The present invention provides a family of binding proteins that bind and neutralize the activity of hepatocyte growth factor (HGF), in particular human HGF. The binding proteins can be used as diagnostic and/or therapeutic agents. With regard to their therapeutic activity, the binding proteins can be used to treat certain HGF responsive disorders, for example, certain HGF responsive tumors.
HEPATOCELLULAR GROWTHFACTOR (HGF) BINDINGPROTEINS

RELATED APPLICATIONS

[0001] This application claims the benefit and priority to U.S. Provisional Application Nos. 60/810,714, filed June 2, 2006, and 60/860,461, 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.


[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 CDR1, CDR2, and CDR3, 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 3D1. The amino acid sequences for each antibody are aligned against one another and the regions defining the signal peptide, CDR1, CDR2, and CDR3 are identified in boxes. The unboxed sequences represent FR sequences.

[0015] Figure 3 is a schematic diagram showing the CDR1, CDR2, and CDR3 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 3D1. The amino acid sequences for each antibody are aligned against one another and the regions defining the signal peptide, CDR1, CDR2, and CDR3 are identified in boxes. The unboxed sequences represent FR sequences.

[0017] Figure 5 is a schematic diagram showing the CDR1, CDR2, and CDR3 sequences for each of the immunoglobulin light chain variable region sequences presented in Figure 4.
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 aU87MG 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.

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.

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.

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**

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 CDRL1-CDRL2 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. CDRLu comprises the amino acid sequence X_1,X_2 Ser X_4 X_5 X_6 X_7 X_8 X_9 X_10 Xn X_12 X_13 X_14 Xis, wherein amino acid X_i is Arg, Lys, or Ser, X_2 is Ala or Thr, X_4 is Glu, Gin, or Ser, X_5 is Asn, Asp, or Ser, X_6 is He 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, Xio is a peptide bond or Gly, X_1 is a peptide bond or Asn, X_12 is a peptide bond, He, or Ser, X_13 is Asn or Tyr, X_14 is He, Leu, Met, or Val, X_15 is Ala, Asn, His, or Ser. CDRL_2 comprises the amino acid sequence Xi6 Xi7 X_1 SX_l X_7 X_8 X_10 X_21 X_22, wherein amino acid Xi6 is Ala, Asp, Arg, Gly, or Val, X_17 is Ala, Thr, or Val, Xis 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. CDRL_3 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 Glu, X_24 is His or Glu, X_25 is Phe, Ser, Tip, or Tyr, X_26 is Asp, He, Ser, Tip, 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.
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_i is Asp, Asn, Ser, or Thr, X_3 is Phe, Ser, Trp, or Tyr, X_4 is lie, Leu, or Met, X_5 is Asn, His, or Ser. CDR_{H2} comprises the amino acid sequence X_6 He X_g X_9 X_{10} Xu Gly X_{13} X_{14} X_5 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, He, or Tyr, X_{33} is Met or Phe, X_{34} is Ala or Asp.

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.

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

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.

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 CDRL1-CDRL2-CDRL3 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 CDRn 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 (LRMR2B88LC). The CDRu 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 (3DH), 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 CDRu comprising the sequence of SEQ ID NO. 8 (1A3), a
CDR.L comprising the sequence of SEQ ID NO. 9 (1A3), and a CDR.H 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.L comprising the sequence of SEQ ID NO. 18 (2B8), a
CDR.H comprising the sequence of SEQ ID NO. 19 (2B8) or SEQ ID NO. 206 (LRMR2B88LC), and a CDR.L 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.L comprising the sequence of SEQ ID NO. 28 (2F8), a
CDRu comprising the sequence of SEQ ID NO. 29 (2F8), and a CDR.L 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 CDRu comprising the sequence of SEQ ID NO. 38 (3B6), a
CDR.L comprising the sequence of SEQ ID NO. 39 (3B6), and a CDR.L comprising the
sequence of SEQ ID NO. 40 (3B6).
In another embodiment, the binding protein comprises an immunoglobulin light chain variable region comprising a CDRn comprising the sequence of SEQ ID NO. 48 (3D11), a CDR_{L2} comprising the sequence of SEQ ID NO. 49 (3D11) and a CDRu comprising the sequence of SEQ ID NO. 51 (3D1).

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

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 CDRu comprising the sequence of SEQ ID NO. 70 (1F3).

In another embodiment, the binding protein comprises an immunoglobulin light chain variable region comprising a CDRu 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).

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.

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 CDRH1-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 CDRm 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 Hvlf.1), SEQ ID NO. 203 (Hu2B8 Hv5a.l 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\(_{m}\) 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 (HuZBS Hvlf.1), SEQ ID NO. 203 (Hu2B8 Hv5a.l 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\(_{m}\) 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 (3D1 1); and a CDR\(_{H3}\) comprising the sequence of SEQ ID NO. 47 (3D1 1).

[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_m 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).
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).

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).

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).

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).

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).

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).

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).

In each of the foregoing embodiments, the binding protein can be an intact antibody, an antigen binding fragment thereof, or a biosynthetic antibody site.
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 Kv3-39.1 light chain variable region), SEQ ID NO. 179 (Hu2B8 Kv3-15.1 light chain variable region), and SEQ ID NO. 199 (LRMR2B8LC light chain variable region); (ii) an immunoglobulin heavy chain variable region selected from the group consisting of SEQ ID NO. 159 (Hu2B8 Hvlf.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), 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.

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 Kv3-39.1 + kappa constant (Km3 allotype (allele 2)), SEQ ID NO. 181 (Hu2B8 Kv3-15.1 + Kappa constant (Km3 allotype (allele 2)), SEQ ID NO. 197 (LR2B8LC + Kappa constant (Km3 allotype (allele I)), and SEQ ID NO. 201 (LRMR2B8LC + Kappa constant (Km3 allotype (allele I)); and (ii) an immunoglobulin heavy chain selected from the group consisting of SEQ ID NO. 163 (Hu2B8 Hvlf.1 + IgGl Constant (Glm(17,l) allotype)), SEQ IDNO. 167 (Hu2B8 Hv5a.1 + IgGl Constant (Glm(17,l) allotype)), SEQ ID NO. 171 (Hu2B8 Hv5-51.1 + IgGl Constant (Glm(17,l) allotype)), SEQ ID NO. 187 (LR2B8HC + IgGl Constant (Glm(3) allotype (allele I)), and SEQ ID NO. 191 (LRMR2B8HC +IgGl Constant (Glm(3) allotype (allele I)). The binding protein can be an intact antibody, an antigen binding fragment thereof, or a biosynthetic antibody site.

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 L2. 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 i X j X k X l X m X n X o X p X q X r X s X t X u X v X w X x X y X z
wherein amino acid X i is Arg or Lys, X j is Ala or Thr, X k is Glu or Gln, X l is Asn, Ser, or Asp, X m is His or Val, X n is Tyr, Asp, or Lys, X o is a peptide bond or Tyr, X p is a peptide
bond or Gly. X q is a peptide bond or Asn, X r is a peptide bond or Ser, X s is Asn or Tyr, X t is is lie or Leu, X u is Ala, Asn, or Ser. CDR2 comprises the amino acid sequence X i6 X j7 X k8
X l9 Leu X m21 X n22, wherein amino acid X j6 is Ala, Asp, Val, or Arg, X j7 is Ala or Val, X k8 is
Asn, Ser, or Thr, X l9 is Arg, Asn, or His, X m21 is Ala, Glu, Val, or Pro, X n22 is Asp or Ser.
CDR3 comprises the amino acid sequence X i23 X j24 X k25 X l26 X m27 X n28 X o29 X p30 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, He, or Trp,
X 27 is Gly or Glu, X 28 is Asp, Phe, or Thr, X 29 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,
CDRH1 and CDR H2, or CDR H1 and CDR H3, or CDR H1 and CDR H2. Optionally, the binding
protein comprises all three CDRs, i.e., CDRH1, CDR H2 and CDR H3. CDRH1 comprises the amino
acid sequence X i Tyr X j3 X k4 X l5 X m6 X n7 X o8 X p9 X q10 X r11 X s12 X t13 X u14 X v15 X w16 X x17 X y18 X z19
wherein amino acid X i is Asp, Asn, Ser, or Thr, X 3 is Phe, Trp, or Tyr, X 4 is lie or Met, X 5 is Asn, His, or Ser. CDR H2 comprises the amino acid
sequence X 6 He X g X h X i X j X k X l X m Lys X n X o X p X q X r X s X t X u X v X w X x X y X z
wherein amino acid X 6 is Lys, Gln, or Tyr, X 8 is Gly, Ser, or Tyr, X 9 is Pro or Ser, X n 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 21 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 X 34 X 35 X 36 X 37 X 38 X 39 X 40 X 41
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\textsubscript{l} 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\textsubscript{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\textsubscript{h} 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\textsubscript{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\textsubscript{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\textsubscript{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 HGF3 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.
In another aspect, the invention provides an isolated binding protein that binds human HGF with a $k_d$ of $4\times10^7$ s$^{-1}$ or lower, $3\times10^5$ s$^{-1}$ or lower, or $2\times10^5$ s$^{-1}$ or lower. The isolated binding proteins can bind human HGF with a $k_d$ from $5\times10^5$ s$^{-1}$ to $0.5\times10^5$ s$^{-1}$, or from $4\times10^5$ s$^{-1}$ to $1.0\times10^5$ s$^{-1}$, or from $3\times10^5$ s$^{-1}$ to $1.5\times10^5$ 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.

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.

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).

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

Binding proteins of the invention can be produced in various ways using approaches known 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.

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.

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 refractive bodies then are solubilized, and the expressed proteins refolded and cleaved by the methods already established for many other recombinant proteins.

If the engineered gene is to be expressed in eukaryotic 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, \( V_L \cdot V_H \) heterodimers, \( V_H \cdot V_L \) or \( V_L \cdot V_H \) 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).

5 HI - 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 169:11 19-1125.

[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.

(I) 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, nasopharyngeal cancer, pancreatic cancer, mesothelioma, melanoma and glioblastoma.

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.

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.

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.

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, Gremophor 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 polyethylene 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 ³H, ¹⁴C, ³²P, ⁵⁷⁷, or ¹²⁵I; 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.
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.

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.

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 $^{99m}$Tc (six hours), a gamma emitter with a half-life of about six hours. Non-radioactive moieties also useful in in vivo imaging include nitrooxide spin labels as well as lanthanide and transition metal ions all of which induce proton relaxation in situ. In addition to immunomaging, 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 (90Yt), $^{131}$Iodine (131I) and $^{11}$Indium (11In). The binding protein can be labeled with 131I, 11In 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.
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

Example 1 — Production of Anti-hHGF Monoclonal Antibodies

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

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

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

All the antibodies were determined to contain a Kappa immunoglobulin light chain and an IgGl immunoglobulin heavy chain.

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

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′ aagcagtgtacagcagtagctacgggg 3′) (SEQ ID NO. 85) and 5′-RACE CDS Primer (5′ UIUIIIIIliiiiiiiiimv 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).

The variable regions of the Kappa and Heavy (IgGl) 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′ oligonucleotide primer mix Universal Primer Mix A (mix of 5′ ctaatacgactcactatagggcaagcaggtgtacaacgcatcg 3′ (SEQ ID NO. 87) and 5′ ctaatacgactcactataggg 3′(SEQ ID NO. 88)) and a 3′ IgGl Constant Region specific primer, either 5′ tattgcagagctttcaacaca 3′ (SEQ ID NO. 89) or 5′ ggcgttgaagctttcagatgggggtctg 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′ cctattcctgtttgaagctttcagaat 3′ (SEQ ID NO. 91) or 5′ cgacttggaacctcagatgt 3′ (SEQ ID NO. 92).

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' GTAAAGCAGCGGCCGTAG 3') (SEQ ID NO. 94), and M13 Reverse primers (5' CAGGAACAGCATCAGACC 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.

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

[0119] Full Length cDNAs for the 1A3, 1D3, and 1F3 IgGl chains were PCR amplified from the cDNA created above using the forward primer

5' ggggacaagtttgtacaaaaaacaggctgccaccaactttggctcagattttcc 3' (start codon underlined) (SEQ ID NO. 96) and the reverse primer 5' gggaccactttgtacagaaagctggtctcatttaccagagggagggaggg 3' (stop codon underlined) (SEQ ID NO. 97). Full Length cDNA for the 2B8 IgGl chain was amplified from the cDNA created above using the forward primer

5' ggggacaagtttgtacaaaaaacaggctgccaccaactttggctcagattttcc 3' (start codon underlined) (SEQ ID NO. 98) and reverse primer 5' gggaccactttgtacagaaagctggtctcatttaccagagggagggaggg 3' (stop codon underlined) (SEQ ID NO. 99).

[0120] Full Length cDNA for the 2B8 Kappa Chain was amplified using the forward primer 5' ggggacaagttgtacaaaaaacaggctgccaccaactttggctcagattttcc 3' (start codon underlined) (SEQ ID NO. 100) and the reverse primer 5' gggaccactttgtacagaaagctggtctcatttaccagagggagggaggg 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

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.

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.

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).

f1) IA3 Heavy Chain Variable Region (SEQ ID NO. 1)

atgaactttg ggctcaeatt gattttcctt gtccttgttt taaaaggtgt gaagtg
61 gtgcagctgg tggagtctgg ggagggctta gtcagcctg gagggtccct gaaactctcc
121 gagaagagc gcaggtggct gcatacatt agtcctggtg gtggtagctc ctactatcca
181 gcaggtgta agggtcggct cacatctcc agagacaatg caaagactcc cctgtacctg
241 caatgagca gtctgagcgtc tggagcaca ggcattgtt actgtgcaag acaaggggat
ggtacctag gggactatgc tatgactac tggggctcaag gaacctcagt cacccgccctc
301 tcag

(2) IA3 Kappa Light Chain Variable Region (SEQ ID NO. 3)

atgaatcttg ggctcaeatt gattttcctt gtccttgttt taaaaggtgt gaagtg
61 gtgcagctgg tggagtctgg ggagggctta gtcagcctg gagggtccct gaaactctcc
121 gagaagagc gcaggtggct gcatacatt agtcctggtg gtggtagctc ctactatcca
181 gcaggtgta agggtcggct cacatctcc agagacaatg caaagactcc cctgtacctg
241 caatgagca gtctgagcgtc tggagcaca ggcattgtt actgtgcaag acaaggggat
ggtacctag gggactatgc tatgactac tggggctcaag gaacctcagt cacccgccctc
301 tcag

atgaatcttg gcacagtgc gcaggtggct gcatacatt agtcctggtg gtggtagctc ctactatcca
61 gcaggtgta agggtcggct cacatctcc agagacaatg caaagactcc cctgtacctg
121 caatgagca gtctgagcgtc tggagcaca ggcattgtt actgtgcaag acaaggggat
ggtacctag gggactatgc tatgactac tggggctcaag gaacctcagt cacccgccctc
181 gcaggtgta agggtcggct cacatctcc agagacaatg caaagactcc cctgtacctg
241 caatgagca gtctgagcgtc tggagcaca ggcattgtt actgtgcaag acaaggggat
ggtacctag gggactatgc tatgactac tggggctcaag gaacctcagt cacccgccctc
301 tcag

gggaccaagc tggaaataaa ac
(3) 2B8 Heavy Chain Variable Region (SEQ ID NO. 11)

```
1  atgggatgga getatatcat cctcttttg tgaacacag ctacagatgt tcaacccag
61  gtccacactgc aacagcttgg ggcgtgaactg tgaagccctg ggaacttcagt gaagcgtc
121  tgcagcgtct tgtgaatact cttccacacc tactgcagtg actggccaga gcaggtcgcc
181  gacagccggcc aagccgacac cccgagacag gcacagggcg ggcggttact atatgttatgt
241  caacgtccag gctcagcatg gcgcgtaccg atgctgactac tggacttgag gcacagccc
301  ggctgacatc tgacattcag ccaacactgc aagctgacac cgttgccagt tgcagccgag
361  ccaacgaggg ccagacctgc actgcagagt tgcagccgtc tggcagtggg tctgggacag
```

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

```
10  atggaatcac agactctggt ttcataatcc atactgctct ggttatatgg tgctgatggg
61  aacattgtaa tgacccaatc tcccaaatcc atgtccatgt cagtaggaga gagggtccacc
121  tgcagcgtca aacagccgac acgactggcgc gcagctgagcc ggcggtccgc
181  ggcgctgcttgccgactgg ggcgtccgagac tcagagcgtc tgcacccgat tggcagtggg
241  gacagccgac agcagctgtc tgcagcgtca agcagcccgt cagccgagct cccgacaccc
301  gcgcgatccg ccaacctggt gcgttctggt tggctggttc ccatgcgtgc ggtgcagtcc
361  gacagagtcc ccaacctgcc gcacaggtctt gcgcgatctc cccgacacac ccaacctgcc
```

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

```
1  atggaatgga gctgggtctt tctcttcctc ctgctgctct ggcgtgtcagc gcaacccgcat
61  gtcagctgca agcagcttgtg ggcgtgtcagc gcaacccgcat ggtccggtctt ggtccggtctt
121  tgcagcgtct tgtgaatact cttccacacc tactgcagtg actggccaga gcaggtcgcc
181  gacagccggcc aagccgacac cccgagacag gcacagggcg ggcggttact atatgttatgt
241  caacgtccag gctcagcatg gcgcgtaccg atgctgactac tggacttgag gcacagccc
301  ggctgacatc tgacattcag ccaacactgc aagctgacac cgttgccagt tgcagccgag
361  ccaacgaggg ccagacctgc actgcagagt tgcagccgtc tggcagtggg tctgggacag
```

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

```
25  atggagacag acacaatct cgtatgggtg cctcttcctc cttctcctc
gcgctgctct gtcagcgtct tgtgaatact cttccacacc tactgcagtg actggccaga gcaggtcgcc
121  atctcctca aacagccgac acgactggcgc gcagctgagcc ggcggtccgc
181  ggcgctgcttgccgactgg ggcgtccgagac tcagagcgtc tgcacccgat tggcagtggg tctgggacag
241  gacagccgac agcagctgtc tgcagcgtca agcagcccgt cagccgagct cccgacaccc
301  gcgcgatccg ccaacctggt gcgttctggt tggctggttc ccatgcgtgc ggtgcagtcc
361  gacagagtcc ccaacctgcc gcacaggtctt gcgcgatctc cccgacacac ccaacctgcc
```
301  cctgtggagg  aggaggatgc  tgcaacctat  tactgtcagc  aaagtattga  ggatcctccc
361  acgttcggtg  ctgggaccaa  gctggagctg

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

1  atggaatggc  ctgtatctt  tctcttcctc  ctgtcagtaa  ctttcggttg  ccactccc
61  gttcagctgc  agcagtctgg  gcctgaaGtg  gttggcctg  ggtcctcagt  gaagatttcc
121  tgcaagctt  cttctcagc  tactggatga  actggggtaa  gccagggcct
181  ggacaggttc  ttcagtggtg  ttgacagatt  tattcctggag  atgggtatag  taactacaat
241  ggaacttca  agggtaaagc  cacactgact  gcagacaaat  cctccagtac  acagctcggg
301  ctgctgaga  actacctgtaa  ctacttgggc  caagggccca  ctcctcagct
361

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

1  ATGgacATGa  ggaacctgcg  tcagtttctt  ggtctctggtt  tccaggtatc
61  aaatgtgaca  tcaagatgac  cacagtctcca  tcttccatgt  atgcatctct  aggagagaga
121  gtaaactcca  aacctcttca  caccatatc  gatcctagc  agatggggtc
181  ccatcagct  ttcagtttcg  ggtgcatttc  ctcctcagct  caccacgctg
301  gagaatgaag  ataggggaaat  ttacctttgt  ctcagttcgt  ggtccagcag
361  gggaggga  ccaagctgga  aataaagc

[0132]  (9~)  3D1 Heavy Chain Variable Region (SEQ ID NO. 41)

1  atggaatggg  eggtgcctgtt  cctctgctgtt  ttgctgatgc  cgccttggag  gatggtactg
61  gtacagcttg  aaggtgtccg  acctgggctg  ggtgctggct  cagactgact  actggtgctcct
121  atgcaagcct  atatgctgt  tataactggg  ccagatctgt  cagccctggg  cacactgac
181  gggagggct  cggatagtct  gggagaatt  tggctgagc  gacactgact  ggggagactc
241  tctctcatt  ccagctgcag  gggagactct  ggtgcctgtc  cggagaattg  ggtgcagcag
301  atggaagtc  tgcatacttg  gagaatggc  ggtgcctgtc  cgccttggag  gatggtactg
361  tctctctggg  aagggaggc  ggtgcctgtc  cgccttggag  gatggtactg
(IG) 3Dl 1 Kappa Light Chain Variable Region (SEQ ID NO. 43)

1 atggattttc aagtgcagat tttcagcttc ctgctaatca ... tttcagtaac tatttcatgt cttgggttcg ccagactcca

181 gagaagaggc tggagtgggt cgcatatatt agtagtggtg gtggtagcac ctactatcca

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

10 1′ atgaaccttg gectcagatt gattttctct gtctttgttt taaaaggtgt gaagtgtgaa

61 gtgcacttg tggagttgg gggaggacta tggtcagctg gagggtcctt gaaacttctc

121 tgcaggtct cttgatcac tttaaaggtgt gtaatattag ctctgtgtgt taccagacta

181 gagaagggc tggagtgggt ctgcatatatt agtagtggtg gtggtagcac ctactatcc

241 gacacttgtag cctcagcact ccaatctactt cctagatgt gagggtcctt gaaacttctc

301 caatagaccag tggagatgtg tggagacactt cctgcatatatt agtagtggtg gtggtagcac ctactatcc

421 tcag

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

1 atgaggtgtc ccacactcaggt ccctggttgtt gctggtatgt gctagtctgt gcagactatcc

20 61 gacactcaggt tgactctgttt cctggtatgt gctggtatgt gctagtctgt gcagactatcc

121 atcatcaggt gacacttactacatcgtgcatgtacttcct cccatcaggt gcactgtgtc

181 ggaatctc cccactcttt ctggtatgt gctggtatgt gctagtctgt gcagactatcc

241 ggtactggtc gctgtgtcgt gctggtatgt gctggtatgt gctagtctgt gcagactatcc

301 gagaggtgtc gctggtatgt gctggtatgt gctagtctgt gcagactatcc
gctggtatgt gctggtatgt gctagtctgt gcagactatcc

361 ggaatctc cccactcttt ctggtatgt gctggtatgt gctagtctgt gcagactatcc

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

1 atgaaccttg ggtgatgtc gtctttgttt taaaaggtgt gaagtgtgag

61 gtcagctcg gggaggctta gccactctgt gagggtcctt gaaacttctc

121 tgtggttttt cctggtatgt gctggtatgt gctagtctgt gcagactatcc

301 ggaagagcct gggaggctt gccactctgt gagggtcctt gaaacttctc

ggaggctt gccactctgt gagggtcctt gaaacttctc
- 33 -

241  gacagtgtga  agggtcgatt  caccatctct  agagacaatg  ccaagaacac  cctgtacctg
301  caaatgagca  gtctgaagtc  tgaggacaca  gccatgtatt  actgtgtaag  acaaggagat
361  ggttactacg  gggactatgc  tatggaactc  tgagggatca  gaacctcagt  caccgtctcc 421  tcag

5  [0137]  ri 4) 1F3 Kappa Light Chain Variable Region (SEQ ID NO. 63)
  1  atgagttgca  ccacactggt  cctgagggttg  ctgctgtctg  ggcttacaga  tgccagaatgtg
  61  gacatccaga  tgactcagtcc  tccagctcc  ctatctgtat  ctgaggaga  aactgtccaccc
  121  atcacaatgct  gacacatgta  gaaatatatta  agtatattagg  catgtgatatc  gcaacacact  taccagatgg  tgtgccatca
  181  gaccaaatatc  etcagctacctg  ggtctgtatg  gcaacacact  taccagatgg  tgtgccatca
  241  gaagattttgc  gggactatactg  tttccctca  agatcaacag  cctgagctct
  301  ggagatttttc  gggactatactg  tttccctca  agatcaacag  cctgagctct
  361  gggaccaaac  tggaaatatta  ac

[0138]  (15) 3A12 Heavy Chain Variable Region (SEQ ID NO. 71)
  1  atgaactttgc  ggtcctgatt  gatattttctct  gttctgtttt  taaagagttgt  gaagtgcgctgaa
  15  gtgcagctgg  tggagtctgg  gggaggctta  gtgcagctcg  gagggtccct
  61  gtgcagctgg  tggagtctgg  gggaggctta  gtgcagctcg  gagggtccct
  121  tggagtctgg  gggaggctta  gtgcagctcg  gagggtccct
  181  gagaagaggc  ttgagttgtg  cgatacatt  aatgtggtgt  gtggtagccac  ctaactatca
  241  gacagtttgta  agggtctctt  caccactcc  agagacaatg  ccaagaacac  cctgtacctg
  301  caaatgagca  gtctgaagtc  tgaggacaca  gccatgtatt  actgtgtaag  acaaggagat
  361  ggttactacg  gggactatgc  tatggaactc  tgagggatca  gaacctcagt  caccgtctcc 421  tcag

[0139]  (16) 3A12 Kappa Light Chain Variable Region (SEQ ID NO. 73)
  1  atgagttgca  ccacactggt  cctgagggttg  ctgctgtctg  ggcttacaga  tgccagaatgtg
  61  gacatccaga  tgactcagtcc  tccagctcc  ctatctgtat  ctgaggaga  aactgtccaccc
  121  atcacaatgct  gacacatgta  gaaatatatta  agtatattagg  catgtgatatc  gcaacacact  taccagatgg  tgtgccatca
  181  gaccaaatatc  etcagctacctg  ggtctgtatg  gcaacacact  taccagatgg  tgtgccatca
  241  gaagattttgc  gggactatactg  tttccctca  agatcaacag  cctgagctct
  301  ggagatttttc  gggactatactg  tttccctca  agatcaacag  cctgagctct
  361  gggaccaaac  tggaaatatta  ac
[0140] **Reference Mouse IgGl Heavy Chain Constant Region (700453) (SEQ ID NO. 81)**

```
1  ccaaaacgac acccccatct gtctatccac tggccccctgg atctgtgc cc a a a ctaact
61  ccatgtgac cctgggatgc ctggtcaagg gctattttcc tga gcc caagtg acatgtgacct
5
121  ggaactctgg atccctgtcc agccgttgc acaccttccc agctgtcctg gatgtgc acc
181  tctacactct gacgcagctca gttctgc acc cctccagcccccttg gacaggtcag
241  ccctgccacg tgcggacagc gacccagc caa aggtga cc aagaaatt gtgcccagg
301  atgtgacctg taagctcttc atatgtacag tttcccccag gatgtgc
361  cccaaagcc caaggtgctg ctcaccattata cccccgagg aggctgcc tggctccgag
421  tagacatcag caaagagatgat cccggagg gcaggtcag cttctgcctgc
481  tgcacacagc tcagacgg tttcctg gacagtt cggcccagc gatgtgc
541  gtgaactttgc catcatgcac caggactggc tcaatggcaa ggagttcaaaa tgcagggtca
601  atgggttgac atatgtacag tttcccccag gatgtgc
661  cctgctctgt gttacatgag gcctgcaca ccaccatac gagaagagct cctctggtaa
721  gtctgacctgc atatgtacag tttcccccag gatgtgc
781  gacgccagc cagccaga ctacagagc ttcagccat catgac ac atgtgtttt
841  agtctctgta cagctccgtg caa cagaagctg ccagaggtc gaggcagga aatacttta
901  cctgtctgtg ctcagaaga ct gcca ggc gagaaga ctcgttctgc
961  ctctgtgat a g
```

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

```
1  ccaaaacgac accccccactgtctatccac tggccccctgg atctgtgc cc a a a ctaact
61  ccatgtgac cctgggatgc ctggtcaagg gctattttcc tga gcc caagtg acatgtgacct
5
121  ggaactctgg atccctgtcc agccgttgc acaccttccc agctgtcctg gatgtgc acc
181  tctacactct gacgcagctca gttctgc acc cctccagcccccttg gacaggtcag
241  ccctgccacg tgcggacagc gacccagc caa aggtga cc aagaaatt gtgcccagg
301  atgtgacctg taagctcttc atatgtacag tttcccccag gatgtgc
361  cccaaagcc caaggtgctg ctcaccattata cccccgagg aggctgcc tggctccgag
421  tagacatcag caaagagatgat cccggagg gcaggtcag cttctgcctgc
481  tgcacacagc tcagacgg tttcctg gacagtt cggcccagc gatgtgc
541  gtgaactttgc catcatgcac caggactggc tcaatggcaa ggagttcaaaa tgcagggtca
```
601 acagtgcagc tttccctgcc cccatcgaga aaaccatctc caaaaccaaa ggcagaccga
661 agctccaca ggtgtacacc attccacctc ccaaggagca...
721 ggtgtacacc attccacctc ccaaggagca gatggccaag gataaagtca
781 cccatcgaga aaaccatctc caaaaccaaa ggcagaccga
841 acctgacctg catgataaca gacttcttcc ctgaagacat tactgacctg tggcagtgga
901 ggtgctccata ggtgtacacc attccacctc ccaaggagca gatggccaag gataaagtca
961 cctgctctgt gttacatgag ggcagaccga...

[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 gggctgtatgc tgcaccaact gatctccat tccaccac tgcgtgca gtaaatcttctg
da
61 gaggctgctc atgtctgtgc tcttgaaca actctccac ccagagac tcaattgcaagt
121 ggaagatgta tggcagtgaa ccagagatg aaggtctgtgc cagttggact gatcagcaga
181 gcaagagag cacctacagc atagagcag gcaagagac gacagacaga cctcaggtt gacagacag gacagacaga
c
241 gacataacag ctagacagt gcggccac gcaagagac actctaccc actagcaga
301 ctctagcag aatgactgtg 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 gggctgtatgc tgcaccaact gatctccat tccaccac tgcgtgca gtaaatcttctg
da
61 gaggctgctc atgtctgtgc tcttgaaca actctccac ccagagac tcaattgcaagt
121 ggaagatgta tggcagtgaa ccagagatg aaggtctgtgc cagttggact gatcagcaga
181 gcaagagag cacctacagc atagagcag gcaagagac gacagacaga cctcaggtt gacagacag gacagacaga
c
241 gacataacag ctagacagt gcggccac gcaagagac actctaccc actagcaga
301 ctctagcag aatgactgtg 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, CDRi, CDR2 and CDR3 are identified by boxes. Figure 3 shows an alignment of the separate CDRi, CDR2 and CDR3 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, CDR1, CDR2 and CDR3 are identified by boxes. Figure 5 shows an alignment of the separate CDR1, CDR2 and CDR3 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>
<thead>
<tr>
<th>SEQ_ID NO.</th>
<th>Protein or Nucleic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Heavy Chain Variable Region 1A3 – nucleic acid</td>
</tr>
<tr>
<td>2</td>
<td>Heavy Chain Variable Region 1A3 – protein</td>
</tr>
<tr>
<td>3</td>
<td>Light (kappa) Chain Variable Region 1A3 – nucleic acid</td>
</tr>
<tr>
<td>4</td>
<td>Light (kappa) Chain Variable Region 1A3 – protein</td>
</tr>
<tr>
<td>5</td>
<td>Heavy Chain CDR1 1A3</td>
</tr>
<tr>
<td>6</td>
<td>Heavy Chain CDR2 1A3</td>
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<tr>
<td>7</td>
<td>Heavy Chain CDR3 1A3</td>
</tr>
<tr>
<td>8</td>
<td>Light (kappa) Chain CDR1 1A3</td>
</tr>
<tr>
<td>9</td>
<td>Light (kappa) Chain CDR2 1A3</td>
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<tr>
<td>10</td>
<td>Light (kappa) Chain CDR3 1A3</td>
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<td>12</td>
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</tr>
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<td>Light (kappa) Chain Variable Region 2B8 – nucleic acid</td>
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<td>Heavy Chain CDR3 2B8</td>
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<td>Heavy Chain CDR2 2F8</td>
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<td>Heavy Chain CDR3 2F8</td>
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<td>Heavy Chain Variable Region 3B6 – protein</td>
</tr>
<tr>
<td>33</td>
<td>Light (kappa) Chain Variable Region 3B6 – nucleic acid</td>
</tr>
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<td>SEQID/NO.</td>
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<td>-------------------------------------------------------------</td>
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<td>50</td>
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<td>Light (kappa) Chain Variable Region 1F3 – nucleic acid</td>
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<td>64</td>
<td>Light (kappa) Chain Variable Region 1F3 – protein</td>
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<tr>
<td>65</td>
<td>Heavy Chain CDR₁ 1F3</td>
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<td>74</td>
<td>Light (kappa) Chain Variable Region 3A12 – protein</td>
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<td>Light (kappa) Chain CDR₂ 3A12</td>
</tr>
<tr>
<td>80</td>
<td>Light (kappa) Chain CDR₃ 3A12</td>
</tr>
</tbody>
</table>
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 3D1 1 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.

10 [0148] (1A3 Heavy Chain Variable Region and IgGl Constant Region) (signal sequence underlined^)

(SEQ ID NO. 122)

```
1  atgaactttg  ggtctcagatt  gattttccttt  gtcttcgfcct  taagaaggtgt  gaaggttgt_gaa
 61  gtgcagccttg  tggagtcttgg  gggaggttta  gtgcagccttg  gagggtctct  gaactctctcc
15  tggctgccct  ttgtaatgta  tgctcctgtg  cttgggttgc  ccaagctcca
20  tggccagttg  tggagtggcct  ggcctcctgg  cggagctggt  cccacttcct
25  tggctccagt  tggagtgggaa  ggtcggcttt  gctggtggct  tggcctggtg
30  tggctccagg  tggagtggga  ggtcggctgg  gctggtgggt  tggcctggtt
35  tggctccctg  tggagtggta  ggtcgggtct  gctggtggat  tggcctcctc
```
(2) Protein Sequence Defining the Full Length IA3 Heavy Chain Sequence (SEQ ID NO. 123)

1  evqlvesggg lvqpgsgslkl scaaeeffts nyymswvrqt peklr1qvwv yspgggsyy
5 61 pasvkgfrft slrdnaktly lqmslksled tamycarcqg dgyygyatnd ywggqtsttv
10 x21 ssaaktppps yplapgsaaq tnmvtlycl vkgqyfepvtt vtmnsagslas qvhtfpaovlq
15 181 sdlytlassv tvgssfrwpse tvtcnvahpa sstkvdkkiv prdcqckpici ctpvepsvsf
20 241 ifppkpkdvl fclll lpkte vwdiskdpp evqfswtvfd vevhtaqtgp reeqfntstr
25 301 svseplpmbh dwlingkfcf rnsaafpap iekltisktkg rpkapqyti pgppkegmakd
30 361 kvaltcmitd ffpeditvweqv gmqpgpaen yrntqpmimt d gsyfvyxln vqksnweagn
35 421 tftcsvglheg lhhnhhteksl shapkg

(3) Nucleic Acid Sequence Encoding the Full Length IA3 Light Chain Sequence (SEQ ID NO. 124)

1  atgagttgtc ccaactcagtt cctggggttg cctgtctgtgtg ggccttacaga tgccagatgt
5 61 gacatccaga tgcatacatc tccagcttccc ctactcttttt ctgtgggaga aacgtctacc
10 121 atccactgtc gacagatgta gatattttgt atcagttta tcaagacttc gacagacaagac
15 181 gggaaatcct ctcaagcttct gccatcagtt gcaccaactt tgcagatgcag tgcagctaca
20 241 aghtctggtg gcacagcttat gtcctccaca gttttccttc atcagatgtac ctgcacccac
25 301 gaagattttg ggcctttatg ctctcaacat ttttgggtga tccctacccaa cttcggaggg
30 361 gggaaccagc tgtaaaataaa acgggtgtat gcgtgccaa tctatccac cccctccacca
35 421 tccagtggac agttacatgc tggaggtggc fcccagttct gttcttggaa caaccttacac
40 481 cccaaagcac ccctgtggtgta gagccagatt gtggagaatg atcctaccag gcagatcctac
45 541 accctgtgga cttgagctag cagccagacg acgactttac caagatgtgg tctagatgg
50 601 ttgaccaagg acaatagatac aqacataaac agctatatct gtagggcgc caacagacac
55 661 tcaacctttac ccaatttgtcag gacgctttacag aggaatcagt tttggttgg

(4) Protein Sequence Defining the Full Length IA3 Light Chain Sequence (SEQ ID NO. 125)

1  diqmtqspas lavsveytv itcraseny nslawyqqk gksqpllyva atnladgyps
5 61 rsfgsrgsttq slskinslqs edfgytyqeh fwwgtptefg gtklmieds atpvsippp
10 121 sseqltsgga swfchnmyn pkdinvkwi dgseryqnyvl nstwqpsdsk tctmsmslltn
15 181 ltkdeyerhn syceathk tncipsvln

(5) Nucleic Acid Sequence Encoding the Full Length 2B8 Heavy Chain Sequence (SEQ ID NO. 126)

1  atggtgatgaa ggttatattac cccctttttttt gtagcaacag cbaacagatg ccaaccttcag
5 61 gtccactgcg acctggctggg ggtgcttaagc gtagccagct ccagctagctt gtagcttgcc
10 121 tggcagcttc ctggcagcttc cttcagcttc ctggacaag tctgctaagt ctggcccagctt
15 181 ggcaacagcc ttggataggt tagggaggttagaatctcctg acgtctctac taactactat
20 241 gagggcttcag ggaggaggttagaagc ctgagctacat gtagccagctt ctgcagcacc agcagcagcttg
25 301 caactcaagc cggctgatctt ggggcttagat gtaagtacag ctcagcaccag gacagcagcagc
30 361 ggctgacatt ttgagctact ccgggaacgg aggctgactacc cagctctctc acddccacta
35 421 acacacccat cctgtcctata ccggctgtgg gtagccagctt cccacactaa cccttcagcagctt
40 451
- 40 -

481 accctgggat gcctgtagca gggctatttc cctgagccag tgacagtgac ctggaactct
541 ggatccctgt ccagcggtgt gcacaccttc ccagctgtcc 5gcctctccag ggttgcaccag gtatgtgttg
601 cttgcgacgc gcggcagcag caccaagttg gacaagaaaa tctgtcccaag ggtttgtggt
661 tttcacttcac ccattgtcaa gagcttcaac aggaatgagt gttag

5

1081
1021
1321
1381

1021 gcccctcttg ccccctatcg gaaacaccag tccaaaccag gaagacagagg caggtgacta
1001 caggtgttca ccattccacc tccaaagagag caggtgacta caggtgacta caggtgacta
1141 ggcacagcag cagacatgct gctggtgatg gccgacagca gcagctgacct gcagctgacct
t1201 ccaagacgcc ccacagacag cagtacgcc cctaggtgact ttactgtcgt
1261 tacagagacc tcaatgtgca gagaagacag tcggagccag gaaacttctt cacactgtct
1321 ggtgttacag gggctgctca caacaccatc actgagaaga gcctctcccc ccctctctgt
1381 aatag

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

1  qvqlqpggae lvkpgstsvl cscasgytft tywvhwvnqr pggglewige inptnghtny
61 nekfkskatl tvdksstagy mqlsltstds savyccanry vgaliydvwq qgtltvsasak
121 ttpsvyypla psaasqntsm vtglcivkgy fpepvtvtn pssalgssvht fpavlsqdlv
181 tissavtvpv tpspetvetc nvhapasskt vdkkiiprdr gckpcictvp evsvsfifpp
241 kpkdvlttl tptkvtcvwd nisddevepf swfvdvevhd taqpcpreeq fsatfrsvse
301 lplmhdwln gkefkrvns aafpaiekt isktkgpaka pqvtpipppk eqmakdsvsl
361 tcmitsffpe ditvewqweg qpaeynkytq pmdttdgysf vysklvnqks nweagntftc
421 svilheghln gbtkelasjsp 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 agactcfcggt tttcatatcc atacctgcct gtttatagtg tgtctgttgag
61 aacattgtgaa tagcctgcast gtaataaccc atgcctgact gtagaaaatc ggtgctgagc
121 ttgagctgca aagccctgtca gastaattgc gactagggct gcagataagc ggtgctgttg
181 ggcagtcgct ctaaatctgc gtaataaggg gactagaaaat ccctgcaagc gcagataagc
241 cgtttgtggct ctaaatctgc gtaataaggg gactagaaaat ccctgcaagc gcagataagc
301 gagactgcttg cagagacact ctcgagggag gtagaatggag atccacactg gctggagggag
361 ggacagcttg cagagacact ctcgagggag gtagaatggag atccacactg gctggagggag
421 ttgaagcttt tcagagcttg ctcgagggag gtagaatggag atccacactg gctggagggag
481 ccaccagc aaatgtgtaa gttgagggag gtagaatggag atccacactg gctggagggag
541 aacacttgga tctgactgta ctcgagggag gtagaatggag atccacactg gctggagggag
601 ttgacatgac agataacatc agctatataa agctatataa gtagaggttg tagg
661 tccactccac cgtatgctca gacgacctac agaatgagact gtagaggttg tagg
(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)

<table>
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<tr>
<th>Protein Region</th>
<th>Sequence</th>
</tr>
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<tbody>
<tr>
<td>Kappa Variable Region</td>
<td>nivmtqspk samsygvert lsckasenw edlayhegg synpptyqf dsgeraqhvl mec</td>
</tr>
<tr>
<td></td>
<td>qagptklyig gtrelkrd nswtdqsdsk stymsatlt</td>
</tr>
<tr>
<td>Kappa Constant Region</td>
<td>tcmitdfp ditvewqwng qpaenykntq pimtdtgysf vysklnvqks nweagnfttc</td>
</tr>
</tbody>
</table>

(9) Nucleic Acid Sequence Encoding the Full Length 2F8 Heavy Chain Sequence (2F8 Heavy Chain Variable Region and IgGl Constant Region) (without signal sequence) (SEQ ID NO. 130)

<table>
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<tr>
<th>Nucleic Region</th>
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<td>atgggcacgg cagggcagg gcaagctgca gataggcctg catctgcac</td>
</tr>
<tr>
<td></td>
<td>gggttacatg tactacatgg cacccagaag gcaagatgc gatcagcact</td>
</tr>
<tr>
<td>Heavy Constant Region</td>
<td>ggcaagcgg ccagggaag gaggtgctag cagggctgca</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Protein Region</th>
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<td>qvqlkqsgae lrvptgtsvm sckasagytf tyyihwv Pqr pqgglewqk igpgsgstyy</td>
</tr>
<tr>
<td></td>
<td>nemfkdkatl tvtdssstagy mqlslsltdc savyfcarrg lgrgfdwqg gttltvssak</td>
</tr>
<tr>
<td>Heavy Constant Region</td>
<td>pgsqggagnt stpsvqvkxwq pqvkyneugq mctgntvqc</td>
</tr>
<tr>
<td></td>
<td>tvlglcgyq fpevtpvsn sgalswey lpmavgslyd</td>
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<td>tvlglcgyq fpevtpvsn sgalswey lpmavgslyd</td>
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[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 acacactct gctatggtg ctgctg ctct ggtgcag cc actccctagt ctccacctg
5 61 gacactgctg tcaccaactc ttcagcttct tgtgtgtgtg tcttctagt gaaaaa ggaacgcag 
10 121 atctctgca agggcatgca aatgtgtcat tgtatgtata taatgata tatcactcat ccagtgtaat
15 181 caagacagcc cagccacgac acacactct ctgctgctgt cctgtacatg ctccactgtg ccactcactg
20 241 gggctccag ccagttctag tcgctgctgctg tcttcttgtg tcttctacat gaaaaa ggggcctcg
25 301 cctctgctag agggcatgca tcgctgctgctg tcttcttgag tcttctacat gaaaaa ggggcctcg
30 361 acctcttgct cgcgcttgca tgtgtgtgtg tcttcttgtg tcttctacat gaaaaa ggggcctcg
35 421 atctctgca agggcatgca tcgctgctgctg tcttcttgag tcttctacat gaaaaa ggggcctcg
40 481 tggcagctcg cttctgctag agggcatgca tcgctgctgctg tcttcttgag tcttctacat gaaaaa ggggcctcg
45 541 cctctgctag agggcatgca tcgctgctgctg tcttcttgag tcttctacat gaaaaa ggggcctcg
50 601 tgcctgtctg ggcgcttgca tcgctgctgctg tcttcttgag tcttctacat gaaaaa ggggcctcg
```

[0159] (12) Protein Sequence Defining the Full Length 2F8 Light Chain Sequence
(Kappa Variable Region and Constant Region) (SEQ ID NO. 133)

```
1 divltqspa slavsgtgn isckasqnsd ydgsyinwv qkpgpptpv liyvnsel kradaptv 
5 61 giparsqsg sgaswefl nmfypkdnv thkstspiv ksfhrncg 
10 121 fippsseqlf slhltlky slhenwyecn tctttcaccct cctggtaaat gggtggttac 
15 181 stliltlky slhenwyecn tctttcaccct cctggtaaat gggtggttac 
20 241 giparsqsg sgaswefl nmfypkdnv thkstspiv ksfhrncg 
25 301 giparsqsg sgaswefl nmfypkdnv thkstspiv ksfhrncg 
30 361 giparsqsg sgaswefl nmfypkdnv thkstspiv ksfhrncg 
35 421 giparsqsg sgaswefl nmfypkdnv thkstspiv ksfhrncg 
40 481 giparsqsg sgaswefl nmfypkdnv thkstspiv ksfhrncg 
45 541 giparsqsg sgaswefl nmfypkdnv thkstspiv ksfhrncg 
50 601 giparsqsg sgaswefl nmfypkdnv thkstspiv ksfhrncg 
```

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

```
1 atggagacag ctgccatctcc tctccctccct ctgctgctgctg ctctgctgact cactcc cactcc 
5 61 gtccaggtgc agcagctcgc gcagctcgc gcagctcgc gcagctcgc gcagctcgc gcagctcgc 
10 121 tgcaggtgct ccagccacct ccagccacct ccagccacct ccagccacct ccagccacct ccagccacct 
15 181 gacccgagcc cccagccacct ccagccacct ccagccacct ccagccacct ccagccacct ccagccacct 
20 241 gacccgagcc cccagccacct ccagccacct ccagccacct ccagccacct ccagccacct ccagccacct 
25 301 gacccgagcc cccagccacct ccagccacct ccagccacct ccagccacct ccagccacct ccagccacct 
30 361 gacccgagcc cccagccacct ccagccacct ccagccacct ccagccacct ccagccacct ccagccacct 
35 421 gacccgagcc cccagccacct ccagccacct ccagccacct ccagccacct ccagccacct ccagccacct 
40 481 gacccgagcc cccagccacct ccagccacct ccagccacct ccagccacct ccagccacct ccagccacct 
45 541 gacccgagcc cccagccacct ccagccacct ccagccacct ccagccacct ccagccacct ccagccacct 
50 601 gacccgagcc cccagccacct ccagccacct ccagccacct ccagccacct ccagccacct ccagccacct 
```

- 42 -
[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  qqvlqsgqag  tvrpgssvki  sckasgyvfs  sywmmwkwq  ppgerewtgg  lypgddnsy  5
21  ngnfkgkatl  tadksstaybe  mqlssltsed  savyfcasqg  glnreyfdw  ggqtltvvs  26
121  akktppsvyp  lagsaagafcn  smvlgclvlk  glyfpepvtv  vmsgalsgvg  htftpavlqd  126
181  lytlsssvtv  ptswpsevtt  tcnvahpaas  tkvdkkkivy  gcgckpcict  vpevssvif  186
241  ppkpkvdlti  tltlkpvctc  vdiskkdpve  qfswfdvdve  vhtatqgtpe  eqfnstfrsv  246
301  selpimhqdw  lngkerfcrv  nsaafpapie  ktkístkgpr  kapqytyipp  pkeqmakdkgv  306
361  altcmitdff  peditvewqw  ngpqacenyn  ttqpinmdtdgs  yfvysklnvq  ksnweagntf  366
421  tcsvlheglh  nhhtekslah  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  ATGGacATGArg  gcaacccctgc  tcaagtttttc  ggaatcctttg  tgcctcctggtt  tccacaggatc  5
21  aaatgtagcaca  tcagttcagc  cccttcctatc  ttcctctgta  ttcctctgta  ttcctctgta  26
121  gacccagagga  aactctctca  gacccctgtc  tctctctttt  cttctctttt  cttctctttt  126
181  gacccagagga  aactctctca  gacccctgtc  tctctctttt  cttctctttt  cttctctttt  186
241  gacccagagga  aactctctca  gacccctgtc  tctctctttt  cttctctttt  cttctctttt  246
301  gacccagagga  aactctctca  gacccctgtc  tctctctttt  cttctctttt  cttctctttt  306
361  gacccagagga  aactctctca  gacccctgtc  tctctctttt  cttctctttt  cttctctttt  366
421  gacccagagga  aactctctca  gacccctgtc  tctctctttt  cttctctttt  cttctctttt  426

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

1  dikmtqgps  myaslgervt  itckasqdiik  sylswfogkq  gksopktiy  vnrliydvgps  5
21  rfsqsggqsd  ssllltslens  edmlginyilq  ydefpsftfg  gktkleirad  aaptsisfpp  26
121  sseqltsgga  swfclnffy  pdkdinkvkk  dgrserqny  nswtdqskd  stymsstlt  126
181  ltdkdey  q18n  sytecaehht  stspivksfn  rnc

[0164] (17) Nucleic Acid Sequence Encoding the Full Length 3D1 1 Heavy Chain Sequence (3D1 1 Heavy Chain Variable Region and IgG1 Constant Region) (signal sequence underlined) (SEQ ID NO. 138)

1  atggccctgcccc  cggggccgcttt  cctcctgccctt  gttccatcctc  caacagctgtg  cccctgcccc  cag  40
21  gtacagctga  aggagtcagg  acctgcccgtt  gttggcctctc  ccaagacgcct  gtccacatcct  46
121  gaaagggagc  tggagtgcat  gggagtagct  gttggcctctc  ccaagacgcct  gtccacatcct  126
181  gaaagggagc  tggagtgcat  gggagtagct  gttggcctctc  ccaagacgcct  gtccacatcct  186
241  gaaagggagc  tggagtgcat  gggagtagct  gttggcctctc  ccaagacgcct  gtccacatcct  246
301  gaaagggagc  tggagtgcat  gggagtagct  gttggcctctc  ccaagacgcct  gtccacatcct  306
361  gaaagggagc  tggagtgcat  gggagtagct  gttggcctctc  ccaagacgcct  gtccacatcct  366
421  gaaagggagc  tggagtgcat  gggagtagct  gttggcctctc  ccaagacgcct  gtccacatcct  426
[0156] (18) Protein Sequence Defining the Full Length 3Dl 1 Heavy Chain Sequence
(3Dl 1 Heavy Chain Variable Region and IgGl Constant Region) (SEQ ID NO. 139)

[0166] (19) Nucleic Acid Sequence Encoding the Full Length 3Dl 1 Light Chain Sequence
(3Dl 1 Kappa Variable Region and Constant Region) (signal sequence underlined) (SEQ ID NO. 140)

[0167] (20) Protein Sequence Defining the Full Length 3Dl 1 Light Chain Sequence (3Dl 1 Kappa Variable Region and Constant Region) (without signal sequence) (SEQ ID NO. 141)
(SEQ ID NO. 142)

1
5

atgaactttg  ggctcagatt  gafctccccct  gtctctgtttt  taaaagtgt  gaaagtga

61
gfcscaagcttg  tgtgaatcttg  ggagggactta  gttcgacccgt  gagggtcctt  gaaacttcc

121
ttgtcaagtgg  tcctgattc  cactagcatg  tacatcgttg  cttcgcctga  ccaactcctc

181
gagagaggtg  tgtgagtttg  cgcacattc  agtgagtggtg  tgtgaggac  ctaactcctca

241
gcaagtctgg  aaggctcct  cccatccct  cggagacagt  ccaagaaac  ccgtacctcc

301
caaatagcca  gctgaggttc  tcggagacca  gcatatctgt  actgtgtag  acaagggcata

10
61

ggttagattc  ggagactctg  tgtggactac  tgggttcaggg  gacaacctcg  catctgtcctc

121
tcgacaaaaa  gcacaacagc  atctgctaac  ccactgcggc  cttgagtcrtg  tcgcaccaact

181
aacattctag  tgacatatgg  cttagctgtg  aaggtgtcatt  ccctctagcc  aqtcagttg

241
acctgggaact  cctgatgcct  gttcagcggc  tcctccctca  ggaatcagtg  ccctgcagtct

15

61
tgctacccaa  agcagcaggc  cgcactcggc  agcgactcag  tggcacaaga  aattgtgcgc

121
aggaggaagg  tgtgtgaaggc  ttgcatgatt  acagctcagc  aagatgt  gatcgcgttc

181
ctcccttccc  aagcagcaag  cctgctcacc  attactcgtg  cttcagcttg  cagctgtgtt

241
gttgtagaca  tcgacagcag  tgaattggag  gttcagcagc  gtcggctgta  aqctgtgtg

301
haggtgeaca  cagtctcagc  gcaacaccgg  gaggacagtt  tcacacagcc  ttcgctgctc

20

61
tgctacccac  gccagtcacg  ttagctcatt  gcagacgatt  tggcactctg  ccaatcgcag

121
tgctcaacagt  cagctttccc  tcgctccctc  cggacacaca  tggacacagc  cagacagcgc

181
ccagagtgcc  ccaagccatc  ccccctcag  cagcatcatt  cagaggtg  caaggttata

241
tttctacattg  cctgctcatt  aggcttcgcl  cttcgcctca  acaagcggc  cagacagcgc

25

61
tgctacccga  agcagcggaga  gacattctc  gctgcatcct  ccctctggag  ccacagagc

121
ttgaaatggcc  agccagcgga  gaaactcctc  gcaatcctct  cagactggc  cagaaatagt

181
tttctacattg  cctgctcatt  aggcttcgcl  cttcgcctca  acaagcggc  cagacagcgc

241
tttctacattg  cctgctcatt  aggcttcgcl  cttcgcctca  acaagcggc  cagacagcgc

30

[0196]
(22) Protein Sequence Defining the Full Length 1D3 Heavy Chain sequence (1D3 Heavy Chain Variable Region and IgGl Constant Region) (without signal sequence) (SEQ ID NO. 143)

1
35
40

1
evqlvesggg  lvqpggsklk  scaangtgfs  dyymswrtq  pekarlewvay  issggsttyy

61
psdkvgrfcti  srdnaknfcly  lqmslkeds  taiyycrvqrg  dgygydyamd  ywqqgttasviv

121
ssakttppsvgp  yplaqgaaq  tnsmvlgcl  vkgypfevpt  vtwnglas  ghvtfpaivq

181
sdltylassv  tspstwpesa  tvctcnvha  sstvkviddp  prdcgcckpl  ctpveasvfv

241
lfppkpklvct  tiltpkvcct  vwdiskdpp  evqsfawfdv  vevhtaqtpq  reeqfstfr

301
svselpimhqv  dlwngkfkc  rnuasaapap  lektsftkg  rpkapqvyl  pppeqmkakk

361
kvalmcld  ffpeditvwe  qngpaaqny  kntgplmdtd  gsyfvyksln  vqksnweagn

421
tftcsvlheg  lnnhtteksl  shapkg

45

[0197]
(23) Nucleic Acid Sequence Encoding the Full Length 1D3 Light Chain Sequence (1D3 Kappa Variable Region and Constant Region)" (sequence signal underlined) (SEQ ID NO. 144)

1
45

1

1

atgaactttg  ccactcaggt  cctggggtct  ccctgctgtg  tcgacctaca  ggtctgagtt

61

gatctccaga  tcctcagcct  tccagccctcc  ctatctgtat  ctgtgaggaga  aacgtctccc

121
atccactcagc  gaaacaggct  gaaatatttc  agtatctagc  ctgtcttctgc  gaacaaacag

181
ggaaaaatcct  ctctacgctcc  aactctgtct  gcaacacaca  tagcagagtt  tggcttgcac

241
aggttcaagt  cgcttggtcct  gaggacacag  tttctccctc  ggatcaacac  cctgcagttc
(24) Protein Sequence Defining the Full Length 1D3 Light Chain Sequence (1D3 Heavy Chain Variable Region and IgGl Constant Region) (without signal sequence) (SEQ ID NO. 145)

[0171]

1 digmtqspas lsvsygetvt itcrtnseny snlawyqqk gkspsqlliya atnladypsp
61 rftsogsgtgq fslrlinslqs edfgyrycqh wagtptf tgg tktklekrad aaptvsifpp
121 sseekltsggs swcflnnfyy pkdinvkwi dgserqngvlns wtdqskd stysmsstlt

15 1tldyeyrhyn sytceamhtk stspivksfn rnc

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

1 atgaacctttg ggctcagatt gatatttcccc ctctctgttt taaaaggtgt gaaggtgt qag
61 gtgcag cgtggtccct gggagcgtta tgcagctcttg gagggtctcct gaaacfcctcc
121 ttggcatctgc gcctgcttcat ggcggacatt cattctccgt cctggacatgt gcctaggtgt
d361 ggtactactc gggactgcgtc ctcgcacttc gggagcgtta ttcgtctctg gttctgactg
d421 tcagccaaa ccgacccccc cttgctcctg ccagcgtctcg ccgacccccc
d481 aactctcttg ggctcagatt gatatttcccc ctctctgttt taaaaggtgt gaaggtgt qag
541 actctctctg ctctgcttcat gtcagctctg gctgcagctct cctggacatgt gcctaggtgt
d601 gacctctctc cctctgcttcat gtcagctctg gctgcagctct cctggacatgt gcctaggtgt
d661 gtcaacetg acgaggttgtgc cccgctgcct gacagctctg gttctgactg gttctgactg
d721 aggtaggtgttg ttgttaagcc tgcgactgtc tgcgaggtgtgt tgcgaggtgtgt tgcgaggtgtgt
d781 ttggccctgg ccgacccccc cttgctcctg ccagcgtctcg ccgacccccc
d841 gtcatctctg ggtcagagac cggaggacct cttggagatgt tgg accctgcttcat
d901 gatctctctc cctctgcttcat gtcagctctg gctgcagctct cctggacatgt gcctaggtgt
d961 gtagctctctc cctctgcttcat gtcagctctg gctgcagctct cctggacatgt gcctaggtgt
d1021 gtcacgcgcgg ccaactgctct gtcctgcttcat gtcctgcttcat gtcctgcttcat
d1081 cctggtctct cctggtctct cctggtctct cctggtctct cctggtctct cctggtctct
d1141 gtacgtctcg cctggtctct cctggtctct cctggtctct cctggtctct cctggtctct
d1201 ttggaggtgct ggcgcggagct ctcggtctct cctggtctct cctggtctct cctggtctct
d1261 tcgctctctc cctggtctct cctggtctct cctggtctct cctggtctct cctggtctct
d1321 ttggaggtgct ggcgcggagct ctcggtctct cctggtctct cctggtctct cctggtctct
d1381 cctggtctct cctggtctct cctggtctct cctggtctct cctggtctct cctggtctct

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

1 evqlvesggg lvqsggsllkl sccaagqftfs nyfmswvrqf pekrlwvay issgggstyy
61 psdvkvgrfii srdnakntly lgmslksksed tamvycbrqrg dgyylydymd ywgqgtstvtt
121 ssaktpppsv yplagpsaqns nsmvtglcg vkygyppepvt vtwngssls gvhfhpavlq
(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  atgatgtgct ccaactcagt cctggggttg cttgctgtgt ggttcataca tgcagaagtgt
25  gaccttcca tccagctcct cttcatcgtct gctatcagtt ctgatgagct ctcagcacaag
30  gctttcttc ctcagctcct gctatcagtt ctgatgagct ctcagcacaag
35  gctttcttc ctcagctcct gctatcagtt ctgatgagct ctcagcacaag
40  gctttcttc ctcagctcct gctatcagtt ctgatgagct ctcagcacaag
45  gctttcttc ctcagctcct gctatcagtt ctgatgagct ctcagcacaag
```

(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  digmtqspas lsvsvgettv itcraseniy snlawyqgkq gksqpdllvd athlpdgypv
25  fsikinslagq edfgyyycgh fwtgpytfgg gtrleikrad aaptvsifpp
30  sseqltsgga swfllnny ptkdinvkwi dgsersqngyl nwstdqdiskd stymssstlt
```

(29) Nucleic Acid Sequence Encoding the Full Length 3AI2 Heavy Chain Sequence (3AI2 Heavy Chain Variable Region and IgGl Constant Region) (signal sequence underlined) (SEQ ID NO. 150)

```
1  atgaaccttg ggctcagatt gatttttctt gttccccctt taaaaggttg gaagttg gaa
25  tggatctgg ggaggcttta gtgcagccttg gagggtcctct gaaatctCG
30  tgtgcagctt tttcatcattc aatgttgtgt ctagtacacag ccaagtacgtg
35  gacgctgtaa agggcttta cccatcagc agagcataag ccagatacagc cccagtcacagc
40  gctttcttc ctcagctcct gctatcagtt ctgatgagct ctcagcacaag
45  gctttcttc ctcagctcct gctatcagtt ctgatgagct ctcagcacaag
50  gctttcttc ctcagctcct gctatcagtt ctgatgagct ctcagcacaag
```

(30) Nucleic Acid Sequence Encoding the Full Length 3AI2 Heavy Chain Sequence (3AI2 Heavy Chain Variable Region and IgGl Constant Region) (signal sequence underlined) (SEQ ID NO. 150)
[0177] (3) Protein Sequence Defining the Full Length 3A12 Heavy Chain Sequence

(3A12 Heavy Chain Variable Region and IgGl Constant Region) (without signal sequence) (SEQIDNO. 151)

1 evqlvesgsg
61 psdvkrifti
121 ssakttppsv
181 sdyltlassv
241 ilpkkpdkvl
301 svspipmhm
361 kvvalcmctld
421 tftcsvlheg

[0178] (3) 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 atgaatgcgg
61 gacatccaga
121 atcacagctc
181 gaaaaatctc
241 aggtctcagc
301 gagatttttg
361 gggacctacaa
421 tcaagtgtgac
481 cGCAAaagaca
541 aacagttagg
601 ttgaccaag

[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 digmtdqpsas
61 rsqsgstgtq
121 sseqltsgaa
181 ltkdeyehln

[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.
Example 3 - Production of Various Recombinant hHGF Proteins

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

<table>
<thead>
<tr>
<th>SEQ_ID/NO.</th>
<th>Protein of Nucleic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>122</td>
<td>1A3 Heavy Variable + IgG1 constant - nucleic acid</td>
</tr>
<tr>
<td>123</td>
<td>1A3 Heavy Variable + IgG1 constant - protein</td>
</tr>
<tr>
<td>124</td>
<td>1A3 Light Variable + constant - nucleic acid</td>
</tr>
<tr>
<td>125</td>
<td>1A3 Light Variable + constant - protein</td>
</tr>
<tr>
<td>126</td>
<td>2B8 Heavy Variable + IgG1 constant - nucleic acid</td>
</tr>
<tr>
<td>127</td>
<td>2B8 Heavy Variable + IgG1 constant - protein</td>
</tr>
<tr>
<td>128</td>
<td>2B8 Light Variable + constant - nucleic acid</td>
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<td>129</td>
<td>2B8 Light Variable + constant - protein</td>
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<td>130</td>
<td>2F8 Heavy Variable + IgG1 constant - nucleic acid</td>
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<td>131</td>
<td>2F8 Heavy Variable + IgG1 constant - protein</td>
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<td>132</td>
<td>2F8 Light Variable + constant - nucleic acid</td>
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<td>133</td>
<td>2F8 Light Variable + constant - protein</td>
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<td>134</td>
<td>3B6 Heavy Variable + IgG1 constant - nucleic acid</td>
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<td>136</td>
<td>3B6 Light Variable + constant - nucleic acid</td>
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<td>1F3 Heavy Variable + IgG1 constant - nucleic acid</td>
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<tr>
<td>147</td>
<td>1F3 Heavy Variable + IgG1 constant - protein</td>
</tr>
<tr>
<td>148</td>
<td>1F3 Light Variable + constant - nucleic acid</td>
</tr>
<tr>
<td>149</td>
<td>1F3 Light Variable + constant - protein</td>
</tr>
<tr>
<td>150</td>
<td>3A12 Heavy Variable + IgG1 constant - nucleic acid</td>
</tr>
<tr>
<td>151</td>
<td>3A12 Heavy Variable + IgG1 constant - protein</td>
</tr>
<tr>
<td>152</td>
<td>3A12 Light Variable + constant - nucleic acid</td>
</tr>
<tr>
<td>153</td>
<td>3A12 Light Variable + constant - protein</td>
</tr>
</tbody>
</table>
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.

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

a. hHGF-Fc

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 HgFc. The overlapping PCR fragments served as template in a second round to amplify hHGF-his-IgFc. The resulting fragment was digested by Nhel and BamHI and cloned into pcDNAs/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.

(I) 5'hHGF Nhel Primer

ACTGGCTAGCATGTGGGTGACCAAACTCCT (SEQ ID NO. 102)

(D) 3' hHGF Notl His Tag Primer

GTGATGGTGATGGTGATGGCGGCCGCATGACTGTGGTACCTTATATG (SEQ ID NO. 103)

(3) 5' HisIgFc Primer

ACTGGCGGCCGCCATCACCATCACCATCAC (SEQ ID NO. 104)

(4) 3' IgFc BamHI Primer

ACTGGATCCTCCTACTATTACCACCGGGGACAG (SEQ ID NO. 105)

b. hHGF-Fc G555E and hHGF-Fc C561R

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.
- 51 -

1) hHGF-Fc (G555E) Sense Primer

\[ \text{CATGATGTCCACGAAAGAGGAGATGAG (SEQ ID NO. 106)} \]

2) hHGF-Fc (G555E1 Anti-sense Primer)

\[ \text{CTCATCTCCTCTTTCGTGGACATCATG (SEQ ID NO. 107)} \]

3) hHGF-Fc (C561R) Sense Primer

\[ \text{GGAAGAGGAGATGAGAAACGCAAACAGGTTCTCAATG (SEQ ID NO. 108)} \]

4) hHGF-Fc (C561IO Anti-sense Primer)

\[ \text{CATTGAGAACCTGTTTGCGTTTCTCATCTCCTTTCC (SEQ ID NO. 109)} \]

c. Mouse-human-mouse chimera Fc

The mouse-human-mouse chimera IgFc construct contains mHGF alpha chain-hHGF, \( \beta \)-chain amino acids Val 495-Leu 585 of human HGF, and mHGF C-terminal beta chain followed by \( \xi \)oxHis tag and IgG-Fc.

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_000601A. 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.

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 Nhel and NotI and cloned into pcDNA5/FRT IgGFc.

1) Fragment 1 Primers for mHGF alpha chain 5'Nhel

\[ \text{5' ATCGGCTAGCATGATGTTGGGGGACAAAC (SEQ ID NO. 110)} \]

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

\[ \text{3' GAATCCCATTTCACAACCCGCAGTTGTTTTTGGG (SEQ ID NO. 111)} \]
d. Construction of hHGF and mhm chimera

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:

CATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACCATCACATC

[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.
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.

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.

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 (1499-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

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% CO₂, 100 µg/mL Zeocin (Invitrogen, Catalog No. R250-01).
(2) Generation of Stable CHO FlpIn Cell Lines

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

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% CO₂ 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

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.

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.

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 (RScD 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>
<thead>
<tr>
<th>Antibody</th>
<th>rhHGF (R&amp;D Systems)</th>
<th>rmHGF (R&amp;D Systems)</th>
<th>mhm-chimera (V495L585)</th>
<th>human-HGF</th>
<th>G555E</th>
<th>C561R</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A3</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>1D3</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>1F3</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>2B8</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>2F8</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>3A12</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>3B6</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>3D11</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

[0215] The results in Table 3 demonstrate that each of the antibodies bind rhHGF 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.

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**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Reduced (Exposure 20 sec)</th>
<th>Non-Reduced (Exposure 20 sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A3</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>1D3</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>1F3</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>2B8</td>
<td>-</td>
<td>1+</td>
</tr>
<tr>
<td>2F8</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>3A12</td>
<td>-</td>
<td>2+</td>
</tr>
<tr>
<td>3B6</td>
<td>3+</td>
<td>2+</td>
</tr>
<tr>
<td>3D11</td>
<td>-</td>
<td>3+</td>
</tr>
</tbody>
</table>

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 3D1 1 did not bind to reduced rhHGF.

**Example 6 —Binding Affinities**

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

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 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 in Table 5.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>$k_a$(l/Ms)</th>
<th>SE(l)</th>
<th>$k_d$(l/s)</th>
<th>SE(s)</th>
<th>$K_D$(pM)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A3</td>
<td>$1.7 \times 10^6$</td>
<td>$7.3 \times 10^4$</td>
<td>$5.2 \times 10^{-5}$</td>
<td>$8.4 \times 10^{-7}$</td>
<td>30.1</td>
<td>5.6</td>
</tr>
<tr>
<td>1D3</td>
<td>$1.7 \times 10^6$</td>
<td>$3.1 \times 10^4$</td>
<td>$8.2 \times 10^{-5}$</td>
<td>$1.7 \times 10^{-6}$</td>
<td>54.2</td>
<td>27.4</td>
</tr>
<tr>
<td>1F3</td>
<td>$1.5 \times 10^6$</td>
<td>$5.0 \times 10^4$</td>
<td>$2.6 \times 10^{-5}$</td>
<td>$6.6 \times 10^{-7}$</td>
<td>18.1</td>
<td>8.2</td>
</tr>
<tr>
<td>2B8</td>
<td>$1.6 \times 10^6$</td>
<td>$2.9 \times 10^4$</td>
<td>$2.1 \times 10^{-5}$</td>
<td>$1.4 \times 10^{-7}$</td>
<td>13.5</td>
<td>4.4</td>
</tr>
<tr>
<td>3A12</td>
<td>$1.6 \times 10^6$</td>
<td>$3.7 \times 10^4$</td>
<td>$1.6 \times 10^{-4}$</td>
<td>$1.6 \times 10^{-5}$</td>
<td>103.0</td>
<td>10.4</td>
</tr>
<tr>
<td>3B6</td>
<td>$2.0 \times 10^6$</td>
<td>$6.5 \times 10^4$</td>
<td>$3.9 \times 10^{-5}$</td>
<td>$3.2 \times 10^{-7}$</td>
<td>17.0</td>
<td>3.4</td>
</tr>
</tbody>
</table>

[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

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)

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 Na₂CO₃ and 34 mM NaHCO₃, 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.

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.

The plates were read on ViCtOr³V instrument (Perkin Elmer) using the Europium method. The IC₅₀ values were calculated and are summarized in Table 6.
The results demonstrate that all the antibodies (i.e., 1D3, 1A3, 2B8, 3A12, 1F3, 3Dl1, and 2F8) other than 3B6 efficiently neutralize HGF binding to c-Met.

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

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.

The resulting cells were incubated at 37°C, 5% CO₂. 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).

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 \( y = \text{bottom} + \frac{(\text{top-bottom})}{(1 + 10^{-\Lambda (\log(EC50-x)*\text{hill slope})})} \) 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.
The results in Table 7 demonstrate that all of the antibodies, 1A3, 1D3, 1F3, 2B8, 2F8, 3A12, 3B6, and 3D1 inhibit HGF induced proliferation in 4MBr-5 cells.

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

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).

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-.

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.

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**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Trial 1</th>
<th>Trial 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A3</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>1D3</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>1F3</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2B8</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>2F8</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3A12</td>
<td>-</td>
<td>/</td>
</tr>
<tr>
<td>3B6</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>3D11</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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

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

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).

PC-3 cells were seeded into individual wells of 96-well Costar tissue culture plates (Corning Catalog No. 3595) at a density of 4.5x10⁴ 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% CO₂, the media was removed, and cells were rinsed once with serum-free F-12K containing 1% penicillin-streptomycin. Cells were then incubated for 24 hours in 100 μL serum-free F-12K containing 1% penicillin-streptomycin.

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.

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 Na₃VO₄ (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% CO₂. 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% CO₂, 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 Na₃VO₄. 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$_3$VO$_4$, 1x protease inhibitor cocktail (Sigma Catalog No. P8340), and 1x phosphatase inhibitor cocktail 2 (Sigma Catalog No. 5726). 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.

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-NI P-Tyr-100 antibody (PerkinElmer Catalog No. ADO159) 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.

Signal then was measured using the Europium protocol on the Victor3 V reader (PerkinElmer). EC50 values were calculated using Prism 4 for Windows (GraphPad Software, Inc., San Diego, CA) and the sigmoidal dose-response equation.

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

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.

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: \( \text{vol. (mm}^3) = \frac{L \times W^2}{2} \). When the tumors grew to approximately 200 mm³, 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.
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 U18 Xenograft Model**

The ability of the antibodies 1A3, 1D3, 1F3 and 2B8 to inhibit tumor growth was tested in an U18 xenograft model. U18 cells (ATCC) were expanded as described in Example 10 (above) with respect to the U87MG cells.

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 mm³. 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 10**

<table>
<thead>
<tr>
<th></th>
<th>Percent Inhibition</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>2B8 vs PBS</td>
<td>93%</td>
<td>0.001</td>
</tr>
<tr>
<td>1A3 vs PBS</td>
<td>73%</td>
<td>0.0075</td>
</tr>
<tr>
<td>1D3 vs PBS</td>
<td>51%</td>
<td>0.075</td>
</tr>
<tr>
<td>1F3 vs PBS</td>
<td>60%</td>
<td>0.027</td>
</tr>
</tbody>
</table>

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.

**Table 11**

<table>
<thead>
<tr>
<th></th>
<th>Percent Inhibition</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>2B8 vs IgG</td>
<td>75%</td>
<td>0.007</td>
</tr>
<tr>
<td>1A3 vs IgG</td>
<td>57%</td>
<td>0.01</td>
</tr>
<tr>
<td>1D3 vs IgG</td>
<td>47%</td>
<td>0.12</td>
</tr>
<tr>
<td>1F3 vs IgG</td>
<td>30%</td>
<td>0.39</td>
</tr>
</tbody>
</table>
Example 12 - Humanization of Murine Monoclonal Antibodies

[0255] This Example describes the humanization of the murine 2BS 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. 169:11 19-1 125; 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 Immunogenetics 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 IgGl (Glm(17,l) allotype) or Kappa (Km(3) allotype (allele 2)) regions (downstream of the variable regions) using standard molecular biology techniques. AU four heavy chain
IgGl antibodies (chimeric 2B8 and 3 humanized heavy chains (Hu2B8 Hvl-f.l, Hu2B8 Hv5-a.l, Hu2B8 Hv5-51.1) were expressed in the possible combinations with all 3 kappa chain antibodies (chimera 2B8 and 2 humanized light chains (Hu2B8 Kvl-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

<table>
<thead>
<tr>
<th>Heavy Chain Variable Region</th>
<th>Light Chain Variable Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chimeric 2B8 (SEQ ID NO: 12)</td>
<td>Chimeric 2B8 (SEQ ID NO: 14)</td>
</tr>
<tr>
<td>Chimeric 2B8 (SEQ ID NO: 12)</td>
<td>Hu2B8 Kv3-15.1 (SEQ IDNO: 179)</td>
</tr>
<tr>
<td>Hu2B8 Hyl-f.l (SEQ ID NO: 159)</td>
<td>Chimeric 2B8 (SEQ ID NO: 14)</td>
</tr>
<tr>
<td>Hu2B8 Hyl-f.l (SEQ ID NO: 159)</td>
<td>Hu2B8 Kv3-15.1 (SEQ IDNO: 179)</td>
</tr>
<tr>
<td>Hu2B8 Hv5-a.l (SEQ ID NO: 165)</td>
<td>Chimeric 2B8 (SEQ ID NO: 14)</td>
</tr>
<tr>
<td>Hu2B8 Hv5-51.1 (SEQ IDNO: 169)</td>
<td>Chimeric 2B8 (SEQ ID NO: 14)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Immunoglobulin Heavy Chain</th>
<th>Immunoglobulin Light Chain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chimeric 2B8 IgGl (SEQ ID NO: 155)</td>
<td>Chimeric 2B8 Kappa (Km(3)) (SEQ ID NO: 157)</td>
</tr>
<tr>
<td>Chimeric 2B8 IgGl (SEQ ID NO: 155)</td>
<td>Hu2B8 Hv1-39.1 + Kappa Constant (Km(3) allotype) (allele 2) (SEQ ID NO: 177)</td>
</tr>
<tr>
<td>Chimeric 2B8 IgGl (SEQ ID NO: 155)</td>
<td>Hu2B8 Hv3-15.1 + Kappa Constant (Km(3) allotype) (allele 2) (SEQ ID NO: 181)</td>
</tr>
<tr>
<td>Hu2B8 Hv1-f.1 + IgGl Constant (Glm(7,1)) allotype (SEQ ID NO: 163)</td>
<td>Chimeric 2B8 Kappa (Km(3)) (SEQ ID NO: 157)</td>
</tr>
<tr>
<td>Hu2B8 Hv1-f.1 + IgGl Constant (Glm(17,1)) allotype (SEQ ID NO: 163)</td>
<td>Hu2B8 Hv1-39.1 + Kappa Constant (Km(3) allotype) (allele 2) (SEQ ID NO: 177)</td>
</tr>
<tr>
<td>Hu2B8 Hv1-f.1 + IgGl Constant (Glm(17,1)) allotype (SEQ ID NO: 163)</td>
<td>Hu2B8 Hv3-15.1 + Kappa Constant (Km(3) allotype) (allele 2) (SEQ ID NO: 181)</td>
</tr>
<tr>
<td>Hu2B8 Hv5-a.1 + IgGl Constant (Glm(17,1)) allotype (SEQ ID NO: 167)</td>
<td>Chimeric 2B8 Kappa (Km(3)) (SEQ ID NO: 157)</td>
</tr>
<tr>
<td>Hu2B8 Hv5-a.1 + IgGl Constant (Glm(17,1)) allotype (SEQ ID NO: 167)</td>
<td>Hu2B8 Hv1-39.1 + Kappa Constant (Km(3) allotype) (allele 2) (SEQ ID NO: 177)</td>
</tr>
<tr>
<td>Hu2B8 Hv5-a.1 + IgGl Constant (Glm(17,1)) allotype (SEQ ID NO: 167)</td>
<td>Hu2B8 Hv3-15.1 + Kappa Constant (Km(3) allotype) (allele 2) (SEQ ID NO: 181)</td>
</tr>
<tr>
<td>Hu2B8 Hv5-51.1 + IgGl Constant (Glm(17,1)) allotype (SEQ ID NO: 171)</td>
<td>Chimeric 2B8 Kappa (Km(3)) (SEQ ID NO: 157)</td>
</tr>
<tr>
<td>Hu2B8 Hv5-51.1 + IgGl Constant (Glm(17,1)) allotype (SEQ ID NO: 171)</td>
<td>Hu2B8 Hv1-39.1 + Kappa Constant (Km(3) allotype) (allele 2) (SEQ ID NO: 177)</td>
</tr>
<tr>
<td>Hu2B8 Hv5-51.1 + IgGl Constant (Glm(17,1)) allotype (SEQ ID NO: 171)</td>
<td>Hu2B8 Hv3-15.1 + Kappa Constant (Km(3) allotype) (allele 2) (SEQ ID NO: 181)</td>
</tr>
</tbody>
</table>

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

5

\[ \text{sh2B8-9 (Glm(17,1))} = \text{hu2B8 Hv5-51.1 (IgGl constant region (Glm(17,1)) allotype) (SEQ ID NO. 171) plus hu2B8 Kv 1-39.1 (Kappa constant region (Km(3) allotype (allele 2))) (SEQ ID NO. 177)} \]

10

\[ \text{sh2B8-12 (Glm(17,1))} = \text{hu2B8 Hv5-51.1 (IgGl constant region (Glm(17,1)) allotype) (SEQ ID NO. 171) plus hu2B8 Kv 3-15.1 (Kappa constant region (Km(3) allotype (allele 2))) (SEQ ID NO. 181).} \]

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.

1. **Nucleic Acid Sequence Encoding the Full Length Chimeric 2B8 Heavy Chain**

   **(Mouse Variable Region and Human IgG1 Constant Region)** (allotype Glm(17.D) (signal sequence underlined) (SEQ ID NO. 154)

   ```
   1 afogggatgga gctatatcafc ctctttttcttg gtagcaacag ctacagtgat ccaccc cag
   61 gttccacctgc agcccgcccc gcggatgctg gtagaagctc cagctacgac ccaccc cag
   121 tgcaggtcact gttcgtcact ctctcaccgc ccaggagaca gcagcggctg ccaccc cag
   181 ggacagggcc ttgagttgat tggagagatt aacctctaca ccagcgcatt taactacaat
   241 gagagcttgc agccagagcg cagactcact gtagaagctc cagctacgac ccaccc cag
   301 caacctcaca gctgcgtcact tggagctcct gcggctgacc ccaggagaca gcagcggctg ccaccc cag
   361 ggagcctgtga cccgcccacc caagcgcact ctacagtgat ccaccc cag
   421 acctccctctt ctctcccttt cgacccaatc ctctggtatca acagaaacca
   ```

2. **Protein Sequence Defining the Full Length Chimeric 2B8 Heavy Chain**

   **(Chimeric 2B8 IgG1 (Glml7,n allotype) (without signal sequence))** (SEQ ID NO. 155)

   ```
   1 qvodqagqae lqvpgtvkll skcsaygtfc t tywhmwnqrg pggggweige intpntghnty
   61 nekfkskatl tvtqkssstays tnlqssltsed savyccarny vsisfvywag gttltvssas
   121 tkgpsvplaa tpskkstsgtt aalgclvkdy tvivnnpkst kvdkhvkpeps cklthhtccpc papelliogps
   181 ysslwttwp ssslqgtqyi cvnhkhpstnt kvdkhvkpeps cklthhtccpc papelliogps
   241 vlfifpppdkd tmlisrtpev tcwivdwhve dpevfhvwvgy dvevhnakt kpreegynst
   301 yrvsvilv1 hqhdvwlngk eykvpksnlp amiplattika kkgpprepvgy tippsrdelt
   361 knvsvlsltclvg kgfypvdslw ewensnymgn nyktppypdl sdgfsflvysk ftvdksrwq
   421 gvnf cscsvnh ealnlnytqk sllspqk
   ```

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 atggatcatc acagccctgtg cttccatattc atactctctc gtatccatgt tcgctgatg
   61 acatctgtaa ttgacccaatc tccaaaaacc atgctccatgt cagtaggaga gagggtcacc
   121 tggagctgca aggccagtga gaatgtggtt tcttatgtat cctggtatca acagaaacca
   ```
- 70 -

181 gcgcagtctc ctaaactgct gatatacggg gcatccaacc ggaacactgg ggtccccgat
241cgcttcacag gcagtggatc tgcaacagat t t σ actctga ... gaaacacaaa gtcagctcct gegaagtacac ctateagggc
301 gtggaactgcc tctgttgtgt gcctgctgaa taacttctat
361 tctgatgagc agttgaaatc tggaactgcc tctgttgtgt gcctgctgaa taacttctat
421 cccagagagg ccaaaagtaca gtggaaggtg gataacgccc t c c σ aatcggg taactcccag
481 nivmtqspks msmsvgervt lsckasenw syvswyqqkp aqspkliyg asnrntgydp
541 rftgsgsatd ftltissvra edladyhcgq synypytfgg gtrleikrtv aapsvfifpp
601 sdeqjksgta swcllnfhy preakvqkwk dnalqsgsq esvteqksdl stysslslt
661 skdadyekhk vyacevthqg lssptksfn rgec

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

20 1 atggactgca cctggagagt cctccttgg gttgcacgcct ctcagggcag
d1 gtccagtgtg tacagtctgg ggtgcaggtg aagaacgctg ggggtacagt gaaatctcc
121 tgcaggttt ctggatccac ttcacagact tactggtgtgc actgggtcga acagggcccct
181 ggaaaagggc ttggactactg gggccagagg aatctcctgct ctggttctcag ttaacctctgct
241 gagaagttcgc ggaactagtt gcagtagttc acctgcagct gatggggagt gtttctctag
25 301 gactctagct acctgtactg gggccagagg aatctcctgct ctggttctcag
361 ggtacacgt ctgactactg gggccagagg aatctcctgct ctggttctcag

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

20 1 cctccaccaa gggcccatcg ctcctctgg tggcagcagc ctcagggcag
d1 gtccagcttg actagttggt ggtgcaggtg aagaacgctg ggggtacagt gaaatctcc
121 tgcaggttt ctggatccac ttcacagact tactggtgtgc actgggtcga acagggcccct
181 ggaaaagggc ttggactactg gggccagagg aatctcctgct ctggttctcag ttaacctctgct
241 gagaagttcgc ggaactagtt gcagtagttc acctgcagct gatggggagt gtttctctag
25 301 gactctagct acctgtactg gggccagagg aatctcctgct ctggttctcag
361 ggtacacgt ctgactactg gggccagagg aatctcctgct ctggttctcag

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

1 evqlvqsgae vkkpgatvks sckvsgytft tywmdhvqqa pgkglewmsquiteapnqny
30 61 nefkqgrvti tadsttdday melslrsed tvavjctnatq vgsifdyqwg gttvys

[0268] (7) Nucleic Acid Sequence Encoding Human IgGl Heavy Chain Constant Region (GIm(1 7.D aIotVPe) (SEQ ID NO. 160)

1 cttccaccaag gggcccatcg ttcctctgg tggcagcagc ctcagggcag
d1 gacagcagc ctgactactg actggtcttc cgaacgcggtg aaggttctggt
121 ggaactcagg cggctctgac acgcgctgc actggtcttc cgaacgcggtg aaggttctggt
181 gactctcct ttcctctccct ctggtctttgc gctctctcag cagctctcag cagctctcag
(8) Protein Sequence Defining Human IgGl Heavy Chain Constant Region

(Ωm(17A) 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 IgGl Heavy Chain sequence.

1 astkgpsvfp lapsskstsg gtaalgclvk dyfpepvpts wnsgaltsgv htfpvqlqs
6 1 glylsswt vspslsqft yienvnhkps nkvdkqkvp ksdkthtcp pcppelllg
20 121 psyflppkp kdltmisrt epctvwdvs hedpevkfhw yvdgvevhna ktkpreeqyn
18 1 styrwsvlt vhlqdlwnkg eykcqknka lpapietikt kakkqprepq vtyllpsrde
24 1 htknqvsrlc Ivkggypdsi avewensngp ennyttppv ldsdgsffly skiltvksrw
30 1 qqugnfcsv mhealhnhyt qksslspgk

(9) Nucleic Acid Sequence Encoding the Full Length Heavy Chain Humanized

(Hv2B8 Hvlf. 1 Variable Region and Human IgGl TGlH7.1 allotype) Heavy Chain Constant Region (signal sequence underlined) (SEQ ID NO. 162)
Protein Sequence Defining the Full Length Heavy Chain Humanized Hu2B8

Hylf.l Variable Region and Human IgGl Heavy Chain Constant Region (Glml(1.7.1) allotype

(without signal sequence) (SEQ ID NO. 163)

1 evqlvqsgae vkkpgatvki sckvsgytft tyw nh.wvqqa pgkglewmge inpfcnghtny
6 1 npsfqghvti sadksistay lqwsslkasd tamyycarny vgsifdywgq gtlvtvssas
12 1 tkpsvflpa psskstsggt aalgcvkdy fepvhtsvvm sglalsgvht fpavlqgsgl
18 1 ylslwtpy sslltqtiy cnv nhksntrq kddkvkepks edktcpcpe papellqggs
24 1 vlfppkpd tllmispeve tcwvdsvhe dpevknfenvy kgvtrhkact kpreeqynst
30 1 ywwvstvlv lqhdwngkey kckvsknkal apiekktisk akgprepfy tflpssrdl etlvdksrwiq
36 1 gnvscsvvmh ealnhnytqk slslspgk

Nucleic Acid Sequence Encoding Humanized Hu2B8 Hv5a.l Heavy Chain Variable Region (signal sequence underlined”) (SEQ ID NO. 164)

1 atggggtcga cagccatctc gcctgcttc ctggctgttc tcaagaggctgctgcaga
6 1 gtgcagctgg tgcagtctgg agcagaggtg aaaaagcccg gggagtctct gaggatctcc
12 1 tgtaagggtt ctggatacag ctttaccacc tactggatgc actgggtgcg ccagatgccc
18 1 gggaaagggc tggaggtgtg gggaggagt aatctacca acggctacar taactacaat
24 1 cgtctcctc aagggcaagt cactacatacg agctacgac ccagatgccc
30 1 cgtctcctc aagggcaagt cactacatacg agctacgac ccagatgccc
36 1 ggtagcatct gggctctctc gcgctctc ctggctgttc tcaagaggctgctgcaga

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

1 evqlvqsgae vkkpgatvki sckvsgytft tyw nh.wvqqa pgkglewmge inpfcnghtny
6 1 npsfqghvti sadksistay lqwsslkasd tamyycarny vgsifdywgq gtlvtvssas
[0274] (13) Nucleic Acid Sequence Encoding the Full Length Humanized Hu2B8 Hv5a.l Heavy Chain Variable Region and Human IgGl (Glml(17.1 allotype)) Heavy Chain Constant Region (signal sequence underlined) (SEQ ID NO. 166)

```
1  atggggctcaat ccgccatctct gcgccctctct ctggcttgct tccaaggagt ctggccgaa
5  gtaagctgctg tcaacctgatt gacatgggtat aaaaagcccg gggagtctct gaggatcccc
10 gcctgcagctgg gccctgacctgg gccatgtatt cctggctgttc ctetgcce
15 gacccagccc gagaacacca ggtgacaccc tccggccgaa ccggctgtcc
20 gcacctctgg cgcgggaccc gcacctctgg gcggtgccga gcggtgccga
25 gcacccagac ccggacggat ccacggcttc gcgtgttctg ctgccccctg
30 ctgccccctg atga
```

[0275] (14) Protein Sequence Defining the Full Length Humanized Hu2B8 Hv5a.l Heavy Chain Variable Region and Human IgGl (Glml(17.1 allotype)) Heavy Chain Constant Region (without signal sequence) (SEQ ID NO. 167)

```
1  evqlvlsgae vkkpgeslri scksgsyst tywmhwvrrqm pgkglewmgpe inptnghnty
61 npsfghvtda sadksistay lwsslaskad fcamyccamy ygsfdlywqg gltvssas
121 tkiqspvplas psksstsggt aalgclvkdy fpepytvswn sgalshtsyt fpaovqssgl
35 1y1smwtyp ssslgqtyii cnvnlhknpsn kvkdkkdevks edkthcpepc papellggps
84 1vllpfpkpd tlmsirptev tcwvdsvese dpevkfnwyyv dgyevhnakt kpreeynst
141 tsthclwngkey hqdwlngkey kcyvkskalp aieptknska kgqrepvyys tlppsreld
361 knysltdlcl kgyslfdysav ewesngpens nykttppvd sdgsfllysk lvdkrsrwqq
421 gnvfsvsmli ecall-i diytqk slsilpgk
```

[0276] (15) Nucleic Acid Sequence Encoding the Full Length Humanized Hu2B8 Hv5-1.1 Heavy Chain Variable Region (signal sequence underlined) (SEQ ID NO. 168)

```
1 atggggctcaat ccgccatctct gcgccctctct ctggcttgct tccaaggagt ctggccgaa
6  gtaagctgctg tcaacctgatt gacatgggtat aaaaagcccg gggagtctct gaggatcccc
121 gcctgcagctgg gccctgacctgg gccatgtatt cctggctgttc ctetgcce
181 gggaaaggcc gggtgcgaa actgggtgcg ccagatgccc
```

- 73 -
[0277] (16) Protein Sequence Defining Humanized HuB28 HvS-51.1 Heavy Chain

Variable Sequence (without signal sequence) (SEQ ID NO. 169)
1 evqlvqsgae vkkpgesli sckgsgysft twvnhwrvqmq pgkglewmg e
61 npsfqqvvti saksdisay lqwsslkasd tamyycarny vgsifdywq g

[0278] (17) Nucleic Acid Sequence Encoding the Full Length Humanized HuB28 HvS-51.1 Heavy Chain Variable Region and Human IgGl (GlM(1.7.1) allotype) Heavy Chain

Constant Region (signal underlined) (SEQ ID NO. 170)
1 atggggtcag ccgccatctct cgcctcctctc tctgggtcgc tctcaaggagt ctgtgccgaa
61 gtgcagcttgg tggatgtcctg aqccaggttg aaaaaagcctt ccggcgagt ccagatcttc
151 cggcgatata caccatgtctg cctgggtca ctgcgcctct gctggactcc gccctgggct
201 gcctgggggcc ggtagctctgg tgcctacctg ctgtggtgac tgacgagctttctgc
251 gcctgggggcc ggtagctctgg tgcctacctg ctgtggtgac tgacgagctttctgc
301 gcctgggggcc ggtagctctgg tgcctacctg ctgtggtgac tgacgagctttctgc
351 gcctgggggcc ggtagctctgg tgcctacctg ctgtggtgac tgacgagctttctgc

[0279] (18) Protein Sequence Defining the Full Length Humanized HuB28 HvS-51.1

Heavy Chain Variable Region and Human IgGl (GlM(1.7.1) allotype) Heavy Chain Constant Region (-without signal sequence) (SEQ ID NO. 171)
1 evqlvqsgae vkkpgesli sckgsgysft twvnhwrvqmq pgkglew mg e
61 npsfqqvvti saksdisay lqwsslkasd tamyycarny vgsifdywq g
121 tggcagcttgg tggatgtcctg aqccaggttg aaaaaagcctt ccggcgagt ccagatcttc
181 cggcgatata caccatgtctg cctgggtca ctgcgcctct gctggactcc gccctgggct
241 gcctgggggcc ggtagctctgg tgcctacctg ctgtggtgac tgacgagctttctgc

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

1 ATGgacATGa gggctccgcg tcagctcctgt gggctectgc tactgtgct ccaggtgcc
61 agatgtgaca tcagatgac ccagctccta tcctcctgt ctgcatctgt aggagacaga
121 gtcatcatc ctggaacgc cagctgagaat ctgcatctgt agagacaga
181 aaaccagaga aagcccttaa gctcctgtat aggagacaga cactgggggtc
241 ccataagtt ccagctcctgc tggatctggg acagatttca ctctcaccat cagcagtctg
301 caactctgaag atttctgt gcgagaggggt acaactctgc gtgcctgtttt
361 gcggagggga ccaagctgga gatcatctga gatcagagtta caactctgcgtttt

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

1 diqmtpqspss lsasvgrvrt itckasenw syvswoycqkp gkapklliyg asrnrtgyps
61 rfsgsgsgtfd fltissqlp edfatycqg synypytfgq gtkleik

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

20 1 gaactgtgcc tgccacatct gtctctatct tcccgtccatc tgatgagcag tgcaaatctg
61 gaactgctct tgtgtgctgt ctgtctgaata acttctatcc cagagagggcc aagtgacagt
121 gggaggtgga taacgctcct cactggggtacactccagga gactgctgagcagcagaca
181 gcagagagac caactcagcg cctagcgcagc cctacgtgaactgctagcagcagcagaca
241 ggccagagcc gagcctgcc cattccagctgctgccagcagcagcagaca
25 301 gtcgactccag cggagagtgt 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.
(23) Nucleic Acid Sequence Encoding the Full Length Humanized Hu2B8 Kv-I-39.1 Light Chain Variable Region and Human Kappa Chain Constant Region (Km(3) allotype)

(allele 2)

(SEQ ID NO. 176)

1 atggacatga gggccccggc tcagctcctg gggcfccctgc tactctggct ccgagggfgcc
61 agagtagctc tccagtagac ccaagtctca tctccctctg ctgcctcttg aggagacaga
121 gcacatctca cttgaaggac caagtcgagat gcgcctgcttg ggtgttcttg gtcacagcag
181 aaaccaggga aagcccttaa gctctcatgc fcacggggcat ccaacccgaa cactgagggtc
241 ccacataagg tcagctggcg cggctcttgtg cagctctctg cacagctcttg cagcagctctg
301 ccacctgtag attttagcaac ttactactgt ggccagagtt acgccctcca agtcacctccag
361 ggccaggagga ccaagtctga gatcaaacga actgtggctg caccatctgt cttcatcttc
ggtgttcttg gtcacagcag

(24) Protein Sequence Defining the Full Length Humanized Hu2B8 Kv-I-39.1 Light Chain Variable Region and Human Kappa Chain Constant Region (Km(3) allotype) (allele 1)

(SEQ ID NO. 177)

1 diqmtqspa lsavgdrtv itkasenw syvswyqqkp gkapkliyg asnrntgyp
61 rf sgsgstgd flisslap edfayyegq synypyrfgq gtlkeikrv apsvffip
121 sdeqlkgsta swllnfy preakvqkwv dnalqgnsq estveqsdsk styslslt
181 lsakdyekkh vyaevthag lsspvtksfn ggcaggtgag

(25) Nucleic Acid Sequence Encoding Humanized Hu2B8 Kv3.1.5 Light Chain Variable Region (signal sequence underlined) (SEQ ID NO. 178)

1 atggagacce cagegcagct tctctctctctgtcctcgagc taccactgga
61 ggaatagtg tagaagctct ctcagcgtct gttcatcttggt cttcagggga aagagccacc
121 cctctcgtca agcagctag tgaagtacctctctgtacac gcagagcactct
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241-aggtactgctg tgcagagcagc ttcagcagctccttgctctc tacaagctagtcaag
301 ggaagattttg cacagtttattc gtctgtgggcact tcaagctagtcaag
361 gggaccaagc tggagatcaac gacagcagagc tacaagctagtcaag
Protein Sequence Defining Humanized Hu2B8 Kv3-15.1 Light Chain Variable Region (without signal sequence) (SEQ ID NO. 179)

1 eivmtqspat lsvapgerat lsckasenw syvswyqkqp gqaprlliyg asnrntgipa

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

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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)

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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**

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<td>Chimeric 2B8 IgG1 (G1m(17,1)) – protein</td>
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<td>Chimeric 2B8 Kappa (Km(3)) – nucleic acid</td>
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<td>Hu2B8 Hv1f.1 Heavy Chain Variable Region – nucleic acid</td>
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<td>Hu2B8 Hv1f.1 Heavy Chain Variable Region – protein</td>
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<td>Hu2B8 Hv1f.1 + IgG1 Constant (G1m(17,1)) allotype – nucleic acid</td>
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<td>Hu2B8 Hv5-51.1 Heavy Chain Variable Region – protein</td>
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<td>Hu2B8 Hv5-51.1 + IgG1 Constant (G1m(17,1)) allotype – nucleic acid</td>
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<td>171</td>
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<td>Hu2B8 Kv1-39.1 Kappa Chain Variable Region – nucleic acid</td>
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<td>Hu2B8 Kv1-39.1 Kappa Chain Variable Region – protein</td>
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<td>Human Kappa Chain Constant Region (Km(3) allotype) (allele 2) – nucleic acid</td>
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<td>Human Kappa Chain Constant Region (Km(3) allotype) (allele 2) – protein</td>
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<td>Hu2B8 Kv3-15.1 + Kappa Constant (Km(3) allotype) (allele 2) – protein</td>
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**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 IgGl heavy chain constant region (Glm(3) allotype (allele I)) was added to the carboxyl terminus of the two human engineered heavy variable regions and the human Kappa constant region (Km(3) allotype (allele I)) 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] (I) Nucleic Acid Sequence Encoding the Humanized LR2B8HC Heavy Chain

Variable Region (signal sequence underlined) (SEQ ID NO. 182)

1 atgggctgtg cattatatt tccttttctg tggctacg ctaccgatgt gcactctcaa
61 gtcaactcg tacaaccagg cgctgaagtc gtaaaacctg gaacatctgt taaactctca
121 tgcaagctgc caggataacag tttcacaact tactggatgc atgggtcag tcaagcccc
181 ggacaagggc tcgaatggt gtggcaagatt aacccacta acggacatac taattataat
241 gaaaaattta agggcaagaac tacaactcac gtgataaat caaactctac agcttatag
301 gaaactttct ccctgagatc agaagatac gccgtctact attgcgccag aaactacgta
361 ggctataat tgcattactg gggcagggc actctctca gtcagagtc a
(2) Protein Sequence Defining Humanized LR2B8HC Heavy Chain Variable Region (without signal sequence) (SEQ ID NO. 183)

1 qvqlvqpgae wkpgtsvkl sckasgytft tywmhwvnqa pgqglewige inptnghtny
6 1 nekfkgkatl tvdkststay melsslrseed tavyycarny vgsifdywq gtiltvss

(3) Nucleic Acid Sequence Encoding the Human IgGl Heavy Chain Constant Region fGlmG allotype) (allele 1) (SEQ ID NO. 184)

1 ccagcacaaa gggcccatcg ttctcccc ccagcggttc ctccagaacg acctctgggg
6 1 gcacagcggc cctgggtcgtgc ctgggtcaaggt aacacctggac gccctcactcac cacccacggca
10 121 ggaactccg ggcctgactc gacgtgtggtg tctcctcactcg cagcctggac aacacctgcga
15 181 gactctcctc cgtcgtggtg tctcctcactcg cagcctggac aacacctgcga
241 acatctgcaa cgtaaatagc cactcttcagc cactcttcagc cactcttcagc cactcttcagc
301 aactttgtga cactactcg cactcttcagc cactcttcagc cactcttcagc cactcttcagc
361 cgtcagtctt cctcttcccc ccagacccct cctagatcct cggacccctcc cgtctcctcg
421 aggtcacatc cgtcgcgtgtgc gacgtgtggtg tctccttcagc cagcctggac aacacctgcga
481 ccagcacaaa gggcccatcg ttctcccc ccagcggttc ctccagaacgacctctgggg
541 gcacagcggc cctgggtcgtgc ctgggtcaaggt aacacctggac gccctcactcac cactcttcagc
601 agtacagctg cgtcgtggtg tctcctcactcg cagcctggac aacacctgcga
661 cgtcagtctt cctcttcccc ccagacccct cctagatcct cggacccctcc cgtctcctcg
721 ccagacccct cctagatcct cggacccctcc cgtcgtcagc gacgtgtggtg tctccttcagc cagcctggac
781 ccagacccct cctagatcct cggacccctcc cgtcgtcagc gacgtgtggtg tctccttcagc cagcctggac
841 ccagacccct cctagatcct cggacccctcc cgtcgtcagc gacgtgtggtg tctccttcagc cagcctggac
901 ccagacccct cctagatcct cggacccctcc cgtcgtcagc gacgtgtggtg tctccttcagc cagcctggac
961 ccagacccct cctagatcct cggacccctcc cgtcgtcagc gacgtgtggtg tctccttcagc cagcctggac

(4) Protein Sequence Defining Human IgGl Heavy Chain Constant Region (GImG) 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 IgGl Heavy Chain sequence.

1 astkgpsvfp lapsskstsg gaalagelvk dyfpepvtvs wnsgaltsgv htpavlqss
6 1 glyslswt vpsslgtqt yicnvnhks ps tkvdkhrvep kscdkhtcep pc papellgg
121 psvflfpkp kdtlnisrtpev tcvwvdvs hedpevkfnw yvdgvevhna ktkpreeqyn
181 styrwsvlt vlhqdwlngk eykckvsnka lpapiektis kakgqprepq vytlppsree
241 mtknqvsltc lvkgfypsdi avewesngqp ennykttppv ldsgsffly skltvdksrw
301 qqqnvfscsv mhealhnhyt qkslslspgk

[0297] (5) Nucleic Acid Sequence Encoding the Full Length Heavy Chain Humanized

5 LR2B8HC Heavy Chain Variable Region and Human  IgGl Heavy Chain Constant Region

(Glm(3) allotype* (allele 1) (signal sequence underlined) (SEQ ID NO. 186)

```
1  atgggttggt  catatattat  actctttctc  gtagccaccg  ccaccgacgt  acactct  cag
```

[0298] (6) Protein Sequence Defining the Full Length Heavy Chain Humanized LR2B8HC

Heavy Chain Variable Region and Human  IgGl Heavy Chain Constant Region (Glm(3)

(allotype) (allele 1) (without signal sequence) (SEQ ID NO. 187)

```
35  1  qvglvqpgae  wkgtsvkl  sckasytft  tywhmwvnqa  pggglewige  inptnghtny
```

[0299] (7) Nucleic Acid Sequence Encoding the Humanized LRMR2B8HC Heavy Chain

Variable Region (signal sequence underlined) (SEQ ID NO. 188)

```
1 atgggtggt  catatattat  actctttctc  tggcagccag  ccaccgacgt  acactct  cag
```
61 gtcaactcgc tacaaccgag cgcgaagtc aagaaaccag gaacatcagt caaactctca
tgtaaagcaa gcggatacac ctttactact tattggatgc ...
1) (without signal sequence) (SEQ ID NO. 191)
qvqlvqpgae vkkpgtsvkl sckasgytft tywmhwvrqa pgqglewige inptnghtny

[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

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

[0302] (IP) Protein Sequence Defining the Full Length Heavy Chain Humanized LRMR2B8HC Heavy Chain Variable Region and Human IgGl Heavy Chain Constant Region (Gl m(3) allotype) (allele 1) (without signal sequence) (SEQ ID NO. 191)
(11) Nucleic Acid Sequence Encoding the Humanized LR2B8LC Light Chain Variable Region (signal sequence underlined) (SEQ ID NO. 192)

```
61  nqkfqgratl  tvdkststay  melsslrsed  tavyycarny  vgsifdywgq  gtltvssas
121 tkgpsvflpa  psskstsggt  aalgelvkdty  fpeptvsvwn  sgaltsgvht  fpavlqssgl
181 ysslswtvp  sslsgtqyti  cnvnhkpsnt  kvdkrvepks  cdkthcepc  papellggps
241 vflfppkpkd  tlmsrtpvep  tcvwdvshe  dpevkfnwyv  dgvevnknt  kpereeqynst
301 kqvlksctcv  kglfpsdiaav  ewesngqpen  nyktpvold  sdgsfflysk  ltvdksrwqq
421 gnvzcsvml  α ealhnyhtqk  sllspgk
```

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

```
10  1 atggaaagtga  agacccttgta  attcatctct  attcttcttt  ggttgtatgg  agcagacggc
61  gacatttactag  tgaaccaatc  cccgcatagt  atggccatga  gtgtaggaga  aagagtcacc
121 cttaattgcag  aagctctccga  aaatgtcgtt  tcatatgtgt  ctttggtatca  acaaaaacc
181 ggccaatcag  ccaaaacctct  catatacggc  gcttcaaaca  gaaacacagg  cgttcccgac
241 agattttagtg  gatccggatctc  accctgctta  gttcaagca  cgttcccgac
301 gaagacgttg  cagactatca  ttgcggacaa  tcttataact  acccttacac  attcggacaa
```

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

```
10  1 gtacggtggc  tgcaccatct  cccgctctcct  gagacggacgc  cgtgaaactctg  cggagtctctct  cggagtctctct  cggagtctctct  cggagtctctct
61  gacatttactag  tgaaccaatc  cccgcatagt  atggccatga  gtgtaggaga  aagagtcacc
121 cttaattgcag  aagctctccga  aaatgtcgtt  tcatatgtgt  ctttggtatca  acaaaaacc
181 ggccaatcag  ccaaaacctct  catatacggc  gcttcaaaca  gaaacacagg
241 gaagacgttg  cagactatca  ttgcggacaa  tcttataact  acccttacac  attcggacaa
301 gttcaagcatc  gggagttgac  gttcaagcatc  gggagttgac
```

(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.

```
10  1 rtvaapsvfi  fppsdeqlks  gtaswllln  nfpvreakvq  wkvdnaqsg  nsqesvteqd
61  skdstyslss  tltskadayk  khkvacev  hallmarkg  sflrgec
```
- 84 -

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

```
1 atggaaagtc agacctctgt atctcatctct attctttcttt ggtttgatatg agccagaggc
5
gacatgttga tgaaccaact cccgatagt atggccatga gttcaagaga aagtagtacc
121 ctatatgca aagctcggga aatgtcgttt tctatatgtg ttttgtatca acaaaacc
181 gcctcaaatc ccaacctctt catatacggc gttcataaaca gaaacagcag ctgttcccgc
241 gatatttgtg gctccaggtc agctatcgca ttttcgggaca aaccttacac atttggacaa
301 gaaagcctga Oagacatgca ttgctgcgaa ctttataact aaccttacac atttggacaa
361 gaaaccccaac tcaagtaattt acgtacaagt ctttcttcat ctttccgcgc
421 tcttcatggtc aggtaactac tttttgacttg tctttgcttg gctttctgtaa tatacttat
481 ccacacagag ccagacagtca gttgaaagtg gtaacgcgca tcaacgcggg taacttcgcag
541 gacagttgct cagacagacg cagcaagagcc acgacactca gcttgcagcc ctttgcagcc
601 ctgaccaaaag caagatgcaaa aagaccaaaa gtttcttcat ggttccgcgc ccatcagccc
661 ctgacagtcgc cgcctcataa cagcttccaa cggggagagt gtttcc
```

[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 B) (SEQ ID NO. 197)

```
1 divmtqspds matnsvgervt lnckasenw syvsyqvpq gqspkliiyg asntmgypd
6 rfsqsgsatd fltissvqa edvadyhcq synypytfgq gtkeikrtv aapsvifpp
12 sdeqkslqta swelllnfy preakvqwkv dnalqsgnsq esvteqksld styssltlt
18 lskadyekkh vyacevthag lssptksfn rgec
```

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

```
1 atggaaatc cc aacccttgt ttgctatctt attctctct ggtttgatatg agccagaggc
6 gacatgttga tgaaccaact cccgatagt atggccatga gttcaagaga aagtagtacc
12 ctatatgca aagctcggga aatgtcgttt tctatatgtg ttttgtatca acaaaacc
18 gcctcaaatc ccaacctctt catatacggc gttcataaaca gaaacagcag ctgttcccgc
24 gatatttgtg gctccaggtc agctatcgca ttttcgggaca aaccttacac atttggacaa
30 gaaagcctga Oagacatgca ttgctgcgaa ctttataact aaccttacac atttggacaa
36 gaaaccccaac tcaagtaattt acgtacaagt ctttcttcat ctttccgcgc
42 tcttcatggtc aggtaactac tttttgacttg tctttgcttg gctttctgtaa tatacttat
48 ccacacagag ccagacagtca gttgaaagtg gtaacgcgca tcaacgcggg taacttcgcag
54 gacagttgct cagacagacg cagcaagagcc acgacactca gcttgcagcc ctttgcagcc
60 ctgaccaaaag caagatgcaaa aagaccaaaa gtttcttcat ggttccgcgc ccatcagccc
66 ctgacagtcgc cgcctcataa cagcttccaa cggggagagt gtttcc
```

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

```
1 diyntqspds lamslgervt lnckasenw syvsyqvpq gqspkliiyg asntmgypd
6 rfsqsgsatd fltissvqa edvadyhcq synypytfgq gtkeikrtv aapsvifpp
12 sdeqkslqta swelllnfy preakvqwkv dnalqsgnsq esvteqksld styssltlt
```


(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     atggaatcc aaccccttgt ttccatcfccct accttctctt ggccttatgg cgccgagcga
5
d1    gacatcgccta agacacaatc ccctgactct ctgtgcatga gcttgccacg aacgactaac
12    ctttaacgctta aagcatcgca aatgcgccga ctgtggtatca ctgtggtatca gccaaacact
18    gtgcaaatgtct ttaaactcttttatattcg gcaagatcgg cgtcccaagc
24    agatttacgcct tttccaggttgc cagcaactgcct ttaccactta caattctcat gcttcaggcc
30    cagacgcacgtg cagactatca ttgcaagaac ctttatatac ctgcttatga ctttcagggcc
36    ggtcaagactac ttgaaattaa acgtgactgt ttgacacccat ctgttcctcat ctcccgccca
42    ttctatgcgc aagttgaaatc ttgcaacttcg ccctggttcttg gcttgctgaa taactttctat
48    cccagagaggg ccagacgttga gttggaaggtt gataacgccca tccatgctgg taactccccag
54    gagatgtgctca cagagcaggg cagacgcctca gcctcagcag caaacctcag cccgttgcag
60    cagactacagcagactacg cgttcaccaaa aggagagagtt gttggtctgg caggaagaac
66    cagactacacagcagactacg cgttcaccaaa aggagagagtt gttggtctgg caggaagaac
```

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

```
1     divmtqspds lamslgervt inckasenw syvswyqkpg qsnpkllyg asnresgyvp
6     rfsgsgsatd fltissqva edvadyhecq synypyrtfeg gtkeikrtv aapsvifpp
12    sdeqksksgta swellmnfy peakvqwkv dnalqgmsq esvteqdskd styissldlt
18    lskadyekhk yvacevthqg issvpdxsn rgec
```

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 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

<table>
<thead>
<tr>
<th>Antibody</th>
<th>CDR1</th>
<th>CDR2</th>
<th>CDR3</th>
<th>Full Length Heavy Chain Variable Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Murine 2B8 Heavy</td>
<td>TYWH (SEQ ID NO: 15)</td>
<td>EINPTGHTNYNEKFKS (SEQ ID NO: 16)</td>
<td>NYVGSIFDY (SEQ ID NO: 17)</td>
<td>SEQ ID NO: 12</td>
</tr>
<tr>
<td>Hu2B8 Hv1f.1</td>
<td>TYWH (SEQ ID NO: 15)</td>
<td>EINPTGHTNYNEKFQG (SEQ ID NO: 202)</td>
<td>NYVGSIFDY (SEQ ID NO: 17)</td>
<td>SEQ ID NO: 159</td>
</tr>
<tr>
<td>Hu2B8 Hv5a.1</td>
<td>TYWH (SEQ ID NO: 15)</td>
<td>EINPTGHTYNPSFQG (SEQ ID NO: 203)</td>
<td>NYVGSIFDY (SEQ ID NO: 17)</td>
<td>SEQ ID NO: 165</td>
</tr>
<tr>
<td>Hu2B8 Hv5-51.1</td>
<td>TYWH (SEQ ID NO: 15)</td>
<td>EINPTGHTYNPSFQG (SEQ ID NO: 203)</td>
<td>NYVGSIFDY (SEQ ID NO: 17)</td>
<td>SEQ ID NO: 169</td>
</tr>
<tr>
<td>LR2B8HC</td>
<td>TYWH (SEQ ID NO: 15)</td>
<td>EINPTGHTNYNEKFQG (SEQ ID NO: 204)</td>
<td>NYVGSIFDY (SEQ ID NO: 17)</td>
<td>SEQ ID NO: 183</td>
</tr>
<tr>
<td>LRMR2B8HC</td>
<td>TYWH (SEQ ID NO: 15)</td>
<td>EINPTGHTNYNQKFGQ (SEQ ID NO: 205)</td>
<td>NYVGSIFDY (SEQ ID NO: 17)</td>
<td>SEQ ID NO: 189</td>
</tr>
</tbody>
</table>
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>
<thead>
<tr>
<th>Antibody</th>
<th>CDR1 (SEQ ID: NO: 18)</th>
<th>CDR2 (SEQ ID: NO: 19)</th>
<th>CDR3 Loop (SEQ ID: NO: 20)</th>
<th>Full Length Light Chain Variable Region (SEQ ID: NO: 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Murine 2B8 Light</td>
<td>KASENVVSYVS</td>
<td>GASNRNT</td>
<td>GQSYNYPY1</td>
<td></td>
</tr>
<tr>
<td>Hu2B8 Kv1-39.1</td>
<td>KASENVVSYVS</td>
<td>GASNRNT</td>
<td>GQSYNYPY1</td>
<td></td>
</tr>
<tr>
<td>Hu2B8 Kv3-15.1</td>
<td>KASENVVSYVS</td>
<td>GASNRNT</td>
<td>GQSYNYPY1</td>
<td></td>
</tr>
<tr>
<td>LR2B8LC</td>
<td>KASENVVSYVS</td>
<td>GASNRRES</td>
<td>GQSYNYPY1</td>
<td></td>
</tr>
<tr>
<td>LRMR2B8LC</td>
<td>KASENVVSYVS</td>
<td>GASNRRES</td>
<td>GQSYNYPY1</td>
<td></td>
</tr>
</tbody>
</table>

C. Binding Affinity of Humanized 2B8 Antibodies

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.

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 BIAevalutate 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.

The results summarized in Figure 8 show that certain combinations of superhumanized heavy chains (Hu2B8 Hv5a,l, Hu2B8 Hv5-51.1 or Hu2B8 Hv1-f.1) and light chains (Hu2B8 Kvl-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

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.

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.
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**

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 (+ IgGl constant region (Glm(3) allotype (allele I)) (SEQ ID NO. 187) plus LR2B8LC (+ Kappa constant region (Km(3) allotype (allele 1))) (SEQ ID NO. 197)

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

- **HE2B8-3** = LRMR2B8HC (+ IgGl constant region (Glm(3) allotype (allele I)) (SEQ ID NO. 191) plus LR2B8LC (+ Kappa constant region (Km(3) allotype (allele 1))) (SEQ ID NO. 197)

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

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 IgGl constant domain (Glm(3) allotype) cDNA was cloned into pEE6.4 (Lonza Biologies, 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 Biologies) using HindIII and EcoRI restriction sites. The hCMV-MIE promoter + full length Hu2B8_Hv5-51.1 + human IgGl constant domain (Glm(3) allotype) cDNA + SV40 poly A fragment (in pEE6.4) was removed by NotI/Sall digestion and inserted into either Kappa chain pEE14.4 vector through NotI/Sall sites, thus creating 2 different expression vectors that each simultaneously express heavy and light chain to make the following antibodies:

\[
\text{sh2B8-9 (Glm(3)) = hu2B8 Hv5-51.1 (+ IgGl constant region (Glm(3) allotype) (allele 2)) (SEQ ID NO. 210)} \]

\[
\text{sh2B8-12 (Glm(3)) = hu2B8 Hv5-51.1 (+ IgGl constant region (Glm(3) allotype) (allele 2)) (SEQ ID NO. 210) plus hu2B8 Kv 3-15.1 (+ Kappa constant region (Km(3) allotype (allele 2))) (SEQ ID NO. 181)} \]

[0325] The nucleic acid sequences encoding and the protein sequences defining the human IgGl Heavy Constant Region Glm(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] (I) **Nucleic Acid Sequence Encoding Human IgGl Heavy Chain Constant Region (QIm(S) allotype') fallele 2** (SEQ ID NO. 207)

````
cctccaccaa gggcccatcg gtcttcccc cgccacccct ctccaagagc acctctgggg
61 gcacagcggc cctgggctgc cggctgacc actacttccc cgaaccggtg acggtgtcgt
ggaactcagg cgccctgacc agcggcgtgc acacccacca cagcttgggc acccagacct
241 acatctgcaa cgtgaatcac aagcccagca acaccaaggt ggacaagaga gttgagccca
````
Protein Sequence Defining Human IgG1 Heavy Chain Constant Region (Glm(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.

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 Glm(3) allotype (allele 2) (signal sequence underlined) (SEQ ID NO. 209)
(4) Protein Sequence Defining the Full Length Heavy Chain Containing Humanized Hu2B8 Hv5-51.1 and the Human IgGl Heavy Chain Constant Region GlmO) allotype (allele 2) Twithout signal sequence) (SEQ ID NO. 210)

[0329] 20
1 evqlvqsgae
61 npsfqgqvi
121 kgpsvplpa
181 ylsswtvp
241 vflfppkpkd
301 yrwsvlyvl
361 knqyslcelv
421 gwiwscevmh
tccctcagca gcgtggtgac cgtgcccctcc cagacagcttgg gcac σ cagac ctacatctgc
601 tccctcagca gcgtggtgac cgtgcccctcc cagacagcttgg gcac σ cagac ctacatctgc
651 aacgtgaatc acaagcccag caacaccaag gtggacaaga ... discussed in Example 3. Each antibody was immobilized o n a carboxymethylated dextran CM5 sensor chip (BIAcore,

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.

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>
<thead>
<tr>
<th>Antibody</th>
<th>2B8</th>
<th>HE2B8-1</th>
<th>HE2B8-2</th>
<th>HE2B8-3</th>
<th>HE2B8-4</th>
<th>sh2B8-9 (G1m(3))</th>
<th>sh2B8-12 (G1m(3))</th>
</tr>
</thead>
<tbody>
<tr>
<td>rhHGF (R&amp;D Systems)</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>mHGF (R&amp;D Systems)</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>MHM chimera (495-585)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>MHM chimera (507-585)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>MHM chimera (499-556)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

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

**Example 1S - Binding Affinities of Humanized 2B8 Variants**

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

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>
<thead>
<tr>
<th>Antibody</th>
<th>$K_D$ (nM)</th>
<th>$K_D$ (µM)</th>
<th>$k_a$</th>
<th>$k_d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2B8</td>
<td>1.4x10^6</td>
<td>1.0x10^-5</td>
<td>7.3</td>
<td>-</td>
</tr>
<tr>
<td>HE2B8-1</td>
<td>2.2x10^6</td>
<td>1.4x10^-3</td>
<td>7.1</td>
<td>5.2</td>
</tr>
<tr>
<td>HE2B8-2</td>
<td>1.8x10^6</td>
<td>9.6x10^-5</td>
<td>5.2</td>
<td>2.7</td>
</tr>
<tr>
<td>HE2B8-3</td>
<td>2.0x10^6</td>
<td>4.1x10^-6</td>
<td>2.0</td>
<td>1.1</td>
</tr>
<tr>
<td>HE2B8-4</td>
<td>1.7x10^6</td>
<td>1.1x10^-3</td>
<td>6.5</td>
<td>1.3</td>
</tr>
<tr>
<td>sh2B8-9 (G1m(17,1))</td>
<td>2.0x10^6</td>
<td>1.7x10^-3</td>
<td>8.1</td>
<td>5.3</td>
</tr>
<tr>
<td>sh2B8-12 (G1m(17,1))</td>
<td>1.9x10^6</td>
<td>2.3x10^-5</td>
<td>12</td>
<td>0.4</td>
</tr>
</tbody>
</table>

[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

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.

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.

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.

Kinetic parameters were determined using the kinetic function of the BIAevaluation software with reference subtraction. Kinetic parameters for each antibody, kₐ (association rate constant), kₙ (dissociation rate constant) and Kₐ (equilibrium dissociation constant) are summarized below in Table 19.
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

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.

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 Na₂CO₃ and 34 mM NaHCO₃, 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 (0.033-25√nM, 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 ViCtOr3V instrument (Perkin Elmer) using the Europium method. The IC₅₀ values were calculated using Prism.

The IC₅₀ values obtained are shown in Table 20.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>IC₅₀ (nM)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>2B8</td>
<td>9.2</td>
<td>1.2</td>
</tr>
<tr>
<td>HE2B8-1</td>
<td>6.0</td>
<td>1.2</td>
</tr>
<tr>
<td>HE2B8-2</td>
<td>5.7</td>
<td>1.1</td>
</tr>
<tr>
<td>HE2B8-3</td>
<td>5.9</td>
<td>1.1</td>
</tr>
<tr>
<td>HE2B8-4</td>
<td>6.5</td>
<td>1.2</td>
</tr>
<tr>
<td>sh2B8-9 (G1m(3))</td>
<td>4.2</td>
<td>-</td>
</tr>
<tr>
<td>sh2B8-12 (G1m(3))</td>
<td>6.8</td>
<td>-</td>
</tr>
</tbody>
</table>

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

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>
<thead>
<tr>
<th>Antibody</th>
<th>IC₅₀ (nM)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>2B8</td>
<td>0.86</td>
<td>0.35</td>
</tr>
<tr>
<td>HE2B8-1</td>
<td>0.47</td>
<td>0.15</td>
</tr>
<tr>
<td>HE2B8-2</td>
<td>0.66</td>
<td>0.13</td>
</tr>
<tr>
<td>HE2B8-3</td>
<td>0.55</td>
<td>0.28</td>
</tr>
<tr>
<td>HE2B8-4</td>
<td>0.58</td>
<td>0.26</td>
</tr>
<tr>
<td>sh2B8-9 (G1m(3))</td>
<td>0.52</td>
<td>0.11</td>
</tr>
<tr>
<td>sh2B8-12 (G1m(3))</td>
<td>0.81</td>
<td>0.22</td>
</tr>
</tbody>
</table>

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

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>
<thead>
<tr>
<th>Antibody</th>
<th>Trial 1</th>
<th>Trial 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>2B8</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>HE2B8-1</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>HE2B8-2</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>HE2B8-3</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>HE2B8-4</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>sh2B8-9 (G1m(3))</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>sh2B8-12 (G1m(3))</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

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

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

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.
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

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% 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.

Near-confluent cells were collected by trypsinization and then 5 x 10⁶ 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 (mm³) = L x W² / 2. When the tumors grew to approximately 200 mm³, 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 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 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.

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.

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

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), the
   binding protein comprising:
   
   (a) an immunoglobulin light chain variable region comprising the structure CDR
    L2-CDR L3, wherein

   (i) CDR L1 comprises the amino acid sequence X1X2 Ser X4X5X6X7XsX9
    Xio Xi1Xi2 Xi3 Xi4 Xi5, wherein amino acid X1 is Arg, Lys, or Ser, X2 is
    Ala or Thr, X4 is Glu, Gln, or Ser, X5 is Asn, Asp, or Ser, X6 is He or
    Val, X7 is Asp, Lys, Ser, Val, or Tyr, Xs is a peptide bond or Tyr, X9 is a
    peptide bond or Asp, Xi0 is a peptide bond or Gly, Xi1 is a peptide bond
    or Asn, Xi2 is a peptide bond, lie, or Ser, Xi3 is Asn or Tyr, Xi4 is lie,
    Leu, Met, or Val, X1s is Ala, Asn, His, or Ser,

   (ii) CDR L2 comprises the amino acid sequence X16X17X18 X19X20 X21 X22,
    wherein amino acid X16 is Ala, Asp, Arg, Gly, or Val, X17 is Ala, Thr, or
    Val, X18 is Asn, Ser, or Thr, X19 is Arg, Asn, Lys, or His, X20 is Leu or
    Arg, X21 is Ala, Asn, Glu, Val, or Pro, X22 is Asp, Ser, or Thr, and

   (iii) CDR L3 comprises the amino acid sequence X23X24X25X26 X27 X28 Pro
    X30Thr, wherein amino acid X23 is Leu, Gly, or Gln, X24 is His or Gln,
    X25 is Phe, Ser, Thr, or Tyr, X26 is Asp, He, Ser, Trp, or Tyr, X27 is Gly,
    Glu, Asn, or Ser, X28 is Asp, Asn, Phe, Thr, or Tyr, X30 is Leu, Phe, Pro,
    or Tyr; and

   (b) an immunoglobulin heavy chain variable region comprising three complementarity
    determining regions,

   wherein the complementarity determining regions of the immunoglobulin light chain
   and immunoglobulin heavy chain define a binding site that binds human HGF.

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

   (a) an immunoglobulin heavy chain variable region comprising the structure CDR
    Hi-CDR H2-CDR H3, wherein
CDR_H_1 comprises the amino acid sequence X_i Tyr X_3 X_4 X_5, wherein amino acid X_i is Asp, Asn, Ser, or Thr, X_3 is Phe, Ser, Trp, or Tyr, X_4 is He, Leu, or Met, X_5 is Asn, His, or Ser.

CDR_H_2 comprises the amino acid sequence X_6 He X_g X_9 X_10 X_n Gly X_13 X_14 X_15 Tyr X_17 X_ig 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_n 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, 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, and

CDR_H_3 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, He, or Tyr, X_33 is Met or Phe, X_34 is Ala or Asp; and

(a) an immunoglobulin light chain variable region comprising three complementarity determining regions,

wherein the complementarity determining regions of the immunoglobulin light chain and immunoglobulin heavy chain define a binding site that binds human HGF.

3. The isolated antibody of claim 2, wherein the immunoglobulin light chain variable region comprises the structure CDR_L_1-CDR_L_2-CDR_L_3, wherein

CDR_L_1 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_n X_12 X_13 X_14 X_15, wherein amino acid X_1 is Arg, Lys, or Ser, X_12 is Ala or Thr, X_4 is Glu, Gln, or Ser, X_5 is Asn, Asp, or Ser, X_6 is He 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, He, or Ser, $X_{13}$ is Asn or Tyr, $X_{14}$ is He, Leu, Met, or Val, $X_{15}$ is Ala, Asn, His, or Ser, 

(ii) CDR L.2 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, and

(iii) CDR.L.3 comprises the amino acid sequence $X_{23}X_{24}X_{25}X_{26}X_{27}X_{28}X_{29}X_{30}$ Pro X30 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, He, Ser, Trp, or Tyr, $X_{27}$ is Gly, Glu, Asn, or Ser, $X_{28}$ is Asp, Asn, Phe, Thr, or Tyr, $X_{29}$ is Leu, Phe, Pro, or Tyr.

4. The binding protein of claim 1, 2, or 3 wherein the complementarity determining regions are interposed between framework regions.

5. The binding protein of claim 4, wherein the CDR sequences are interposed between human or humanized framework regions.

6. An isolated nucleic acid comprising a nucleotide sequence encoding the immunoglobulin light chain variable region of claim 1.

7. An expression vector containing a nucleotide sequence of claim 6.

8. A host cell containing the expression vector of claim 7.

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

   (a) growing the host cell of claim 8 under conditions so that the host cell expresses the immunoglobulin light chain variable region; and

   (b) harvesting the immunoglobulin light chain variable region.

10. The method of claim 9, wherein, after step (b), the immunoglobulin light chain variable region is covalently linked to an immunoglobulin heavy chain variable region, so that the light chain and heavy chain variable regions together bind human HGF.

11. An isolated nucleic acid comprising a nucleotide sequence encoding the immunoglobulin heavy chain variable region of claim 2.

12. An expression vector containing a nucleotide sequence of claim 11.
13. A host cell containing the expression vector of claim 12.

14. A method of producing a binding protein, the method comprising:
   (a) growing the host cells of claim 13 under conditions so that the host cell
       expresses the immunoglobulin heavy chain variable region; and
   (b) harvesting the immunoglobulin heavy chain variable region.

15. The method of claim 14, wherein, after step (b), the immunoglobulin heavy chain
    variable region is covalently linked to an immunoglobulin light chain variable region, so that
    the light and heavy chain variable regions together define a binding site that binds human HGF.

16. An isolated binding protein that binds human hepatocyte growth factor (HGF)
    comprising:
    (a) an immunoglobulin light chain variable region comprising the structure CDR_L1-
        CDRL_2-CDRL_3, wherein
        (i) 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); and
        (ii) CDRL_2 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), and SEQ ID NO. 79 (3A12); and
        (iii) CDRu 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); 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.
17. The binding protein of claim 16, wherein the immunoglobulin light chain variable region comprises
(i) a CDRL1 comprising the sequence SEQ ID NO. 8 (1A3),
(ii) a CDR L2 comprising the sequence SEQ ID NO. 9 (1A3), and
(iii) a CDRu comprising the sequence SEQ ID NO. 10 (1A3).

18. The binding protein of claim 16, wherein the immunoglobulin light chain variable region comprises
(i) a CDR L1 comprising the sequence SEQ ID NO. 18 (2B8),
(ii) a CDR L2 comprising the sequence SEQ ID NO. 19 (2B8), and
(iii) a CDR L3 comprising the sequence SEQ ID NO. 20 (2B8).

19. The binding protein of claim 16, wherein the immunoglobulin light chain variable region comprises
(iv) a CDRL1 comprising the sequence SEQ ID NO. 28 (2F8),
(v) a CDRL2 comprising the sequence SEQ ID NO. 29 (2F8), and
(vi) a CDRL3 comprising the sequence SEQ ID NO. 30 (2F8).

20. The binding protein of claim 16, wherein the immunoglobulin light chain variable region comprises
(i) a CDR L1 comprising the sequence SEQ ID NO. 38 (3B6),
(ii) a CDR L2 comprising the sequence SEQ ID NO. 39 (3B6), and
(iii) a CDR L3 comprising the sequence SEQ ID NO. 40 (3B6).

21. The binding protein of claim 16, wherein the immunoglobulin light chain variable region comprises
(i) a CDR L1 comprising the sequence SEQ ID NO. 48 (3DII),
(ii) a CDR L2 comprising the sequence SEQ ID NO. 49 (3D1), and
(iii) a CDR L3 comprising the sequence SEQ ID NO. 50 (3D11).
22. The binding protein of claim 16, wherein the immunoglobulin light chain variable region comprises
   (i) a CDRu comprising the sequence SEQ ID NO. 58 (1D3),
   (ii) a CDR_L2 comprising the sequence SEQ ID NO. 59 (1D3), and
   (iii) a CDR_L3 comprising the sequence SEQ ID NO. 60 (1D3).

23. The binding protein of claim 16, wherein the immunoglobulin light chain variable region comprises
   (i) a CDRn comprising the sequence SEQ ID NO. 68 (1F3),
   (ii) a CDR_L2 comprising the sequence SEQ ID NO. 69 (1F3), and
   (iii) a CDR_L3 comprising the sequence SEQ ID NO. 70 (1F3).

24. The binding protein of claim 16, wherein the immunoglobulin light chain variable region comprises
   (i) a CDRL_1 comprising the sequence SEQ ID NO. 78 (3A12),
   (ii) a CDR_L2 comprising the sequence SEQ ID NO. 79 (3A12), and
   (iii) a CDR_L3 comprising the sequence SEQ ID NO. 80 (3A12).

25. The binding protein of claim 16, wherein CDR_L1, CDR_L2, and CDR_L3 are interposed between human or humanized immunoglobulin framework regions.

26. The binding protein of claim 16, wherein the binding protein is an antibody or an antigen binding fragment thereof.

27. The binding protein of claim 26, wherein the antibody is a monoclonal antibody.

28. An isolated binding protein that binds human hepatocyte growth factor (HGF) comprising:
   (a) an immunoglobulin heavy chain variable region comprising the structure CDRH1-CDRH2-CDRH3, wherein
   (i) CDRH_1 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); 

(ii) CDRH₂ 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 Hvlf.l), and SEQ ID NO. 203 (Hu2B8 Hv5a.l and Hu2B8 Hv5-51.1); and 

(iii) CDRH₃ 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); 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.

29. The binding protein of claim 28, wherein the immunoglobulin heavy chain variable region comprises 

(i) a CDRm comprising the sequence SEQ ID NO. 5 (1A3), 

(ii) a CDRH₂ comprising the sequence SEQ ID NO. 6 (1A3), and 

(iii) a CDRH₃ comprising the sequence SEQ ID NO. 7 (1A3).

30. The binding protein of claim 28, wherein the immunoglobulin heavy chain variable region comprises 

(i) a CDRH₁ comprising the sequence SEQ ID NO. 15 (2B8), 

(ii) a CDRH₂ comprising the sequence SEQ ID NO. 16 (2B8), SEQ ID NO. 202 (Hu2B8 Hvlf.l), or SEQ ID NO. 203 (Hu2B8 Hv5a.l and Hu2B8 Hv5-51.1), and 

(iii) a CDRH₃ comprising the sequence SEQ ID NO. 17 (2B8).
31. The binding protein of claim 28, wherein the immunoglobulin heavy chain variable region comprises
   (i) a CDRH1 comprising the sequence SEQ ID NO. 25 (2F8),
   (ii) a CDRH2 comprising the sequence SEQ ID NO. 26 (2F8), and
   (iii) a CDRH3 comprising the sequence SEQ ID NO. 27 (2F8).

32. The binding protein of claim 28, wherein the immunoglobulin heavy chain variable region comprises
   (i) a CDRH1 comprising the sequence SEQ ID NO. 35 (3B6),
   (ii) a CDRH2 comprising the sequence SEQ ID NO. 36 (3B6), and
   (iii) a CDRH3 comprising the sequence SEQ ID NO. 37 (3B6).

33. The binding protein of claim 28, wherein the immunoglobulin heavy chain variable region comprises
   (i) a CDRH1 comprising the sequence SEQ ID NO. 45 (3DII),
   (ii) a CDRH2 comprising the sequence SEQ ID NO. 46 (3D11), and
   (iii) a CDRH3 comprising the sequence SEQ ID NO. 47 (3D11).

34. The binding protein of claim 28, wherein the immunoglobulin heavy chain variable region comprises
   (i) a CDRH1 comprising the sequence SEQ ID NO. 55 (1D3),
   (ii) a CDRH2 comprising the sequence SEQ ID NO. 56 (1D3), and
   (iii) a CDRH3 comprising the sequence SEQ ID NO. 57 (1D3).

35. The binding protein of claim 28, wherein the immunoglobulin heavy chain variable region comprises
   (i) a CDRH1 comprising the sequence SEQ ID NO. 65 (1F3),
   (ii) a CDRH2 comprising the sequence SEQ ID NO. 66 (1F3), and
   (iii) a CDRH3 comprising the sequence SEQ ID NO. 67 (1F3).
36. The binding protein of claim 28, wherein the immunoglobulin heavy chain variable region comprises
   (i) a CDRm comprising the sequence SEQ ID NO. 75 (3A12),
   (ii) a CDR\textsubscript{H2} comprising the sequence SEQ ID NO. 76 (3A12), and
   (iii) a CDR\textsubscript{H3} comprising the sequence SEQ ID NO. 77 (3A12).

37. The binding protein of claim 28, wherein CDR\textsubscript{H1}, CDR\textsubscript{H2}, and CDR\textsubscript{H3} are interposed between human or humanized immunoglobulin framework regions.

38. The binding protein of claim 28, wherein the binding protein is an antibody of an antigen binding fragment thereof.

39. The binding framework of claim 38, wherein the antibody is a monoclonal antibody.

40. An isolated nucleic acid comprising a nucleotide sequence encoding the immunoglobulin light chain variable region of claim 16.

41. An expression vector containing the nucleic acid sequence of claim 40.

42. A host cell containing the expression vector of claim 41.

43. A method of producing a binding protein, the method comprising:
   (i) growing the host cell of claim 42 under conditions so that the host cell expresses the immunoglobulin light chain variable region; and
   (ii) harvesting the immunoglobulin light chain variable region.

44. The method of claim 43, wherein, after step (b), the immunoglobulin light chain variable region is covalently linked to an immunoglobulin heavy chain variable region, so that the light and heavy chain variable regions together bind human HGF.

45. An isolated nucleic acid comprising a nucleotide sequence encoding the immunoglobulin heavy chain variable region of claim 28.

46. An expression vector containing the nucleic acid sequence of claim 45.

47. A host cell containing the expression vector of claim 46.
48. A method of producing a binding protein, the method comprising:
   (i) growing the host cell of claim 47 under conditions so that the host cell expresses the
   immunoglobulin heavy chain variable region; and
   (ii) harvesting the immunoglobulin heavy chain variable region.

49. The method of claim 48, wherein, after step (b), the immunoglobulin heavy chain
   variable region is covalently linked to an immunoglobulin light chain variable region, so that
   the light and heavy chain variable regions together define a binding site capable of binding
   human HGF.

50. An isolated binding protein that binds human hepatocyte growth factor (HGF)
   comprising:
   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 (3DII) residues 21-127 of SEQ ID NO. 54 (1D3), residues 21-127 of SEQ ID
   NO. 64 (1F3), residues 21-127 of SEQ ID NO. 74 (3A12), SEQ ID NO. 173 (Hu2B8 Kvl-39.1
   Kappa chain variable region), and SEQ ID NO. 179 (Hu2B8 Kv3-15.1 Kappa chain
   variable region); and

   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), residues 20-141 of SEQ ID NO. 72 (3A12), SEQ ID NO. 159 (Hu2B8 Hvlf.1
   heavy chain variable region), SEQ ID NO. 165 (Hu2B8 Hv5a.1 heavy chain variable
   region), and SEQ ID NO. 169 (Hu2B8 Hv5-51.1 heavy chain variable region).

51. The binding protein of claim 50, wherein the immunoglobulin light chain variable
   region comprises the amino acid sequence of residues 21-127 of SEQ ID NO. 4 (1A3), and the
   immunoglobulin heavy chain variable region comprises the amino acid sequence of residues of
   20-141 of SEQ ID NO. 2 (1A3).
52. The binding protein of claim 50, wherein the immunoglobulin light chain variable region comprises the amino acid sequence of residues 21-127 of SEQ ID NO. 14 (2B8), and the immunoglobulin heavy chain variable region comprises the amino acid sequence of residues 20-137 of SEQ ID NO. 12 (2B8).

53. The binding protein of claim 50, wherein the immunoglobulin light chain variable region comprises the amino acid sequence of residues 20-131 of SEQ ID NO. 24 (2F8), and the immunoglobulin heavy chain variable region comprises the amino acid sequence of residues 20-137 of SEQ ID NO. 22 (2F8).

54. The binding protein of claim 50, wherein the immunoglobulin light chain variable region comprises the amino acid sequence of residues 23-129 of SEQ ID NO. 34 (3B6), and the immunoglobulin heavy chain variable region comprises the amino acid sequence of residues 20-139 of SEQ ID NO. 32 (3B6).

55. The binding protein of claim 50, wherein the immunoglobulin light chain variable region comprises the amino acid sequence of residues 23-128 of SEQ ID NO. 44 (3D11), and the immunoglobulin heavy chain variable region comprises the amino acid sequence of residues 20-132 of SEQ ID NO. 42 (3D11).

56. The binding protein of claim 50, wherein the immunoglobulin light chain variable region comprises the amino acid sequence of residues 21-127 of SEQ ID NO. 54 (1D3), and the immunoglobulin heavy chain variable region comprises the amino acid sequence of residues 20-141 of SEQ ID NO. 52 (1D3).

57. The binding protein of claim 50, wherein the immunoglobulin light chain variable region comprises the amino acid sequence of residues 21-127 of SEQ ID NO. 64 (1F3), and the immunoglobulin heavy chain variable region comprises the amino acid sequence of residues 20-141 of SEQ ID NO. 62 (1F3).

58. The binding protein of claim 50, wherein the immunoglobulin light chain variable region comprises the amino acid sequence of residues 21-127 of SEQ ID NO. 74 (3A12), and the immunoglobulin heavy chain variable region comprises the amino acid sequence of residues 20-141 of SEQ ID NO. 72 (3A12).
59. An isolated binding protein that binds human hepatocyte growth factor (HGF) comprising:
   an immunoglobulin light chain variable region selected from the group consisting of
   SEQ ID NO. 173 (Hu2B8 Kvl-39.1 light chain variable region), and SEQ ID NO. 179
   (Hu2B8 Kv3-15.1 light chain variable region); and
   an immunoglobulin heavy chain variable region selected from the group consisting of
   SEQ ID NO. 159 (Hu2B8 Hvlf.1 heavy chain variable region), SEQ ID NO. 165 (Hu2B8
   Hv5a.l heavy chain variable region), and SEQ ID NO. 169 (Hu2B8 Hv5-51.1 heavy chain
   variable region).

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

61. The binding protein of claim 50, 59 or 60, wherein the binding protein is an antibody or
an antigen binding fragment thereof.

62. The binding protein of claim 61, wherein the antibody is a monoclonal antibody.

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

   (i) an immunoglobulin light chain variable region comprising three
complementarity determining regions; and

   (ii) an immunoglobulin heavy chain variable region comprising three
complementarity determining regions,
wherein the complementarity determining regions of the immunoglobulin light chain and the immunoglobulin heavy chain together define a binding site that binds reduced human HGF.

64. The binding protein of claim 63, wherein the binding protein is an antibody or an antigen binding fragment thereof.

65. The binding protein of claim 64, wherein the antibody is a monoclonal antibody.

66. The binding protein of claim 63, wherein the complementarity determining regions are interposed between framework regions.

67. The binding protein of claim 63, wherein the immunoglobulin heavy chain is IgGl.

68. The binding protein of claim 63, wherein the binding protein binds to human HGF containing a cysteine to arginine substitution at position 561 or a glycine to glutamate substitution at position 555.

69. The binding protein of claim 63, wherein the binding protein binds the α-chain of human HGF.

70. The binding protein of claim 63, wherein the immunoglobulin light chain variable region comprises at least one complementarity determining region (CDR) selected from the group consisting of CDR1, CDR2, and CDR3.

wherein CDR1 comprises the amino acid sequence X1 X2 Ser X4 X5 X6 X7 X8 X9 X10
Xn X11 X12 X13 X14 X15 X16 X17 X18 X19 X20 X21 X22 X23 X24 X25 X26 X27 X28 X29 X30
wherein amino acid X1 is Arg or Lys, X2 is Ala or Thr, X4 is Glu or Gln, X5 is Asn, Ser, or Asp, X6 is Ile or Val, X7 is Tyr, Asp, or Lys, X8 is a peptide bond or Tyr, X9 is a peptide bond or Ser, X10 is a peptide bond or Gly, X11 is a peptide bond or Asn, X12 is a peptide bond or Ser, X13 is Asn or Tyr, X14 is His or Leu, X15 is Ala, Asn, or Ser,

wherein CDR2 comprises the amino acid sequence X16 X17 X18 X19 X20 X21 X22
wherein amino acid X16 is Ala, Asp, Val, or Arg, X17 is Ala or Val, X18 is Asn, Ser, or Thr, X19 is Arg, Asn, or His, X21 is Ala, Glu, Val, or Pro, X22 is Asp or Ser, and

wherein CDR3 comprises the amino acid sequence X23 X24 X25 X26 X27 X28 X29 X30
Thr, wherein amino acid X23 is Leu or Gln, X24 is His or Gln, X25 is Phe, Ser, or Tyr, X26 is Asp, He, or Trp, X27 is Gly or Glu, X28 is Asp, Phe, or Thr, X29 is Phe, Pro, or Tyr.
71. The binding protein of claim 63 or 70, wherein the immunoglobulin heavy chain variable region comprises at least one CDR selected from the group consisting of CDRn, CDR₁, 2 and CDR₃, wherein CDRᵢ comprises the amino acid sequence Xᵢ Tyr X₃ X₄ X₅, wherein amino acid Xᵢ is Asp, Asn, Ser, or Thr, X₃ is Phe, Hip, or Tyr, X₄ is He or Met, X₅ is Asn, His, or Ser, wherein CDRm comprises the amino acid sequence X₆ He X₈ X₉ Gly Xn Gly X₁₃ X₁₄ X₁₅ Tyr X₁₇ Xig X₁₉ X₂₀ Lys X₂₂, wherein amino acid X₆ is Lys, Gln, or Tyr, X₈ is Gly, Ser, or Tyr, X₉ is Pro or Ser, Xn is Asp, Gly, or Ser, X₁₃ is Asp or Ser, X₁₄ is Ser or Thr, X₁₅ is Asn or Tyr, X₁₇ is Asn or Pro, X₁₉ is Ala, Asp, Gly, or Glu, X₁₉ is Asn, Met, or Ser, X₂₀ is Phe or Val, X₂₂ is Asp or Gly, and

wherein CDRᵣ₀ comprises the amino acid sequence X₂₃ X₂₄ X₂₅ X₂₆ X₂₇ X₂₈ X₂₉ X₃₀ X₃₁ X₃₂ X₃₃ Asp Tyr, wherein amino acid X₂₃ is Arg or Gln, X₂₄ is Gly or Leu, X₂₅ is Asp, Gly, or a peptide bond, X₂₆ is Gly or a peptide bond, X₂₇ is a peptide bond or Tyr, X₂₈ is Leu, a peptide bond or Tyr, X₂₉ is Gly, Arg or Leu, X₃₀ is Asp, Gly or Glu, X₃₁ is Tyr, Arg or Asn, X₃₂ is Ala, Gly or Tyr, X₃₃ is Met or Phe.

72. The binding protein of claim 70, wherein the immunoglobulin light chain comprises

(i) a CDRn 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 CDR₁ 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 CDRu 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).

73. The binding protein of claim 72, wherein the CDR sequences are interposed between human or humanized framework regions.

74. The binding protein of claim 72, wherein the immunoglobulin light chain variable region comprises 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).

75. The binding protein of claim 71, wherein the immunoglobulin heavy chain variable region comprises

(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).

(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).

76. The binding protein of claim 75, wherein the CDR sequences are interposed between human or humanized framework regions.

77. The binding protein of claim 75, wherein 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).

78. An isolated nucleic acid comprising a nucleotide sequence encoding the immunoglobulin light chain variable region of claim 63, 70, 72, or 74.

79. An expression vector containing the nucleic acid sequence of claim 78.

80. A host cell containing the expression vector of claim 79.

81. An isolated nucleic acid comprising a nucleotide sequence encoding the immunoglobulin heavy chain variable region of claim 63, 71, 75, or 77.

82. An expression vector containing the nucleic acid sequence of claim 81.
A host cell containing the expression vector of claim 82.

An isolated binding protein that binds human hepatocyte growth factor (HGF) comprising an immunoglobulin light chain variable region and an immunoglobulin heavy chain variable region, wherein 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 \(2\text{F8}\) and an immunoglobulin heavy chain variable region of residues 20-137 of SEQ ID NO. 22 \(2\text{F8}\),

(ii) an antibody having an immunoglobulin light chain variable region of residues 23-129 of SEQ ID NO. 34 \(3\text{B6}\) and an immunoglobulin heavy chain variable region of residues 20-139 of SEQ ID NO. 32 \(3\text{B6}\), and

(iii) an antibody having an immunoglobulin light chain variable region of residues 23-128 of SEQ ID NO. 44 \(3\text{D11}\) and an immunoglobulin heavy chain variable region of residues 20-132 of SEQ ID NO. 42 \(3\text{D11}\).

The binding protein of claim 84, wherein the binding protein binds the same epitope of HGF as one of the reference antibodies.

An isolated binding protein that binds human hepatocyte growth factor (HGF) with a \(k_d\) of \(4\times10^{-5}\) s\(^{-1}\) or lower.

The binding protein of claim 86, wherein the \(k_d\) is \(3\times10^{-5}\) s\(^{-1}\) or lower.

The binding protein of claim 87, wherein the \(k_d\) is \(2\times10^{-5}\) s\(^{-1}\) or lower.

An isolated binding protein that specifically binds human hepatocyte growth factor (HGF) with a \(K_D\) of 20 pM or lower.

The binding protein of claim 89, wherein the \(K_D\) is 10 pM or lower.

The binding protein of claim 90, wherein the \(K_D\) is 5 pM or lower.

An isolated binding protein that binds human hepatocyte growth factor (HGF), wherein the antibody binds to human HGF with lower \(K_D\) at 37°C than at 25°C.
93. The binding protein of claim 92, wherein the binding protein has a $K_D$ less than 5 pM at 37°C.

94. A method of inhibiting or reducing proliferation of a tumor cell comprising exposing the cell to an effective amount of the binding protein of claim 1, 2, 3, 16, 28, 50, 59, 60, 63, 70, 71, 72, 74, 75, 77, 84, 86, or 89 to inhibit or reduce proliferation of the tumor cell.

95. A method of inhibiting or reducing proliferation of a tumor cell comprising exposing the cell to an effective amount of a binding protein that inhibits or reduces proliferation of the tumor cell, wherein the binding protein specifically binds human HGF but does not substantially reduce the ability of human HGF to bind to c-Met.

96. The method of claim 95, wherein the binding protein comprises the binding protein of claim 22, 23, 24, 34, 35, 36, 56, 57, 58, 84, 86 or 89.

97. The method of claim 94 or 95, wherein the tumor cell is a human tumor cell.

98. 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 claim 1, 2, 3, 16, 28, 50, 59, 60, 63, 84, 86, 89 or 92 to inhibit or reduce proliferation of the tumor.

99. A method of treating a tumor in a mammal, the method comprising administering an effective amount of the binding protein of claim 1, 2, 3, 16, 28, 50, 59, 60, 63, 84, 86, 89 or 92.

100. The method of claim 98 or 99, wherein the mammal is a human.
<table>
<thead>
<tr>
<th>Antibody</th>
<th>Signal Peptide</th>
<th>CDR1</th>
<th>CDR2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A3</td>
<td><strong>KWPGRLFVTVLKGK</strong></td>
<td><strong>VQLVESGGLVQPSLGKSLCASKSPTFS</strong></td>
<td><strong>GYSMTYRVRTPKLRQMLYWTSFGSSYTPAPVSKVGRPTISRDNAKNTLYL</strong></td>
</tr>
<tr>
<td>2B8</td>
<td><strong>KGAAYLFLVATTVHVS</strong></td>
<td><strong>VQLQGFGHVRHNSLCLKSASSGFTPS</strong></td>
<td><strong>PYNIPHTNYHVEYKQKATLTVKDSSTAYM</strong></td>
</tr>
<tr>
<td>2F8</td>
<td><strong>KMQSWYPLFTLSYTVKHC</strong></td>
<td><strong>VQLQQGARLVRPGTSVNMCSASKAAGFTPS</strong></td>
<td><strong>TYTHWVQRPQGLLEWIKLGPGSSYTMFQKATLTVKDSSTAYM</strong></td>
</tr>
<tr>
<td>3B6</td>
<td><strong>KMNCPYFLFVSVTEGYSV</strong></td>
<td><strong>VQLQQGSAELVRPGSSVSLKSCASKAAGFTPS</strong></td>
<td><strong>SYTHMVQRPQGLLEWIKLYPDGSSYNGFQKATLTVKDSSTAYM</strong></td>
</tr>
<tr>
<td>3D11</td>
<td><strong>MAVYPFLCLVAPPSCL</strong></td>
<td><strong>VQLQKSGPGLVAPSGSLTCTVTSGFLYSQLNLNRQPQGLLEWIKVTWAG</strong></td>
<td><strong>QNTNYSLSMLTIRKDNSKQVFL</strong></td>
</tr>
<tr>
<td>1D3</td>
<td><strong>KMNPLFLVLVGGK</strong></td>
<td><strong>VQLQQGSGGGLQPSLGKSLCASKSPTFS</strong></td>
<td><strong>GYSMTYRVRTPKLRQMLYWTSFGSSYTPAPVSKVGRPTISRDNAKNTLYL</strong></td>
</tr>
<tr>
<td>1F3</td>
<td><strong>KMNPLLFLVLVGGK</strong></td>
<td><strong>VQLQQGSGGGLQPSLGKSLCASKSPTFS</strong></td>
<td><strong>GYSMTYRVRTPKLRQMLYWTSFGSSYTPAPVSKVGRPTISRDNAKNTLYL</strong></td>
</tr>
<tr>
<td>3A12</td>
<td><strong>KMNPLFLVLVGGK</strong></td>
<td><strong>VQLQQGSGGGLQPSLGKSLCASKSPTFS</strong></td>
<td><strong>GYSMTYRVRTPKLRQMLYWTSFGSSYTPAPVSKVGRPTISRDNAKNTLYL</strong></td>
</tr>
</tbody>
</table>

**CDR3**

(1A3 cont.) QMSSLKSEDTAMYCASGSGHGYGTYAMDVGCGGTSVTVSS (SEQ ID: 2)
(2B8 cont.) QLSSLTSNASVYCCASNYVFSGIDWNGQOTTVS (SEQ ID: 12)
(2F8 cont.) QLSSLTSNASVYCCASNYLQRGFDWNGQOTTVS (SEQ ID: 22)
(3B6 cont.) QLSSLTSNASVYCCASNYLQREYFWNGQOTTVS (SEQ ID: 32)
(3D11 cont.) QMSSLQCDTFAMYCASEVSAEVWSWQOTTVS (SEQ ID: 42)
(1D3 cont.) QMSSLKSDTFAMYCVVQDGGYGDYAMNVQGTSVTVSS (SEQ ID: 52)
(1F3 cont.) QMSSLKSDTFAMYCVVQDGGYGDYAMNVQGTSVTVSS (SEQ ID: 62)
(3A12 cont.) QMSSLKSDTFAMYCVVQDGGYGDYAMNVQGTSVTVSS (SEQ ID: 72)

**FIG. 2**
# Heavy Chain CDR Amino Acid Alignments

<table>
<thead>
<tr>
<th>Antibody</th>
<th>CDR1</th>
<th>CDR2</th>
<th>CDR3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A3</td>
<td>NYMNS (SEQ ID NO: 5)</td>
<td>YISPGGSYYPASVKG (SEQ ID NO: 6)</td>
<td>Q3DGYG FYAMDY (SEQ ID NO: 7)</td>
</tr>
<tr>
<td>2B8</td>
<td>TNYMH (SEQ ID NO: 15)</td>
<td>EINPTKGNHTYNENKFKS (SEQ ID NO: 16)</td>
<td>N3DGYG FYAMDY (SEQ ID NO: 17)</td>
</tr>
<tr>
<td>2F8</td>
<td>TYYIH (SEQ ID NO: 25)</td>
<td>KISPGGSYYPASVKG (SEQ ID NO: 26)</td>
<td>R3DGYG FYAMDY (SEQ ID NO: 27)</td>
</tr>
<tr>
<td>3B6</td>
<td>SYNAM (SEQ ID NO: 35)</td>
<td>QIYPGGDSYYNENFKS (SEQ ID NO: 36)</td>
<td>O3DGYG FYAMDY (SEQ ID NO: 37)</td>
</tr>
<tr>
<td>3D11</td>
<td>SYLHE (SEQ ID NO: 45)</td>
<td>VYNAG-GNTYNSLMS (SEQ ID NO: 46)</td>
<td>E3DGYG FYAMDY (SEQ ID NO: 47)</td>
</tr>
<tr>
<td>1D3</td>
<td>DYVMN (SEQ ID NO: 55)</td>
<td>YISSGGSTYYPDSVKG (SEQ ID NO: 56)</td>
<td>Q3DGYG FYAMDY (SEQ ID NO: 57)</td>
</tr>
<tr>
<td>1F3</td>
<td>YFMS (SEQ ID NO: 65)</td>
<td>YISSGGSTYYPDSVKG (SEQ ID NO: 66)</td>
<td>Q3DGYG FYAMDY (SEQ ID NO: 67)</td>
</tr>
<tr>
<td>3A12</td>
<td>YFMS (SEQ ID NO: 75)</td>
<td>YISSGGSTYYPDSVKG (SEQ ID NO: 76)</td>
<td>Q3DGYG FYAMDY (SEQ ID NO: 77)</td>
</tr>
</tbody>
</table>

**FIG. 3**
Complete Light (Kappa) Chain Variable Region Amino Acid Alignments

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Signal Peptide</th>
<th>CDR1</th>
<th>CDR2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A3</td>
<td>MSVPTQVLGLLLMLTDCIQTPSASLSVSGLGTIVTTCGASENIV-----SNLAWYQQKQ5KSPQ1L4VVPATNLADGVPSFSGSGTQLK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2B8</td>
<td>MEOQTITPLTSLLLWCAQSIGTMQSKSMSSVGLGTIVTTCGASENVV-----SYVSYWYQQKPAQ5KSPKLTVYASNRMTLVQGDRTQGSGTQATPLT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2F8</td>
<td>MEQVTVLGLLMLTDCIQTPSASLSVSGLGTIVTTCGASNIVV-----SNLAWYQQKQ5KSPQ1L4VVPATNLADGVPSFSGSGTQLK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3B6</td>
<td>MDARFAVQPLG1LLWNPQ1KQDIQMTQSKSMSSVGLGTIVTTCGASDEIK-----SYLWSWYQQKQ5KSPKLTVYANLFDQVPSFSGSGTQLK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3D11</td>
<td>MEOCVLQVFLFLISASVLIQG1QITQPSAMKYPDQEVIVTTCGASSVS-----SYLWSWYQQKQ5KSPQ1L4VVPATNLADGVPSFSGSGTQLK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1D3</td>
<td>MSVPTQVLGLLMLTDCIQTPSASLSVSGLGTIVTTCGASENVV-----SNLAWYQQKQ5KSPQ1L4VVPATNLADGVPSFSGSGTQLK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1F3</td>
<td>MSVPTQVLGLLMLTDCIQTPSASLSVSGLGTIVTTCGASENVV-----SNLAWYQQKQ5KSPQ1L4VVPATNLADGVPSFSGSGTQLK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3A12</td>
<td>MSVPTQVLGLLMLTDCIQTPSASLSVSGLGTIVTTCGASENVV-----SNLAWYQQKQ5KSPQ1L4VVPATNLADGVPSFSGSGTQLK</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CDR3

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Signal Peptide</th>
<th>CDR3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A3 cont.</td>
<td>INSQSEDFGTYYCHFGDPTTPFGGKLKEIK (SEQ ID NO: 4)</td>
<td></td>
</tr>
<tr>
<td>2B8 cont.</td>
<td>ISSVRAEDLADYCHGSSNYTPTPFGGTRLEIK (SEQ ID NO: 14)</td>
<td></td>
</tr>
<tr>
<td>2F8 cont.</td>
<td>IIPVEBERAATYYCHQQSEDQTPTPFGGTLKLK (SEQ ID NO: 24)</td>
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</tr>
<tr>
<td>3B6 cont.</td>
<td>ITSLENESEGTYCHQQDRFGTPFPGGTLKBK (SEQ ID NO: 34)</td>
<td></td>
</tr>
<tr>
<td>3D11 cont.</td>
<td>ISSMEAIRDAATYYCHWSSNDTPTPFGGTLKLK (SEQ ID NO: 44)</td>
<td></td>
</tr>
<tr>
<td>1D3 cont.</td>
<td>INSQSEDFGKYCHFGDPTTPFGGKLKEIK (SEQ ID NO: 54)</td>
<td></td>
</tr>
<tr>
<td>1F3 cont.</td>
<td>INSQSEDFGKYCHFGDPTTPFGGKLKEIK (SEQ ID NO: 64)</td>
<td></td>
</tr>
<tr>
<td>3A12 cont.</td>
<td>INSQSEDFGKYCHFGDPTTPFGGKLKEIK (SEQ ID NO: 74)</td>
<td></td>
</tr>
</tbody>
</table>

FIG. 4
### Light (Kappa) Chain CDR Amino Acid Alignments

<table>
<thead>
<tr>
<th>Antibody</th>
<th>CDR1</th>
<th>CDR2</th>
<th>CDR3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A3</td>
<td>RASENY----SNLA (SEQ ID NO: 8)</td>
<td>AATNLAD (SEQ ID NO: 9)</td>
<td>QHFWGTPYTT (SEQ ID NO: 10)</td>
</tr>
<tr>
<td>1D3</td>
<td>RTHENY-----SNLA (SEQ ID NO: 58)</td>
<td>AATNLAD (SEQ ID NO: 59)</td>
<td>QHFWGTPYTT (SEQ ID NO: 60)</td>
</tr>
<tr>
<td>2B8</td>
<td>KASENVV-----EVSV (SEQ ID NO: 18)</td>
<td>GASNRNT (SEQ ID NO: 19)</td>
<td>QSSYNPYTT (SEQ ID NO: 20)</td>
</tr>
<tr>
<td>2F6</td>
<td>KASQSVYDYGNSYIN (SEQ ID NO: 28)</td>
<td>VASNLIES (SEQ ID NO: 29)</td>
<td>QssiedpTT (SEQ ID NO: 30)</td>
</tr>
<tr>
<td>3D11</td>
<td>SASSSVS------YMI (SEQ ID NO: 48)</td>
<td>DTSKLAB (SEQ ID NO: 49)</td>
<td>QWSSNPLT (SEQ ID NO: 50)</td>
</tr>
<tr>
<td>3B6</td>
<td>KASQDK------SYLS (SEQ ID NO: 38)</td>
<td>KVNRIVD (SEQ ID NO: 39)</td>
<td>LQYDEPPFT (SEQ ID NO: 40)</td>
</tr>
<tr>
<td>1F3</td>
<td>RASENY----SNLA (SEQ ID NO: 68)</td>
<td>DATHLPD (SEQ ID NO: 69)</td>
<td>QHFWGTPYTT (SEQ ID NO: 70)</td>
</tr>
<tr>
<td>3A12</td>
<td>RASENY-----ZHLA (SEQ ID NO: 78)</td>
<td>AATKLAD (SEQ ID NO: 79)</td>
<td>QHFWGTPYTT (SEQ ID NO: 80)</td>
</tr>
</tbody>
</table>

**FIG. 5**
FIG. 6
<table>
<thead>
<tr>
<th>Kappa</th>
<th>Heavy</th>
<th>$ka$ (1/Ms)</th>
<th>STDEV</th>
<th>$kd$ (1/s)</th>
<th>STDEV</th>
<th>KD (pM)</th>
<th>STDEV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chimeric 2B8</td>
<td>Chimeric 2B8</td>
<td>2.3x10^6</td>
<td></td>
<td>2.7x10^-5</td>
<td></td>
<td>11.6</td>
<td></td>
</tr>
<tr>
<td>Hu2B8_Kv1-39.1</td>
<td>Chimeric 2B8</td>
<td>2.8x10^6</td>
<td></td>
<td>3.9x10^-5</td>
<td></td>
<td>13.6</td>
<td></td>
</tr>
<tr>
<td>Hu2B8_Kv3-15.1</td>
<td>Chimeric 2B8</td>
<td>3.1x10^6</td>
<td></td>
<td>1.7x10^-5</td>
<td></td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>Chimeric 2B8</td>
<td>Hu2B8_Hv1-f.1</td>
<td>2.4x10^6</td>
<td></td>
<td>1.6x10^-3</td>
<td></td>
<td>662.5</td>
<td></td>
</tr>
<tr>
<td>Chimeric 2B8</td>
<td>Hu2B8_Hv5-a.1</td>
<td>2.4x10^6</td>
<td></td>
<td>1.1x10^-5</td>
<td></td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>Chimeric 2B8</td>
<td>Hu2B8_Hv5-51.1</td>
<td>2.1x10^6</td>
<td></td>
<td>3.4x10^-5</td>
<td></td>
<td>16.3</td>
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</tr>
<tr>
<td>Hu2B8_Kv1-39.1</td>
<td>Hu2B8_Hv1-f.1</td>
<td>7.1x10^6</td>
<td></td>
<td>2.1x10^-3</td>
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<td>294.0</td>
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<tr>
<td>Hu2B8_Kv1-39.1</td>
<td>Hu2B8_Hv5-a.1</td>
<td>2.6x10^6</td>
<td></td>
<td>3.8x10^-5</td>
<td></td>
<td>14.7</td>
<td></td>
</tr>
<tr>
<td>Hu2B8_Kv1-39.1</td>
<td>Hu2B8_Hv5-51.1</td>
<td>2.0x10^6</td>
<td>4.2x10^5</td>
<td>1.7x10^-5</td>
<td>1.4x10^-5</td>
<td>8.1</td>
<td>5.3</td>
</tr>
<tr>
<td>Hu2B8_Kv3-15.1</td>
<td>Hu2B8_Hv1-f.1</td>
<td>7.8x10^6</td>
<td></td>
<td>3.7x10^-3</td>
<td></td>
<td>465.6</td>
<td></td>
</tr>
<tr>
<td>Hu2B8_Kv3-15.1</td>
<td>Hu2B8_Hv5-a.1</td>
<td>2.2x10^6</td>
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<td>5.9x10^-5</td>
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<td>26.9</td>
<td></td>
</tr>
<tr>
<td>Hu2B8_Kv3-15.1</td>
<td>Hu2B8_Hv5-51.1</td>
<td>1.9x10^6</td>
<td>4.7x10^5</td>
<td>2.3x10^-5</td>
<td>6.3x10^-6</td>
<td>12.0</td>
<td>0.4</td>
</tr>
</tbody>
</table>

**Fig. 8**