant Region (G1m(3) allotype) (allele 1) – nucleic acid
185	Human IgG1 Heavy Chain Constant Region (G1m(3) allotype) (allele 1) – protein
186	LR2B8HC + IgG1 Constant (G1m(3) allotype) (allele 1) – nucleic acid
187	LR2B8HC + IgG1 Constant (G1m(3) allotype) (allele 1) – protein
188	LRMR2B8HC Heavy Chain Variable Region – nucleic acid
189	LRMR2B8HC Heavy Chain Variable Region – protein
190	LRMR2B8HC + IgG1 Constant (G1m(3) allotype) (allele 1) – nucleic acid
191	LRMR2B8HC + IgG1 Constant (G1m(3) allotype) (allele 1) – protein
192	LR2B8LC Light Chain Variable Region – nucleic acid
193	LR2B8LC Light Chain Variable Region – protein
194	Human Kappa Chain Constant Region (Km(3) allotype) (allele 1) – nucleic acid
195	Human Kappa Chain Constant Region (Km(3) allotype) (allele 1) – protein
196	LR2B8LC + Kappa Constant (Km(3) allotype) (allele 1) – nucleic acid
197	LR2B8LC + Kappa Constant (Km(3) allotype) (allele 1) – protein
198	LRMR2B8LC Light Chain Variable Region – nucleic acid
199	LRMR2B8LC Light Chain Variable Region – protein
200	LRMR2B8LC + Kappa Constant (Km(3) allotype) (allele 1) – nucleic acid
201	LRMR2B8LC + Kappa Constant (Km(3) allotype) (allele 1) – protein

[0314] Table 15 summarizes the heavy chain CDR sequences (Kabat Definition) of the humanized 2B8 antibodies prepared by humanization procedure 1 and by humanization procedure 2 described herein above in this Example.

TABLE 15

Antibody	CDR1	CDR2	CDR3	Full Length Heavy Chain Variable Region
Murine 2B8 Heavy	TYWMH (SEQ ID NO: 15)	EINPTNGHTNYNEKFKS (SEQ ID NO: 16)	NYVGSIFDY (SEQ ID NO: 17)	SEQ ID NO: 12
Hu2B8 Hv1f.1	TYWMH (SEQ ID NO: 15)	EINPTNGHTNYNEKFQG (SEQ ID NO: 202)	NYVGSIFDY (SEQ ID NO: 17)	SEQ ID NO: 159
Hu2B8 Hv5a.1	TYWMH (SEQ ID NO: 15)	EINPTNGHTNYNPSFQG (SEQ ID NO: 203)	NYVGSIFDY (SEQ ID NO: 17)	SEQ ID NO: 165
Hu2B8 Hv5-51.1	TYWMH (SEQ ID NO: 15)	EINPTNGHTNYNPSFQG (SEQ ID NO: 203)	NYVGSIFDY (SEQ ID NO: 17)	SEQ ID NO: 169
LR2B8HC	TYWMH (SEQ ID NO: 15)	EINPTNGHTNYNEKFKG (SEQ ID NO: 204)	NYVGSIFDY (SEQ ID NO: 17)	SEQ ID NO: 183
LRMR2B8HC	TYWMH (SEQ ID NO: 15)	EINPTNGHTNYNQKFQG (SEQ ID NO: 205)	NYVGSIFDY (SEQ ID NO: 17)	SEQ ID NO: 189

[0315] Table 16 summarizes the light chain CDR sequences (Kabat Definition) of the humanized 2B8 antibodies prepared by humanization procedure 1 and by humanization procedure 2 described herein above in this Example.

TABLE 16

Antibody	CDR1	CDR2	CDR3	Full-Length Light Chain Variable Region
Murine 2B8 Light	KASENVVSYVS (SEQ ID NO: 18)	GASNRNT (SEQ ID NO: 19)	GQSYNYPYT (SEQ ID NO: 20)	SEQ ID NO: 14
Hu2B8 Kv1-39.1	KASENVVSYVS (SEQ ID NO: 18)	GASNRNT (SEQ ID NO: 19)	GQSYNYPYT (SEQ ID NO: 20)	SEQ ID NO: 173
Hu2B8 Kv3-15.1	KASENVVSYVS (SEQ ID NO: 18)	GASNRNT (SEQ ID NO: 19)	GQSYNYPYT (SEQ ID NO: 20)	SEQ ID NO: 179
LR2B8LC	KASENVVSYVS (SEQ ID NO: 18)	GASNRNT (SEQ ID NO: 19)	GQSYNYPYT (SEQ ID NO: 20)	SEQ ID NO: 193
LRMR2B8LC	KASENVVSYVS (SEQ ID NO: 18)	GASNRES (SEQ ID NO: 206)	GQSYNYPYT (SEQ ID NO: 20)	SEQ ID NO: 199

C. Binding Affinity of Humanized 2B8 Antibodies

[0316] Antigen-binding affinity and kinetics of interaction were assessed by surface plasmon resonance technology using a BIAcore T100 instrument. Mouse anti-human immunoglobulins (Jackson ImmunoResearch Labs, 209-005-098) were immobilized on carboxymethylated dextran CM4 sensor chips (BIAcore, Catalog No. BR-1005-34) by amine coupling (BIAcore, Catalog No. BR-1000-50) using a standard coupling protocol according to manufacturer's recommendations. The analyses were performed at 25°C using PBS (GIBCO, Catalog No. 14040-133) containing 0.05% surfactant P20 (BIAcore, Catalog No. BR-1000-54), 2 mg/mL BSA (EMD, Catalog No. 2930) and 10 mg/mL CM-Dextran Sodium salt (Fluka, Catalog No. 86524) as running buffer.

[0317] The antibodies were captured on individual flow cell at a flow rate of 10 μL/min. Injection time was variable for each antibody to yield approximately 20 RU of antibody captured for each cycle. Buffer or HGF (R&D Systems, Catalog No. 294-HGN-025) diluted in running buffer was injected sequentially over a reference surface (no antibody captured) and the active surface (antibody to be tested) for 2 minutes at 60 μL/min. The dissociation phase was monitored for 15 or 90 minutes, depending on concentration. The surface then was regenerated with 10 mM Glycine-HCl, pH 2.0 (BIAcore, Catalog No. BR-1003-55) injected for

3 minutes at a flow rate of 60 μL/min before another cycle was initiated. HGF concentrations tested were 1.88, 3.75 and 7.5 nM. Determination of kinetic parameters was achieved using the kinetic function of the BIAevalutation software with reference subtraction. Kinetic parameters for each antibody, k_a (association rate constant), k_d (dissociation rate constant) and K_D (equilibrium dissociation constant) are summarized in Figure 8.

[0318] The results summarized in Figure 8 show that certain combinations of superhumanized heavy chains (Hu2B8 Hv5a.1, Hu2B8 Hv5-51.1 or Hu2B8 Hv1-f.1) and light chains (Hu2B8 Kv1-39.1 or Hu2B8 Kv3-15.1) retain similar binding affinity (K_D) to HGF as chimeric 2B8 (mouse variable regions with human constant regions) and 2B8 (Table 5).

D. Mutually Exclusive Binding Assay

[0319] Mutually exclusive binding to HGF was assessed by surface plasmon resonance technology using a BIAcore T100 instrument. Mouse anti-human immunoglobulins (Jackson ImmunoResearch Labs, 209-005-098) were immobilized on carboxymethylated dextran CM5 sensor chips (BIAcore, Catalog No. BR-1006-68) by amine coupling (BIAcore, Catalog No. BR-1000-50) using a standard coupling protocol according to manufacturer's recommendations. The analyses were performed at 25°C using PBS (GIBCO, Catalog No. 14040-133) containing 0.05% surfactant P20 (BIAcore, #BR-1000-54), 2 mg/mL BSA (EMD, Catalog No. 2930) and 10 mg/ml CM-Dextran Sodium salt (Fluka, Catalog No. 86524) as running buffer.

[0320] The humanized antibodies were captured on an individual flow cell at a flow rate of 30 μL/min. Injection time was variable for each antibody to yield approximately 150 RU of antibody captured for each cycle. HGF (R&D Systems, Catalog No. 294-HGN-025) diluted in running buffer at a final concentration of 7.5 μg/mL was injected for 90 sec at 30 μL/min over the captured humanized antibodies. Binding of HGF was monitored before subsequent injection of mouse 2B8 antibody or polyclonal goat anti-HGF antibody (R & D Systems, AF294) for 3 min at 30 μL/min. The surface then was regenerated with 10mM Glycine-HCl, pH 2.0 (BIAcore, Catalog No. BR-1003-55) injected for 3 min at a flow rate of 60 μL /min before another antibody was tested. The results are summarized in Figure 9.

[0321] Results summarized in Figure 9 show that both humanized 2B8 antibodies and chimeric 2B8 antibodies prevent murine 2B8 from binding HGF. These results demonstrate that the humanized antibodies still bind the same HGF epitope as the original 2B8 antibody.

Example 13 – Production of Humanized 2B8 Variants

a. HUMAN ENGINEERED™ Antibodies

[0322] Codon- and expression-optimized low risk and low-plus-moderate risk Human Engineered light chain (LR2B8LC and LRMR2B8LC, respectively) and heavy chains (LR2B8HC and LRMR2B8HC, respectively) were cloned in-phase into XOMA's transient antibody expression vectors, which contain human Kappa and Gamma-1 constant regions modules. The four Human Engineered 2B8 variants were produced by transient transfection in HEK293E cells. The following four antibodies were produced:

HE2B8-1 = LR2B8HC (+ IgG1 constant region (G1m(3) allotype (allele 1)) (SEQ ID NO. 187) plus LR2B8LC (+ Kappa constant region (Km(3) allotype (allele 1))) (SEQ ID NO. 197)

HE2B8-2 = LR2B8HC (+ IgG1 constant region (G1m(3) allotype (allele 1)) (SEQ ID NO. 187) plus LRMR2B8LC (+ Kappa constant region (Km(3) allotype (allele 1))) (SEQ ID NO. 201)

HE2B8-3 = LRMR2B8HC (+ IgG1 constant region (G1m(3) allotype (allele 1)) (SEQ ID NO. 191) plus LR2B8LC (+ Kappa constant region (Km(3) allotype (allele 1))) (SEQ ID NO. 197)

HE2B8-4 = LRMR2B8HC (+ IgG1 constant region (G1m(3) allotype (allele 1)) (SEQ ID NO. 191) plus LRMR2B8LC (+ Kappa constant region (Km(3) allotype (allele 1))) (SEQ ID NO. 201)

[0323] The light and heavy chains were co-transfected into XOMA's suspension adapted HEK293E cells grown in IS293 media (Irvine Scientific, Irvine, CA) using 2 liter shake flasks. After 24 hours in the shake flasks, 200 mL of transfected cells were centrifuged, resuspended in 40 mL of fresh medium and transferred to Integra flasks (Wilson Wolf Manufacturing Inc., MN) for production. After incubation for seven days, the cell suspensions were removed from the Integra flasks, centrifuged and the culture supernatants retained. Antibodies in the culture supernatants were purified on protein A spin columns (Pro-Chem), dialyzed against PBS,

concentrated and sterile filtered.

b. SUPERHUMANIZED™ Antibodies

[0324] Full length Hu2B8_Hv5-51.1 + human IgG1 constant domain (G1m(3) allotype) cDNA was cloned into pEE6.4 (Lonza Biologics, Berkshire, UK) using HindIII and EcoRI restriction sites. Full length Hu2B8_Kv1-39.1 variable region + human Kappa constant domain cDNA and full length Hu2B8_Kv3-15.1 variable region + human Kappa constant domain cDNA were each cloned into pEE14.4 (Lonza Biologics) using HindIII and EcoRI restriction sites. The hCMV-MIE promoter + full length Hu2B8_Hv5-51.1 + human IgG1 constant domain (G1m(3) allotype) cDNA + SV40 poly A fragment (in pEE6.4) was removed by NotI/SalI digestion and inserted into either Kappa chain pEE14.4 vector through NotI/SalI sites, thus creating 2 different expression vectors that each simultaneously express heavy and light chain to make the following antibodies:

sh2B8-9 (G1m(3)) = hu2B8 Hv5-51.1 (+ IgG1 constant region (G1m(3) allotype) (allele 2)) (SEQ ID NO. 210) plus hu2B8 Kv 1-39.1 (+ Kappa constant region (Km(3) allotype (allele 2))) (SEQ ID NO: 177)

sh2B8-12 (G1m(3)) = hu2B8 Hv5-51.1 (+ IgG1 constant region (G1m(3) allotype) (allele 2)) (SEQ ID NO. 210) plus hu2B8 Kv 3-15.1 (+ Kappa constant region (Km(3) allotype (allele 2))) (SEQ ID No. 181)

[0325] The nucleic acid sequences encoding and the protein sequences defining the human IgG1 Heavy Constant Region G1m(3) allotype (allele 2) and each of the full length heavy chain sequences are set forth below. The light chain sequences were the same as described in Example 12.

[0326] (1) Nucleic Acid Sequence Encoding Human IgG1 Heavy Chain Constant Region (G1m(3) allotype) (allele 2) (SEQ ID NO. 207)

1 cctccaccaa gggcccatcg gtcttccccc tggcaccctc ctccaagagc acctctgggg
61 gcacagcggc cctgggctgc ctggtcaagg actacttccc cgaaccggtg acggtgtcgt
121 ggaactcagg cgccctgacc agcggcgtgc acaccttccc ggctgtccta cagtcctcag
181 gactctactc cctcagcagc gtggtgaccg tgccctccag cagcttgggc acccagacct
241 acatctgcaa cgtgaatcac aagcccagca acaccaaggt ggacaagaga gttgagccca
301 aatcttgtga caaaactcac acatgcccac cgtgcccagc acctgaactc ctgggggggac

361 cgtcagtctt cctcttcccc ccaaaaccca aggacaccct catgatctcc cggacccctg
421 aggtcacatg cgtggtggtg gacgtgagcc acgaagaccc tgaggtcaag ttcaactggt
481 acgtggacgg cgtggaggtg cataatgcca agacaaagcc gcgggaggag cagtacaaca
541 gcacgtaccg tgtggtcagc gtcctcaccg tcctgcacca ggactggctg aatggcaagg
601 agtacaagtg caaggtctcc aacaaagccc tcccagcccc catcgagaag accatctcca
661 aagccaaagg gcagccccga gaaccacagg tgtacaccct gcccccatcc cgggaggaga
721 tgaccaagaa ccaggtcagc ctgacctgcc tggtcaaagg cttctatccc agcgacatcg
781 ccgtggagtg ggagagcaat gggcagccgg agaacaacta caagaccacg cctcccgtgc
841 tggactccga cggctccttc ttcctctaca gcaagctcac cgtggacaag agcaggtggc
901 agcaggggaa cgtcttctca tgctccgtga tgcatgaggc tctgcacaac cactacacgc
961 agaagagcct ctccctgtct ccgggtaaat ga

[0327] (2) Protein Sequence Defining Human IgG1 Heavy Chain Constant Region (G1m(3) allotype) (allele 1 or 2) (SEQ ID NO. 208). The first amino acid is derived from translation of the last nucleotide of variable region and the beginning two nucleotides of the IgG1 Heavy Chain sequence.

1 astkgpsvfp lapsskstsg gtaalgclvk dyfpepvtvs wnsgaltsgv htfpavlqss
61 glyslssvvt vpssslgtqt yicnvnhkps ntkvdkrvep kscdkthtcp pcpapellgg
121 psvflfppkp kdtlmisrtp evtcvvvdvs hedpevkfnw yvdgvevhna ktkpreeqyn
181 styrvvsvlt vlhqdwlngk eykckvsnka lpapiektis kakgqprepq vytlppsree
241 mtknqvsltc lvkgfypsdi avewesngqp ennykttppv ldsdgsffly skltvdksrw
301 qqgnvfscsv mhealhnhyt qkslslspgk

[0328] (3) Nucleic Acid Sequence Encoding the Full Length Chain Containing Humanized Hu2B8 Hv5-51.1 Heavy Chain Variable Region and the Human IgG1 Heavy Chain Constant Region G1m(3) allotype (allele 2) (signal sequence underlined) (SEQ ID NO. 209)

1 atggggtcaa ccgccatcct cgccctcctc ctggctgttc tccaaggagt ctgtgccgaa
61 gtgcagctgg tgcagtctgg agcagaggtg aaaaagcccg gggagtctct gaagatctcc
121 tgtaagggtt ctggatacag ctttaccacc tactggatgc actgggtgcg ccagatgccc
181 gggaaaggcc tggagtggat gggggagatt aatcctacca acggtcatac taactacaat
241 ccgtccttcc aaggccaggt caccatctca gctgacaagt ccatcagcac tgcctacctg
301 cagtggagca gcctgaaggc ctcggacacc gccatgtatt actgtgcgag aaactatgtt
361 ggtagcatct ttgactactg gggccaagga accctggtca ccgtctcctc agcctccacc
421 aagggcccat cggtcttccc cctggcaccc tcctccaaga gcacctctgg gggcacagcg
481 gccctgggct gcctggtcaa ggactacttc cccgaaccgg tgacggtgtc gtggaactca
541 ggcgccctga ccagcggcgt gcacaccttc ccggctgtcc tacagtcctc aggactctac
601 tccctcagca gcgtggtgac cgtgccctcc agcagcttgg gcacccagac ctacatctgc
661 aacgtgaatc acaagcccag caacaccaag gtggacaaga gagttgagcc caaatcttgt

```
 721 gacaaaactc acacatgccc accgtgccca gcacctgaac tcctgggggg accgtcagtc
 781 ttcctcttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca
 841 tgcgtggtgg tggacgtgag ccacgaagac cctgaggtca agttcaactg gtacgtggac
 901 ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac
 961 cgtgtggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag
1021 tgcaaggtct ccaacaaagc cctcccagcc cccatcgaga agaccatctc caaagccaaa
1081 gggcagcccc gagaaccaca ggtgtacacc ctgcccccat cccgggagga gatgaccaag
1141 aaccaggtca gcctgacctg cctggtcaaa ggcttctatc ccagcgacat cgccgtggag
1201 tgggagagca atgggcagcc ggagaacaac tacaagacca cgcctcccgt gctggactcc
1261 gacggctcct tcttcctcta cagcaagctc accgtggaca agagcaggtg gcagcagggg
1321 aacgtcttct catgctccgt gatgcatgag gctctgcaca accactacac gcagaagagc
1381 ctctccctgt ctccgggtaa atga
```

[0329] (4) Protein Sequence Defining the Full Length Heavy Chain Containing Humanized Hu2B8 Hv5-51.1 and the Human IgG1 Heavy Chain Constant Region G1m(3) allotype (allele 2) (without signal sequence) (SEQ ID NO. 210)

```
  1 evqlvqsgae vkkpgeslki sckgsgysft tywmhwvrqm pgkglewmge inptnghtny
 61 npsfqgqvti sadksistay lqwsslkasd tamyycarny vgsifdywgq gtlvtvssas
121 tkgpsvfpla psskstsggt aalgclvkdy fpepvtvswn sgaltsgvht fpavlqssgl
181 yslssvvtvp ssslgtqtyi cnvnhkpsnt kvdkrvepks cdkthtcppc papellggps
241 vflfppkpkd tlmisrtpev tcvvvdvshe dpevkfnwyv dgvevhnakt kpreeqynst
301 yrvvsvltvl hqdwlngkey kckvsnkalp apiektiska kgqprepqvy tlppsreemt
361 knqvsltclv kgfypsdiav ewesngqpen nykttppvld sdgsfflysk ltvdksrwqq
421 gnvfscsvmh ealhnhytqk slslspgk
```

[0330] Each dual expression vector was transfected into 293T cells for transient expression using DMEM 10% fetal bovine serum. Forty-eight hours after transfection, cells were washed with and then replaced with serum free medium, IS GRO™ (Irvine Scientific, Santa Ana, CA) containing 4mM L-Glutamine. Supernatant was harvested daily and replaced with fresh media for 10 days. The culture supernatants were centrifuged, filtered (0.45μm) and concentrated 10-100 fold. Antibodies were purified on ProSep vA resin (Millipore), dialyzed against PBS, concentrated and sterile filtered.

Example 14 – Binding Characteristics of Humanized 2B8 Variants

[0331] The humanized antibodies produced in Example 13 were characterized by their ability to bind hHGF and the recombinant HGF proteins produced in Example 3.

[0332] The antibodies were analyzed by surface-plasmon resonance using a BIAcore T100 instrument to assess their ability to bind hHGF and the fusion proteins discussed in Example 3. Each antibody was immobilized on a carboxymethylated dextran CM5 sensor chip (BIAcore, Catalog No. BR-1006-68) by amine coupling (BIAcore, Catalog No. BR-1000-50) using a standard coupling protocol according to manufacturer's instructions.

[0333] Analyses were performed at 25°C using PBS (GIBCO, Catalog No. 14040-133) containing 0.05% surfactant P20 (BIAcore, Catalog No. R-1000-54), 2 mg/mL BSA (EMD, Catalog No. 2930) and 10 mg/mL CM-Dextran Sodium salt (Fluka, Catalog No. 86524) as running buffer. Supernatant containing different HGF fusion proteins or supernatant from cells transfected with empty vector were injected over each antibody at a flow rate of 30 μL/min for 3 minutes. The resulting binding was determined as resonance units (RU) over baseline 30 seconds after the end of injection. Binding was compared to human HGF (R&D Systems, Catalog No. 294-HGN-025) diluted in running buffer. Non-specific binding was monitored by comparing binding to a control surface. The results are summarized in the Table 17.

TABLE 17

Antibody	rhHGF (R&D Systems)	rmHGF (R&D Systems)	MHM chimera (495-585)	MHM chimera (507-585)	MHM chimera (499-556)
2B8	Yes	No	Yes	Yes	Yes
HE2B8-1	Yes	No	Yes	Yes	Yes
HE2B8-2	Yes	No	Yes	Yes	Yes
HE2B8-3	Yes	No	Yes	Yes	Yes
HE2B8-4	Yes	No	Yes	Yes	Yes
sh2B8-9 (G1m(3))	Yes	No	Yes	Yes	Yes
sh2B8-12 (G1m(3))	Yes	No	Yes	Yes	Yes

[0334] The results in Table 17 demonstrate that each of the humanized 2B8-based antibodies bind rhHGF and all three mouse-human-mouse chimeras.

Example 15 – Binding Affinities of Humanized 2B8 Variants

[0335] The binding affinities and kinetics of interaction of the antibodies listed in Table 15 were measured by surface plasmon resonance.

[0336] Mouse anti-human immunoglobulins (Jackson Labs, Catalog No. 209-005) were immobilized on carboxymethylated dextran CM4 sensor chips (BIAcore, Catalog No. BR-1006-68) by amine coupling (BIAcore, Catalog No. BR-1000-50) using a standard coupling protocol according to manufacturer's instructions. The analyses were performed at 25°C using PBS (GIBCO, Catalog No. 14040-133) containing 0.05% surfactant P20 (BIAcore, Catalog No.

BR-1000-54), and 2 mg/mL BSA (EMD, Catalog No. 2930).

[0337] The antibodies were captured in an individual flow cell at a flow rate of 10 μL/min. Injection time was variable for each antibody to yield approximately 20 RU of antibody captured for each cycle. Buffer or HGF (R&D Systems, Catalog No. 294-HGN-025) diluted in running buffer was injected sequentially over a reference surface (no antibody captured) and the active surface (antibody to be tested) for 2 minutes at 60 μL/min. The dissociation phase was monitored for 15 or 90 minutes, depending on concentration. The surface then was regenerated with 10mM Glycine-HCl, pH 2.2 (BIAcore, Catalog No. BR-1003-54) injected for 3 minutes at a flow rate of 60 μL/min before another cycle was initiated. HGF concentrations tested were 0.46 nM to 7.5 nM.

[0338] Kinetic parameters were determined using the kinetic function of the BIAevalutation™ software with reference subtraction. Kinetic parameters for each antibody, k_a (association rate constant), k_d (dissociation rate constant) and K_D (equilibrium dissociation constant) are summarized in Table 18.

TABLE 18

Antibody	k_a (1/Ms)	k_d (1/s)	K_D (pM)	SD
2B8	$1.4x10^6$	$1.0x10^{-5}$	7.3	-
HE2B8-1	$2.2x10^6$	$1.4x10^{-5}$	7.1	5.2
HE2B8-2	$1.8x10^6$	$9.6x10^{-6}$	5.2	2.7
HE2B8-3	$2.0x10^6$	$4.1x10^{-6}$	2.0	1.1
HE2B8-4	$1.7x10^6$	$1.1x10^{-5}$	6.5	1.3
sh2B8-9 (G1m(17,1)	$2.0x10^6$	$1.7x10^{-5}$	8.1	5.3
sh2B8-12 (G1m(17,1)	$1.9x10^6$	$2.3x10^{-5}$	12	0.4

[0339] These data show that the humanized antibodies have fast association rates (k_a), very slow dissociation rates (k_d), and very high affinities (K_D). In particular, the antibodies have affinities ranging from 2.0-12pM.

Example 16 – Comparison of Binding Affinities at 25°C and 37°C

[0340] The binding affinities and kinetics of interaction of antibody HE2B8-4, sh2B8-9, sh2B8-12, and murine 2B8 were measured by surface plasmon resonance under different conditions.

[0341] Mouse anti-human immunoglobulins (Jackson Labs, Catalog No. 209-005) or rabbit anti-mouse immunoglobulins (BIAcore, Catalog No. BR-1005-14) were immobilized on carboxymethylated dextran CM4 sensor chips (BIAcore, Catalog No. BR-1006-68) by amine coupling (BIAcore, Catalog No. BR-1000-50) using a standard coupling protocol according to manufacturer's instructions. In the case of 25°C measurements for sh2b8-9 and sh2B8-12, a CM5 sensor chip (BIAcore, Catalog No. BR-1006-68) was used. The analyses were performed at 25°C and 37°C using PBS (GIBCO, Catalog No. 14040-133) containing 0.05% surfactant P20 (BIAcore, Catalog No. BR-1000-54), and 2 mg/mL BSA (EMD, Catalog No. 2930) as running buffer.

[0342] The antibodies were captured in an individual flow cell at a flow rate of 10 μL/min. Injection time was variable for each antibody to yield approximately 20 RU of antibody captured for each cycle. Buffer or HGF (R&D Systems, Catalog No. 294-HGN-025) diluted in running buffer was injected sequentially over a reference surface (no antibody captured) and the active surface (antibody to be tested) for 2 minutes at 60 μL/min. The dissociation phase was monitored for 15 or 90 minutes, depending on concentration. The surface of mouse anti-human immunoglobulins sensor chips was then regenerated with 10mM Glycine-HCl, pH 2.2 (BIAcore, Catalog No. BR-1003-54) injected for 3 minutes at a flow rate of 60 μL/min before another cycle was initiated. The surface of rabbit anti-mouse immunoglobulins sensor chips was regenerated with 10mM Glycine-HCl, pH 1.7 (BIAcore, Catalog No. BR-1003-54) injected for 3 minutes at a flow rate of 60 μL/min before another cycle was initiated. HGF concentrations tested were 0.46 nM to 7.5 nM.

[0343] Kinetic parameters were determined using the kinetic function of the BIAevaluation software with reference subtraction. Kinetic parameters for each antibody, k_a (association rate constant), k_d (dissociation rate constant) and K_D (equilibrium dissociation constant) are summarized below in Table 19.

TABLE 19

Antibody	Temp. (° C)	k_a (1/Ms)	k_d (1/s)	K_D (pM)
2B8	25	$1.6x10^6$	$2.1x10^{-5}$	13.5
2B8	37	$2.8 x10^6$	$1.3x10^{-5}$	4.5
HE2B8-4	25	$2.0x10^6$	$1.2x10^{-5}$	5.6
HE2B8-4	37	$3.1x10^6$	$1.0x10^{-5}$	3.3
sh2B8-9 (G1m(17,1))	25	$2.0x10^6$	$1.7x10^{-5}$	8.1
sh2B8-9 (G1m(3))	37	$2.5x10^6$	$1.4x10^{-5}$	5.8
sh2B8-12 (G1m(17,1))	25	$1.9x10^6$	$2.3x10^{-5}$	12.0
sh2B8-12 (G1m(3))	37	$2.4x10^6$	$1.1x10^{-5}$	4.8

[0344] As expected, the association rate constants increased with an increase in the temperature. Surprisingly, the dissociation constants did not change significantly with a corresponding increase in temperature. Consequently, the overall equilibrium dissociation constants (K_D) were approximately 1.4 to 3 times smaller (higher affinity) at physiological temperature (37° C).

Example 17 – Neutralization Activity of Humanized 2B8 Variants

[0345] The antibodies described in Example 14 were characterized for their ability to (a) inhibit the binding of hHGF to c-Met, and (b) inhibit HGF stimulated BrdU incorporation in 4MBr-5 cells.

[0346] HGF-Met Binding Inhibition Assay (Neutralization Assay) was performed as described in as follows. The antibodies were tested by ELISA for their ability to inhibit hHGF binding to c-Met. Specifically, Wallac 96-well DELFIA assay plates (Wallac Inc., Catalog No. AAAND-0001) were coated with 100 µL of 6.25 µg/mL HGF (R&D Systems, Catalog No. 294-HGN-025) in carbonate coating buffer (15 mM Na_2CO_3 and 34 mM $NaHCO_3$, pH 9.0) for 16 hours at 4°C. The plates then were blocked with 200 µL of 5% non-fat dry milk in PBS for 1 hour at room temperature. The antibodies were prepared in a separate plate by adding increasing concentrations of the antibodies under investigation (0.033-250nM, 2-fold-serial dilution) to 2nM biotinylated c-Met in 5% non-fat dry milk in PBS. c-Met (R&D Systems, Catalog No. 358-MT/CF) is biotinylated according to manufacturer's instruction at 10:1 biotin to c-Met ratio (Pierce, Catalog No. 21335). 100 µL of sample per well was transferred to the assay plate and incubated for 2 hours at room temperature. The resulting plates were washed

three times with PBS-0.1% Tween 20, and incubated for 1 hour at room temperature with Eu-labeled Streptavidin (Wallac, Catalog No. 1244-360) diluted 1:1000 in DELFIA assay buffer (Wallac, Catalog No. 4002-0010). The resulting plates were washed 3 times with DELFIA wash solution (Wallac, Catalog No. 4010-0010) and incubated with 100 μL/well DELFIA enhancement solution (Wallac #4001-0010) for 15 minutes at room temperature with agitation. The plates were read on Victor^{3}V instrument (Perkin Elmer) using the Europium method. The IC_{50} values were calculated using Prism.

[0347] The IC_{50} values obtained are shown in Table 20.

TABLE 20

Antibody	IC_{50} (nM)	SD
2B8	9.2	1.2
HE2B8-1	6.0	1.2
HE2B8-2	5.7	1.1
HE2B8-3	5.9	1.1
HE2B8-4	6.5	1.2
sh2B8-9 (G1m(3))	4.2	-
sh2B8-12 (G1m(3)	6.8	-

[0348] These results from Table 20 demonstrate that the humanized antibodies tested efficiently neutralize HGF binding to c-Met.

[0349] The antibodies in Table 17 were also tested in the cell proliferation assay described in Example 7(b). The results are summarized below in Table 21.

TABLE 21

Antibody	IC_{50} (nM)	SD
2B8	0.86	0.35
HE2B8-1	0.47	0.15
HE2B8-2	0.66	0.13
HE2B8-3	0.55	0.28
HE2B8-4	0.58	0.26
sh2B8-9 (G1m(3))	0.52	0.11
sh2B8-12 (G1m(3))	0.81	0.22

[0350] The results from Table 21 demonstrate that all the humanized antibodies tested inhibit HGF-induced proliferation of 4MBr-5 cells.

Example 18 – Anti-Scatter Activity of Humanized 2B8 Variants

[0351] The antibodies in Table 17 were tested in the anti-scatter assay described in Example 8. The results are summarized below in Table 22.

TABLE 22

Inhibition of HGF-induced MDCK Cell Scattering		
Antibody	Trial 1	Trial 2
2B8	++	++
HE2B8-1	++	++
HE2B8-2	++	++
HE2B8-3	++	++
HE2B8-4	++	++
sh2B8-9 (G1m(3))	++	++
sh2B8-12 (G1m(3))	++	++

- No Inhibition
+++ Very strong, nearly complete inhibition
++ Strong inhibition
+ Detectable inhibition

[0352] The results in Table 22 demonstrate that all the humanized antibodies tested inhibited HGF-induced scattering to the same extent as the murine monoclonal antibody 2B8.

Example 19 – Inhibition of HGF-stimulated c-Met Phosphorylation

[0353] The antibodies in Table 17 were tested in the c-Met phosphorylation assay described in Example 9. The results are summarized below in Table 23.

TABLE 23

Antibody	Average of Two Trials	Standard Deviation
2B8	0.91	0.02
he2B8-1	0.80	0.04
he2B8-2	0.88	0.15
he2B8-3	0.79	0.05
he2B8-4	0.75	0.14
sh2B8-9 (G1m(3))	0.93	0.03
sh2B8-12 (G1m(3))	0.81	0.07

[0354] The results in Table 23 demonstrate that all the humanized antibodies tested are potent inhibitors of HGF-induced c-Met phosphorylation in PC-3 cells.

Example 20 – Tumor Inhibition in U87MG Xenograft Model

[0355] The ability of the humanized monoclonal antibodies of the invention to inhibit tumor growth was tested in an U87MG xenograft model. U87MG cells (ATCC) were expanded in culture at 37°C in an atmosphere containing 5% CO_2 and 95% air, using a medium comprising Dulbecco's Modified Eagle medium (DMEM) with 10% fetal bovine serum, 100 units/mL penicillin and 100 µg/mL streptomycin. The cells were subcultured and maintained by detaching the cells from the wall of the culture dish using trypsin-EDTA.

[0356] Near-confluent cells were collected by trypsinization and then 5 x 10^6 cells in 50% Matrigel (BD Biosciences; catalog no. 356237) were injected subcutaneously into the upper dorsal area between the shoulder blades of 7-week old female ICR SCID mice (Taconic Labs). The long (L) and short (W) diameters (mm) of tumors were measured with a caliper. Tumor volume (vol.) was calculated as: volume (mm^3) = $L \times W^2 / 2$. When the tumors grew to approximately 200 mm^3, the tumor-bearing mice were randomized into 5 groups of 10 mice each. One group received PBS and one group received human IgG control. Each of the other 4 groups received one of the humanized antibodies (HE2B8-1, HE2B8-2, HE2B8-3, and HE2B8-4). All the antibodies were dosed at 0.25 mg/kg body weight, twice per week, by intra-peritoneal injections of 5 doses. Tumor volumes and mouse body weights were recorded twice per week. Tumor growth inhibition was analyzed using Student's t-test.

[0357] The humanized antibodies tested were active *in vivo*. There was 57% tumor growth inhibition for HE2B8-1 with a p value of 0.02, 61% tumor growth inhibition for HE2B8-2 with a p value of 0.02, 85% tumor growth inhibition for HE2B8-3, with a p value of 0.0004, and 74% tumor growth inhibition for HE2B8-4 with a p value of 0.001. No significant body weight loss was observed.

[0358] A subsequent study was performed as described above in female NCR nude mice (Taconic Labs) bearing subcutaneous U87MG tumors inoculated in the flank. Each group (10 mice each) received one of the following treatments at 0.5 mg/kg: PBS vehicle control, huIgG control, HE2B8-4, or sh2B8-9. Treatment was given intra-peritoneal twice weekly for a minimum of 5 weeks. Each treatment group demonstrated similar tumor regression with tumor growth inhibition of 113% for sh2B8-9 and 115% for HE2B8-4, and a minimum tumor growth delay of 30 days. Both treatments were well-tolerated with no significant body weight loss.

INCORPORATION BY REFERENCE

[0359] The entire disclosure of each of the patent documents and scientific articles referred to herein is incorporated by reference for all purposes.

EQUIVALENTS

[0360] The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting on the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are intended to be embraced therein.

WHAT IS CLAIMED IS:

1. An isolated binding protein that binds human hepatocyte growth factor (HGF) comprising:

(a) an immunoglobulin light chain variable region comprising the structure CDR_{L1}-CDR_{L2}-CDR_{L3}, wherein

(i) CDR_{L1} comprises amino acid sequence SEQ ID NO. 18 **(2B8)**,

(ii) CDR_{L2} comprises amino acid sequence SEQ ID NO. 206 **(LRMR2B8LC)**, and

(iii) CDR_{L3} comprises amino acid sequence SEQ ID NO. 20 **(2B8)**; and

(b) an immunoglobulin heavy chain variable region, wherein the immunoglobulin light chain variable region and the immunoglobulin heavy chain variable region together define a single binding site for binding human HGF.

2. The binding protein of claim 1, wherein CDR_{L1}, CDR_{L2}, and CDR_{L3} are interposed between human or humanized immunoglobulin framework regions.

3. The binding protein of claim 1 or 2, wherein the binding protein is an antibody or an antigen binding fragment thereof.

4. The isolated binding protein of any one of claims 1-3 comprising the immunoglobulin light chain variable region of SEQ ID NO. 193 **(LR2B8LC light chain variable region)** or SEQ ID NO. 199 **(LRMR2B8LC light chain variable region)**.

5. The isolated binding protein of claim 4 comprising the immunoglobulin light chain sequence of SEQ ID NO. 197 **(LR2B8LC + Kappa Constant (km(3) allotype) (allele 1))** or SEQ ID NO. 201 **(LRMR2B8LC + Kappa Constant (km(3) allotype) (allele 1))**.

6. An isolated binding protein that binds human hepatocyte growth factor (HGF) comprising

(a) an immunoglobulin heavy chain variable region comprising the structure CDR_{H1}-CDR_{H2}-CDR_{H3}, wherein

(i) CDR_{H1} comprises amino acid sequence SEQ ID NO. 15 **(2B8)**,

(ii) CDR_{H2} comprises a sequence selected from the group consisting of SEQ ID NO. 204 **(LR2B8HC)** and SEQ ID NO. 205 **(LRMR2B8HC)**, and

(iii) CDR_{H3} comprises amino acid sequence SEQ ID NO. 17 **(2B8)**, and

(b) an immunoglobulin light chain variable region, wherein the immunoglobulin heavy chain variable region and the immunoglobulin light chain variable region together define a single binding site for binding human HGF.

7. The binding protein of claim 6, wherein CDR_{H1}, CDR_{H2}, and CDR_{H3} are interposed between human or humanized immunoglobulin framework regions.

8. The binding protein of claim 7, wherein the binding protein is an antibody or an antigen binding fragment thereof.

9. The isolated binding protein of any one of claims 6-8 comprising the immunoglobulin heavy chain variable region of SEQ ID NO. 183 **(LR2B8HC heavy chain variable region)** or SEQ ID NO. 189 **(LRMR2B8HC heavy chain variable region)**.

10. The isolated binding protein of claim 9 comprising the immunoglobulin heavy chain sequence of SEQ ID NO. 187 **(LR2B8HC + IgG1 Constant (G1m(3) allotype) (allele 1))** or SEQ ID NO. 191 **(LRMR2B8HC + IgG1 Constant (G1m(3) allotype) (allele 1))**.

11. The isolated binding protein of claim 6 further comprising an immunoglobulin light chain variable region comprising the structure CDR_{L1}-CDR_{L2}-CDR_{L3}, wherein

(i) CDR_{L1} comprises amino acid sequence SEQ ID NO. 18 **(2B8)**,

(ii) CDR_{L2} comprises amino acid sequence SEQ ID NO. 19 **(2B8)** or SEQ ID NO. 206 **(LRMR2B8LC)**, and

(iii) CDR_{L3} comprises amino acid sequence SEQ ID NO. 20 **(2B8)**,

wherein the immunoglobulin light chain variable region and the immunoglobulin heavy chain variable region together define a single binding site for binding human HGF.

12. The binding protein of any one of claims 1, 6 or 11, wherein the antibody is a monoclonal antibody.

13. An isolated nucleic acid comprising a nucleotide sequence encoding the immunoglobulin light chain variable region of any one of claims 1-5.

14. An isolated nucleic acid comprising a nucleotide sequence encoding the immunoglobulin heavy chain variable region of any one of claims 6-10.

15. An expression vector containing the nucleic acid sequence of claim 13 or 14.

16. A host cell containing the expression vector of claim 15.

17. A method of producing a binding protein, the method comprising:

(i) growing the host cell of claim 16 under conditions so that the host cell expresses the immunoglobulin light chain variable region and/or the immunoglobulin heavy chain variable region; and

(ii) harvesting the immunoglobulin light chain variable region and/or the immunoglobulin heavy chain variable region.

18. The isolated binding protein of any one of claims 1-11, wherein the binding protein binds human hepatocyte growth factor with a k_d of $4.0x10^{-5}$ s^{-1} or lower.

19. The isolated binding protein of claim 18, wherein the k_d is $3.0x10^{-5}$ s^{-1} or lower.

20. The isolated binding protein of claim 19, wherein the k_d is $2.0x10^{-5}$ s^{-1} or lower.

21. The isolated binding protein of any one of claims 1-11, wherein the binding protein binds human hepatocyte growth factor with a K_D of 20 pM or lower.

22. The isolated binding protein of claim 21, wherein the K_D is 10 pM or lower.

23. The isolated binding protein of claim 22, wherein the K_D is 5 pM or lower.

24. A method of inhibiting or reducing proliferation of a tumor cell comprising exposing the cell to an effective amount of the binding protein of any one of claims 1-11 to inhibit or reduce proliferation of the tumor cell.

25. The method of claim 24, wherein the tumor cell is a human tumor cell.

26. A method of inhibiting or reducing tumor growth in a mammal, the method comprising exposing the mammal to an effective amount of the binding protein of any one of claims 1-11 to inhibit or reduce proliferation of the tumor.

27. A method of treating a tumor in a mammal, the method comprising administering an effective amount of the binding protein of any one of claims 1-11.

28. The method of claim 26 or 27, wherein the mammal is a human.