Title: ANTI-RON ANTIBODIES

Abstract: Monoclonal antibodies that bind and inhibit activation of human RON (Recepteur d'Origine Nantais) are disclosed. The antibodies can be used to treat certain forms of cancer that are associated with activation of RON.
ANTI-RON ANTIBODIES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of and priority to U.S. Provisional Application Serial No. 61/466,679, filed March 23, 2011, and U.S. Provisional Application Serial No. 61/361,808, filed July 6, 2010; the contents of each application are hereby incorporated by reference in their entirety.

FIELD OF THE INVENTION

The field of the invention is molecular biology, immunology and oncology. More particularly, the field is therapeutic antibodies.

BACKGROUND

Recepteur d'Origine Nantais (RON), also known as Macrophage Stimulating Protein Receptor (MSP R, or MST1-R), is a member of the MET family of receptor tyrosine kinases that binds the ligand known as Macrophage Stimulating Protein (MSP). RON is composed of a 40 kDa extracellular a chain and a 150 kDa transmembrane β chain. The β chain is responsible for the intrinsic kinase activity, and the extracellular portions of the two chains function together as the ligand binding domain (Wagh et al., 2008, ADV. CANCER RES. 100:1-33).

MSP binding to RON activates multiple downstream signaling pathways and mediates multiple cellular activities. RON pathway dysregulation is involved in inflammatory response, wound healing and liver regeneration. RON signaling can sustain tumor growth, survival, motility, invasion and angiogenesis in certain malignancies. The RON protein exists in several splice variants, some of which are tumorigenic in animal models of cancer. One such splice variant is delta 160 RON, which lacks exons 5 and 6 (Lu et al., 2007, CANCER LETT. 257:157-164).

When activated by ligand binding, RON activates the PI3K/AKT pathway and the MAPK pathway. RON also affects cells through interactions with other receptors, e.g., c-Met, integrins and EGFR. To date, no activating mutations in RON exons have been reported. Alternative splicing and overexpression appear to be the main mechanisms for constitutive
activation of the receptor. Several small molecule inhibitors have been reported that inhibit multiple receptor tyrosine kinases, including RON, examples of which include EXCEL-2880, (Qian et al. 2009, CANCER RES. 69:8009-8016) and BMS-77607 (Schroeder et al. 2009 J. MED CHEM. 52:1251-1254). A dual c-met/RON inhibitor has also been reported, Amgen compound 1 (Zhang et al., 2008, CANCER RES. 68:6680-6687). A recent publication describes a selective RON small molecule inhibitor (Raeppel et al., 2010 BIOORG MED CHEM LETT 20:2745-9). Several antibodies that inhibit human RON activity have been reported (Huet et al., US 2009/0226442; Pereira et al., US 2009/0136510; Zhu et al., WO 2006/020258; Pereira et al., WO 2005/120557; and commercial antibody MAB691, R&D Systems, Minneapolis, MN).

[N0006] Naturally occurring antibodies are multimeric proteins that contain four polypeptide chains (FIG. 1). Two of the polypeptide chains are called heavy chains (H chains), and two of the polypeptide chains are called light chains (L chains). The immunoglobulin heavy and light chains are connected by an interchain disulfide bond. The immunoglobulin heavy chains are connected by interchain disulfide bonds. A light chain consists of one variable region (\(V_L\) in FIG. 1) and one constant region (\(C_L\) in FIG. 1). The heavy chain consists of one variable region (\(V_H\) in FIG. 1) and at least three constant regions (\(C_H^1, C_H^2\) and \(C_H^3\) in FIG. 1). The variable regions determine the specificity of the antibody. Each variable region comprises three hypervariable regions also known as complementarity determining regions (CDRs) flanked by four relatively conserved framework regions (FRs). The three CDRs, referred to as CDR\(_1\), CDR\(_2\), and CDR\(_3\), contribute to the antibody binding specificity. Naturally occurring antibodies have been used as starting material for engineered antibodies, such as chimeric antibodies and humanized antibodies.

[N0007] Although antibodies that bind RON are known in the art, there is still a need for improved RON antibodies that can be used as therapeutic agents.

SUMMARY

[N0008] The invention is based, in part, upon the discovery of a family of antibodies that specifically bind human RON. The antibodies contain RON binding sites based on the CDRs of the antibodies. The antibodies can be used as therapeutic agents. When used as therapeutic agents, the antibodies are engineered, e.g., humanized, to reduce or eliminate an immune response when administered to a human patient.
[0009] The antibodies prevent or inhibit the activation of (i.e., neutralize) human RON. In some embodiments, the antibodies prevent RON from binding to its ligand, MSP, thereby neutralizing RON activity. In certain embodiments, the antibodies prevent RON activation without inhibiting RON binding to MSP. The antibodies can be used to inhibit the downstream signaling of the breast tumor cell line T47D. Furthermore, when administered to a mammal, the antibodies can inhibit or reduce tumor growth in the mammal.

[0010] These and other aspects and advantages of the invention will become apparent upon consideration of the following figures, detailed description, and claims. As used herein, "including" means without limitation, and examples cited are non-limiting.

DESCRIPTION OF THE DRAWINGS

[0011] The invention can be more completely understood with reference to the following drawings.

[0012] FIG. 1 (prior art) is a schematic representation of a typical naturally-occurring antibody.

[0013] FIG. 2 is a sequence alignment showing the amino acid sequence of the complete immunoglobulin heavy chain variable region of antibodies 07F01, 12B11, 17F06, 18H09 and 29B06. The amino acid sequences for each antibody are aligned against one another, and CDR1, CDR2, and CDR3, are identified in boxes. The unboxed sequences represent framework (FR). Alignment positioning (gaps) are based on Kabat numbering, rather than an alignment algorithm such as Clustal sequences.

[0014] FIG. 3 is a sequence alignment showing the CDR1, CDR2, and CDR3 sequences for each of the immunoglobulin heavy chain variable region sequences in FIG. 2.

[0015] FIG. 4 is a sequence alignment showing the amino acid sequence of the complete immunoglobulin light chain variable region of antibodies 07F01, 12B11, 17F06, 18H09 and 29B06. The amino acid sequences for each antibody are aligned against one another, and CDR1, CDR2, and CDR3, are identified in boxes. The unboxed sequences represent framework (FR) sequences. Alignment positioning (gaps) are based on Kabat numbering, rather than an alignment algorithm such as Clustal sequences.

[0016] FIG. 5 is a sequence alignment showing the CDR1, CDR2, and CDR3 sequences for each of the immunoglobulin light chain variable region sequences in FIG. 4.
[0017] FIG. 6 is a graph showing dose-response curves for inhibition of the MSP-RON binding interaction by antibodies 17F06 (A), 07F01 (○), 12B11 (●), 18H09 (■), and 29B06 (x), as measured by electrochemiluminescence assay.

[0018] FIG. 7 is a graph showing dose-response curves for inhibition of MSP-dependent phosphorylation of ERK by antibodies 17F06 (A), 07F01 (○), 12B11 (●), 18H09 (■), and 29B06 (x) by ELISA assay.

[0019] FIG. 8 is a histogram summarizing results from an experiment measuring inhibition of MSP induced HPAF-II cell migration by antibodies 07F01, 18H09, 29B06, 12B11, 17F06 and an IgG negative control (murine IgG) by transwell assay.

[0020] FIG. 9 is a graph summarizing data on inhibition of growth of a wild-type (wt) RON-dependent in vivo tumor model by antibodies 07F01 (○), 12B11 (●), 18H09 (■), 29B06 (•), and a murine IgG control (o). The antibodies and IgG control were dosed at 20 mg/kg twice per week intraperitoneally.

[0021] FIG. 10 is a graph summarizing data on inhibition of growth of a delta 160 RON-dependent in vivo tumor model by antibodies 17F06 (A), 07F01 (○), 12B11 (●), 18H09 (■), 29B06 (•), and a murine IgG control (o). The antibodies and IgG control were dosed at 20 mg/kg twice per week intraperitoneally.

[0022] FIG. 11 is a graph summarizing data on inhibition of growth of an NCI-H358 xenograft tumor model by antibody 29B06 (•) and a murine IgG control (o). The antibody and IgG control were dosed at 40 mg/kg (abbreviated as "mpk") three per week intraperitoneally.

[0023] FIG. 12A is a schematic diagram showing the amino acid sequences of the complete immunoglobulin heavy chain variable region of 07F01 (SEQ ID NO: 2) and the complete heavy chain variable regions denoted as Chimeric 07F01 C102S (SEQ ID NO: 133), Sh07F01 Hv3-48 (SEQ ID NO: 135), and Sh07F01 Hv3-48 D28T T60A L63V E65G (SEQ ID NO: 137). The amino acid sequences for each heavy chain variable region are aligned against one another, and Complementary Determining Sequences (CDR) (Kabat definition), CDR1, CDR2, and CDR3, are identified in boxes. The unboxed sequences represent framework (FR) sequences.

[0024] FIG. 12B is a schematic diagram showing the amino acid sequences of the complete immunoglobulin heavy chain variable region of 29B06 (SEQ ID NO: 42) and the complete heavy chain variable regions denoted as Sh29B06 Hv4-59 (SEQ ID NO: 143), Hu29B06 Hv4-59 (SEQ ID NO: 144), and Sh29B06 Hv4-59 D160E (SEQ ID NO: 145).
59 (SEQ ID NO: 145), and Hu29B06 Hv4-59 D27G T30S M48I I67V Y78F (SEQ ID NO:
147). The amino acid sequences for each heavy chain variable region are aligned against one
another, and CDR_1, CDR_2, and CDR_3 sequences (Kabat definition) are identified in boxes. The
unboxed sequences represent framework (FR) sequences.

5 [0025] **FIG. 13A** is a schematic diagram showing the CDR_1, CDR_2, and CDR_3 sequences
(Kabat definition) for each of the variable region sequences shown in **FIG. 12A**.

[0026] **FIG. 13B** is a schematic diagram showing the CDR_1, CDR_2, and CDR_3 sequences
(Kabat definition) for each of the variable region sequences shown in **FIG. 12B**.

[0027] **FIG. 14A** is a schematic diagram showing the amino acid sequences of the
complete light chain variable region of 07F01 (SEQ ID NO: 4) and the complete light chain
variable regions denoted as HE L 07F01 Kv1-9 (SEQ ID NO: 139) and Sh07F01 Kv1-9 F1
(SEQ ID NO: 141). The amino acid sequences for each light chain variable region are aligned
against one another, and CDR_1, CDR_2, and CDR_3 sequences (Kabat definition) are identified in
boxes. The unboxed sequences represent framework (FR) sequences.

10 [0028] **FIG. 14B** is a schematic diagram showing the amino acid sequences of the complete
light chain variable region of 29B06 (SEQ ID NO: 44) and the complete light chain variable
region denoted as Sh29B06 Kv2-28 (SEQ ID NO: 149). The amino acid sequences for each
light chain variable region are aligned against one another, and CDR_1, CDR_2, and CDR_3
sequences (Kabat definition) are identified in boxes. The unboxed sequences represent
framework (FR) sequences.

15 [0029] **FIG. 15A** is a sequence alignment showing the CDR_1, CDR_2, and CDR_3 sequences
(Kabat definition) for each of the variable region sequences shown in **FIG. 14A**.

[0030] **FIG. 15B** is a sequence alignment showing the CDR_1, CDR_2, and CDR_3 sequences
(Kabat definition) for each of the variable region sequences shown in **FIG. 14B**.

20 [0031] **FIG. 16** is a histogram summarizing results from an experiment measuring
inhibition of MSP induced HPAF-II cell migration by anti-RON antibodies Sh29B06-78 and
Sh07F01-62, an IgG negative control (human IgG), and a no MSP control by transwell assay.

25 [0032] **FIG. 17** is a histogram summarizing results from an experiment measuring
inhibition of MSP induced HPAF-II cell invasion by anti-RON antibodies Sh29B06-78 and
Sh07F01-62 and an IgG negative control (human IgG) at 0 and 1 nM MSP.
FIG. 18 is a graph summarizing data on inhibition of growth of an NCI-H358 xenograft tumor model by anti-RON antibodies mu07F01 (○), Sh07F01-62 (A), mu29B06 (●), RON8 (■), and Sh29B06-78 (▲), and a human IgG control (+).

FIG. 19 depicts Western blots summarizing results from an experiment measuring RON receptor degradation by anti-RON antibodies mu07F01, Sh07F01-62, mu29B06, RON8, and Sh29B06-78.

DETAILED DESCRIPTION

The anti-RON antibodies disclosed herein are based on the antigen binding sites of certain monoclonal antibodies that have been selected on the basis of binding and neutralizing the activity of human RON. The antibodies contain immunoglobulin variable region CDR sequences that define a binding site for human RON.

In view of the neutralizing activity of these antibodies, they are useful for modulating the growth and/or proliferation of certain types of cancer cells. When used as a therapeutic agent, the antibodies can be engineered to minimize or eliminate an immune response when administered to a human patient. In some embodiments, the antibodies are fused or conjugated to other moieties, such as effector molecules (e.g., other proteins or small molecule therapeutics), a detectable label or a toxin moiety. Various features and aspects of the invention are discussed in more detail below.

As used herein, unless otherwise indicated, the term "antibody" means an intact antibody (e.g., an intact monoclonal antibody) or antigen-binding fragment of an antibody (e.g., an antigen-binding fragment of a monoclonal antibody), including an intact antibody or antigen-binding fragment that has been modified, engineered or chemically conjugated, or that is a human antibody. Examples of antibodies that have been modified or engineered are chimeric antibodies, humanized antibodies, and multispecific antibodies (e.g., bispecific antibodies). Examples of antigen-binding fragments include Fab, Fab', F(ab')2, Fv, single chain antibodies (e.g., scFv), minibodies and diabodies. An antibody conjugated to a toxin moiety is an example of a chemically conjugated antibody.

1. Antibodies That Bind RON

The antibodies disclosed herein comprise: (a) an immunoglobulin heavy chain variable region comprising the structure CDRH1-CDRH2-CDRH3 and (b) an immunoglobulin light chain variable region comprising the structure CDRL1-CDR L2-CDR L3, wherein the heavy...
chain variable region and the light chain variable region together define a single binding site for binding human RON protein.

[0039] In some embodiments, the antibody comprises: (a) an immunoglobulin heavy chain variable region comprising the structure CDR_{H1}-CDR_{H2}-CDR_{H3} and (b) an immunoglobulin light chain variable region, wherein the heavy chain variable region and the light chain variable region together define a single binding site for binding human RON. A CDR_{H1} comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 5 (07F01), SEQ ID NO: 51 (07F01), SEQ ID NO: 124 (Sh07F01 Hv3-48 D28T T60A L63V E65G), SEQ ID NO: 15 (12B11), SEQ ID NO: 53 (12B11), SEQ ID NO: 25 (17F06), SEQ ID NO: 55 (17F06), SEQ ID NO: 35 (18H09), SEQ ID NO: 57 (18H09), SEQ ID NO: 45 (29B06), SEQ ID NO: 59 (29B06), and SEQ ID NO: 126 (Sh29B06 Hv4-59, Hu29B06 Hv4-59 D27G T30S M48I I67V Y78F); a CDR_{H2} comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 6 (07F01), SEQ ID NO: 16 (12B11), SEQ ID NO: 26 (17F06), SEQ ID NO: 36 (18H09), SEQ ID NO: 46 (29B06), and SEQ ID NO: 122 (Sh07F01 Hv3-48 D28T T60A L63V E65G); and a CDR_{H3} comprises an amino acid sequence selected from the group consisting of SEQ ID NO:7 (07F01), SEQ ID NO: 17 (12B11), SEQ ID NO: 27 (17F06), SEQ ID NO: 37 (18H09), SEQ ID NO: 47 (29B06), and SEQ ID NO: 123 (Chimeric 07F01 C102S, Sh07F01 Hv3-48, Sh07F01 Hv3-48 D28T T60A L63V E65G). Throughout the specification a particular SEQ ID NO. is followed in parentheses by the antibody that was the origin of that sequence. For example, "SEQ ID NO: 5 (07F01)" means that SEQ ID NO: 5 comes from antibody 07F01.

[0040] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising the amino acid sequence of SEQ ID NO: 5 (07F01), SEQ ID NO: 51 (07F01), or SEQ ID NO: 124 (Sh07F01 Hv3-48 D28T T60A L63V E65G); a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 6 (07F01) or SEQ ID NO: 122 (Sh07F01 Hv3-48 D28T T60A L63V E65G), and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 7 (07F01) or SEQ ID NO: 123 (Chimeric 07F01 C102S, Sh07F01 Hv3-48, Sh07F01 Hv3-48 D28T T60A L63V E65G).

[0041] In some embodiments, the heavy chain variable region comprises a CDR_{H1} comprising the amino acid sequence of SEQ ID NO: 5 (07F01), a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 122 (Sh07F01 Hv3-48 D28T T60A L63V E65G), and a CDR_{H3}
comprising the amino acid sequence of SEQ ID NO: 123 (Chimeric 07F01 C102S, Sh07F01 Hv3-48, Sh07F01 Hv3-48 D28T T60A L63V E65G).

[0042] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDR H1 comprising the amino acid sequence of SEQ ID NO: 15 (12B11) or SEQ ID NO: 53 (12B11), a CDR H2 comprising the amino acid sequence of SEQ ID NO: 16 (12B11), and a CDR H3 comprising the amino acid sequence of SEQ ID NO: 17 (12B11).

[0043] In some embodiments, the heavy chain variable region comprises a CDR H1 comprising the amino acid sequence of SEQ ID NO: 25 (17F06) or SEQ ID NO: 55 (17F06), a CDR H2 comprising the amino acid sequence of SEQ ID NO: 26 (17F06), and a CDR H3 comprising the amino acid sequence of SEQ ID NO: 27 (17F06).

[0044] In some embodiments, the heavy chain variable region comprises a CDR H1 comprising the amino acid sequence of SEQ ID NO: 35 (18H09) or SEQ ID NO: 57 (18H09), a CDR H2 comprising the amino acid sequence of SEQ ID NO: 36 (18H09), and a CDR H3 comprising the amino acid sequence of SEQ ID NO: 37 (18H09).

[0045] In some embodiments, the heavy chain variable region comprises a CDR H1 comprising the amino acid sequence of SEQ ID NO: 45 (29B06), SEQ ID NO: 59 (29B06), or SEQ ID NO: 126 (Sh29B06 Hv4-59, Hu29B06 Hv4-59 D27G T30S M48I I67V Y78F), a CDR H2 comprising the amino acid sequence of SEQ ID NO: 46 (29B06), and a CDR H3 comprising the amino acid sequence of SEQ ID NO: 47 (29B06).

[0046] In some embodiments, the heavy chain variable region comprises a CDR H1 comprising the amino acid sequence of SEQ ID NO: 45 (29B06) or SEQ ID NO: 126 (Sh29B06 Hv4-59, Hu29B06 Hv4-59 D27G T30S M48I I67V Y78F), a CDR H2 comprising the amino acid sequence of SEQ ID NO: 46 (29B06), and a CDR H3 comprising the amino acid sequence of SEQ ID NO: 47 (29B06).

[0047] Preferably, the CDR H1, CDR H2, and CDR H3 sequences are interposed between human or humanized immunoglobulin FRs. The antibody can be an intact antibody or an antigen-binding antibody fragment.

[0048] In some embodiments, the antibody comprises (a) an immunoglobulin light chain variable region comprising the structure CDR L1-CDR L2-CDR L3, and (b) an immunoglobulin heavy chain variable region, wherein the IgG light chain variable region and the IgG heavy
chain variable region together define a single binding site for binding human RON. A CDR L 1 comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 8 (07F01), SEQ ID NO: 18 (12B11), SEQ ID NO: 28 (17F06), SEQ ID NO: 38 (18H09), SEQ ID NO: 48 (29B06), and SEQ ID NO: 130 (HE L 07F01 Kvl-9, Sh07F01 Kvl-9 Fl); a CDR L 2 comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 9 (07F01), SEQ ID NO: 19 (12B11), SEQ ID NO: 29 (17F06), SEQ ID NO: 39 (18H09), SEQ ID NO: 49 (29B06), and SEQ ID NO: 131 (HE L 07F01 Kvl-9, Sh07F01 Kvl-9 Fl); and a CDR L 3 comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 10 (07F01), SEQ ID NO: 20 (12B11), SEQ ID NO: 30 (17F06), SEQ ID NO: 40 (18H09), and SEQ ID NO: 50 (29B06).

[0049] In some embodiments, the antibody comprises an immunoglobulin light chain variable region comprising a CDR L 1 comprising the amino acid sequence of SEQ ID NO: 8 (07F01) or SEQ ID NO: 130 (HE L 07F01 Kvl-9, Sh07F01 Kvl-9 Fl), a CDR L 2 comprising the amino acid sequence of SEQ ID NO: 9 (07F01) or SEQ ID NO: 131 (HE L 07F01 Kvl-9, Sh07F01 Kvl-9 Fl), and a CDR L 3 comprising the amino acid sequence of SEQ ID NO: 10 (07F01).

[0050] In some embodiments, the antibody comprises an immunoglobulin light chain variable region comprising a CDR L 1 comprising the amino acid sequence of SEQ ID NO: 130 (HE L 07F01 Kvl-9, Sh07F01 Kvl-9 Fl); a CDR L 2 comprising the amino acid sequence of SEQ ID NO: 131 (HE L 07F01 Kvl-9, Sh07F01 Kvl-9 Fl); and a CDR L 3 comprising the amino acid sequence of SEQ ID NO: 10 (07F01).

[0051] In some embodiments, the antibody comprises an immunoglobulin light chain variable region comprising a CDR L 1 comprising the amino acid sequence of SEQ ID NO: 18 (12B11); a CDR L 2 comprising the amino acid sequence of SEQ ID NO: 19 (12B11); and a CDR L 3 comprising the amino acid sequence of SEQ ID NO: 20 (12B11).

[0052] In some embodiments, the antibody comprises an immunoglobulin light chain variable region comprising a CDR L 1 comprising the amino acid sequence of SEQ ID NO: 28 (17F06); a CDR L 2 comprising the amino acid sequence of SEQ ID NO: 29 (17F06); and a CDR L 3 comprising the amino acid sequence of SEQ ID NO: 30 (17F06).

[0053] In some embodiments, the antibody comprises an immunoglobulin light chain variable region comprising a CDR L 1 comprising the amino acid sequence of SEQ ID NO: 38
(18H09); a CDR \(_{L2}\) comprising the amino acid sequence of SEQ ID NO: 39 (18H09); and a CDR \(_{L3}\) comprising the amino acid sequence of SEQ ID NO: 40 (18H09).

[0054] In some embodiments, the antibody comprises an immunoglobulin light chain variable region comprising a CDR \(_{L1}\) comprising the amino acid sequence of SEQ ID NO: 48 (29B06); a CDR \(_{L2}\) comprising the amino acid sequence of SEQ ID NO: 49 (29B06); and a CDR \(_{L3}\) comprising the amino acid sequence of SEQ ID NO: 50 (29B06).

[0055] Preferably, the CDR \(_{L1}\), CDR \(_{L2}\), and CDR \(_{L3}\) sequences are interposed between human or humanized immunoglobulin FRs. The antibody can be an intact antibody or an antigen-binding antibody fragment.

[0056] In some embodiments, the antibody comprises: (a) an immunoglobulin heavy chain variable region comprising the structure CDR \(_{H1}\)-CDR \(_{H2}\)-CDR \(_{H3}\) and (b) an immunoglobulin light chain variable region comprising the structure CDR \(_{L1}\)-CDR \(_{L2}\)-CDR \(_{L3}\), wherein the heavy chain variable region and the light chain variable region together define a single binding site for binding human RON. The CDR \(_{H1}\) is an amino acid sequence selected from the group consisting of SEQ ID NO: 5 (07F01), SEQ ID NO: 51 (07F01), SEQ ID NO: 124 (Sh07F01 Hv3-48 D28T T60A L63V E65G), SEQ ID NO: 15 (12B11), SEQ ID NO: 53 (12B11), SEQ ID NO: 25 (17F06), SEQ ID NO: 55 (17F06), SEQ ID NO: 35 (18H09), SEQ ID NO: 57 (18H09), SEQ ID NO: 45 (29B06), SEQ ID NO: 59 (29B06), and SEQ ID NO: 126 (Sh29B06 Hv4-59, Hu29B06 Hv4-59 D27G T30S M48I I67V Y78F); the CDR \(_{H2}\) is an amino acid sequence selected from the group consisting SEQ ID NO: 6 (07F01), SEQ ID NO: 16 (12B11), SEQ ID NO: 26 (17F06), SEQ ID NO: 36 (18H09), SEQ ID NO: 46 (29B06), and SEQ ID NO: 122 (Sh07F01 Hv3-48 D28T T60A L63V E65G); and the CDR \(_{H3}\) is an amino acid sequence selected from the group consisting of SEQ ID NO: 7 (07F01), SEQ ID NO: 17 (12B11), SEQ ID NO: 27 (17F06), SEQ ID NO: 37 (18H09), SEQ ID NO: 47 (29B06), and SEQ ID NO: 123 (Chimeric 07F01 C102S, Sh07F01 Hv3-48, Sh07F01 Hv3-48 D28T T60A L63V E65G). The CDR \(_{L1}\) is an amino acid sequence selected from the group consisting of SEQ ID NO: 8 (07F01), SEQ ID NO: 18 (12B11), SEQ ID NO: 28 (17F06), SEQ ID NO: 38 (18H09), SEQ ID NO: 48 (29B06), and SEQ ID NO: 130 (HE L 07F01 Kvl-9, Sh07F01 Kvl-9 Fl); the CDR \(_{L2}\) is an amino acid sequence selected from the group consisting of SEQ ID NO: 9 (07F01), SEQ ID NO: 19 (12B11), SEQ ID NO: 29 (17F06), SEQ ID NO: 39 (18H09), SEQ ID NO: 49 (29B06), and SEQ ID NO: 131 (HE L 07F01 Kvl-9, Sh07F01 Kvl-9 Fl); and the CDR \(_{L3}\) is an amino acid sequence selected from the group consisting of SEQ ID NO: 10
(07F01), SEQ ID NO: 20 (12B11), SEQ ID NO: 30 (17F06), SEQ ID NO: 40 (18H09), and
SEQ ID NO: 50 (29B06).

[0057] The antibodies disclosed herein comprise an immunoglobulin heavy chain variable
region and an immunoglobulin light chain variable region. In some embodiments, the antibody
comprises an immunoglobulin heavy chain variable region selected from the group consisting
of SEQ ID NO: 2 (07F01), SEQ ID NO: 12 (12B11), SEQ ID NO: 22 (17F06), SEQ ID NO: 32
(18H09), SEQ ID NO: 42 (29B06), SEQ ID NO: 133 (Chimeric 07F01 C102S), SEQ ID NO:
135 (Sh07F01 Hv3-48), SEQ ID NO: 137 (Sh07F01 Hv3-48 D28T T60A L63V E65G), SEQ
ID NO: 143 (Sh29B06 Hv4-59), SEQ ID NO: 145 (Hu29B06 Hv4-59), and SEQ ID NO: 147
(Hu29B06 Hv4-59 D27G T30S M48I I67V Y78F), and an immunoglobulin light chain
variable region.

[0058] In other embodiments, the antibody comprises an immunoglobulin light chain
variable region selected from the group consisting of SEQ ID NO: 4 (07F01), SEQ ID NO: 14
(12B11), SEQ ID NO: 24 (17F06), SEQ ID NO: 34 (18H09), SEQ ID NO: 44 (29B06), SEQ
ID NO: 139 (HE L 07F01 Kvl-9), SEQ ID NO: 141 (Sh07F01 Kvl-9 Fl), and SEQ ID NO:
149 (Sh29B06 Kv2-28), and an immunoglobulin heavy chain variable region.

[0059] In some embodiments, the antibody comprises an immunoglobulin heavy chain
variable region selected from the group consisting of SEQ ID NO: 2 (07F01), SEQ ID NO: 12
(12B11), SEQ ID NO: 22 (17F06), SEQ ID NO: 32 (18H09), SEQ ID NO: 42 (29B06), SEQ
ID NO: 133 (Chimeric 07F01 C102S), SEQ ID NO: 135 (Sh07F01 Hv3-48), SEQ ID NO:
137 (Sh07F01 Hv3-48 D28T T60A L63V E65G), SEQ ID NO: 143 (Sh29B06 Hv4-59), SEQ
ID NO: 145 (Hu29B06 Hv4-59), and SEQ ID NO: 147 (Hu29B06 Hv4-59 D27G T30S M48I
I67V Y78F), and an immunoglobulin light chain variable region selected from the group
consisting of SEQ ID NO: 4 (07F01), SEQ ID NO: 14 (12B11), SEQ ID NO: 24 (17F06), SEQ
ID NO: 34 (18H09), SEQ ID NO: 44 (29B06), SEQ ID NO: 139 (HE L 07F01 Kvl-9), SEQ
ID NO: 141 (Sh07F01 Kvl-9 Fl), and SEQ ID NO: 149 (Sh29B06 Kv2-28).

[0060] In some embodiments, the antibody comprises an immunoglobulin heavy chain
variable region comprising the amino acid sequence of SEQ ID NO: 2 (07F01), and an
immunoglobulin light chain variable region comprising the amino acid sequence of
SEQ ID NO: 4 (07F01).
In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 12 (12B11), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 14 (12B11).

In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 22 (17F06), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 24 (17F06).

In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 32 (18H09), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 34 (18H09).

In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 42 (29B06), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 44 (29B06).

In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 137 (Sh07F01 Hv3-48 D28T T60A L63V E65G), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 139 (HE L 07F01 Kv1.9).

In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 147 (Hu29B06 Hv4-59 D27G T30S M48I I67V Y78F), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 149 (Sh29B06 Kv2-28).

In certain embodiments, the antibodies disclosed herein comprise an immunoglobulin heavy chain and an immunoglobulin light chain. In some embodiments, the antibody comprises an immunoglobulin heavy chain selected from the group consisting of SEQ ID NO: 93 (07F01), SEQ ID NO: 97 (12B11), SEQ ID NO: 101 (17F06), SEQ ID NO: 105 (18H09), SEQ ID NO: 109 (29B06), SEQ ID NO: 156 (Chimeric 07F01 C102S IgGl), SEQ ID NO: 160 (Chimeric 29B06 IgGl), SEQ ID NO: 164 (Sh07F01 Hv3-48 IgGl), SEQ ID NO: 166 (Sh07F01 Hv3-48 D28T T60A L63V E65G IgGl), SEQ ID NO: 172 (Sh29B06
Hv4-59 IgGl), SEQ ID NO: 174 (Hu29B06 Hv4-59 IgGl), and SEQ ID NO: 176 (Hu29B06 Hv4-59 D27G T30S M48I I67V Y78F IgGl), and an immunoglobulin light chain.

[0068] In other embodiments, the antibody comprises an immunoglobulin light chain selected from the group consisting of SEQ ID NO: 95 (07F01), SEQ ID NO: 99 (12B11), SEQ ID NO: 103 (17F06), SEQ ID NO: 107 (18H09), SEQ ID NO: 111 (29B06), SEQ ID NO: 158 (Chimeric 07F01 Kappa), SEQ ID NO: 162 (Chimeric 29B06 Kappa), SEQ ID NO: 168 (HE L 07F01 Kvl-9 Kappa), SEQ ID NO: 170 (Sh07F01 Kvl-9 Fl Kappa), and SEQ ID NO: 178 (Sh29B06 Kvl-9 Kappa), and an immunoglobulin heavy chain.

[0069] In some embodiments, the antibody comprises (i) an immunoglobulin heavy chain selected from the group consisting of SEQ ID NO: 93 (07F01), SEQ ID NO: 97 (12B11), SEQ ID NO: 101 (17F06), SEQ ID NO: 105 (18H09), SEQ ID NO: 109 (29B06), SEQ ID NO: 156 (Chimeric 07F01 C102S IgGl), SEQ ID NO: 160 (Chimeric 29B06 IgGl), SEQ ID NO: 164 (Sh07F01 Hv3-48 IgGl), SEQ ID NO: 166 (Sh07F01 Hv3-48 D28T T60A L63V E65G IgGl), SEQ ID NO: 172 (Sh29B06 Hv4-59 IgGl), and SEQ ID NO: 176 (Hu29B06 Hv4-59 D27G T30S M48I I67V Y78F IgGl), and (ii) an immunoglobulin light chain selected from the group consisting of SEQ ID NO: 95 (07F01), SEQ ID NO: 99 (12B11), SEQ ID NO: 103 (17F06), SEQ ID NO: 107 (18H09), SEQ ID NO: 111 (29B06), SEQ ID NO: 158 (Chimeric 07F01 Kappa), SEQ ID NO: 162 (Chimeric 29B06 Kappa), SEQ ID NO: 168 (HE L 07F01 Kvl-9 Kappa), SEQ ID NO: 170 (Sh07F01 Kvl-9 Fl Kappa), and SEQ ID NO: 178 (Sh29B06 Kvl-28 Kappa).

[0070] In some embodiments, the antibody comprises an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 93 (07F01), and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 95 (07F01).

[0071] In some embodiments, the antibody comprises an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 97 (12B11), and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 99 (12B11).

[0072] In some embodiments, the antibody comprises an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 101 (17F06), and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 103 (17F06).
In some embodiments, the antibody comprises an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 105 (18H09), and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 107 (18H09).

In some embodiments, the antibody comprises an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 109 (29B06), and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 111 (29B06).

In some embodiments, the antibody comprises an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 166 (Sh07F01 Hv3-48 D28T T60A L63V E65G IgGl), and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 168 (HE L 07F01 Kvl-9 Kappa).

In some embodiments, the antibody comprises an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 176 (Hu29B06 Hv4-59 D27G T30S M48I 167V Y78F IgGl), and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 178 (Sh29B06 Kv2-28 Kappa).

In certain embodiments, an isolated antibody that binds human RON comprises an immunoglobulin heavy chain variable region comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% identical to the entire variable region or the framework region sequence of SEQ ID NO: 2 (07F01), SEQ ID NO: 12 (12B11), SEQ ID NO: 22 (17F06), SEQ ID NO: 32 (18H09), SEQ ID NO: 42 (29B06), SEQ ID NO: 133 (Chimeric 07F01 C102S), SEQ ID NO: 135 (Sh07F01 Hv3-48), SEQ ID NO: 137 (Sh07F01 Hv3-48 D28T T60A L63V E65G), SEQ ID NO: 143 (Sh29B06 Hv4-59), SEQ ID NO: 145 (Hu29B06 Hv4-59), or SEQ ID NO: 147 (Hu29B06 Hv4-59 D27G T30S M48I 167V Y78F).

In certain embodiments, an isolated antibody that binds human RON comprises an immunoglobulin light chain variable region comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% identical to the entire variable region or the framework region sequence of SEQ ID NO: 4 (07F01), SEQ ID NO: 14 (12B11), SEQ ID NO: 24 (17F06), SEQ ID NO: 34 (18H09), SEQ ID NO: 44 (29B06), SEQ ID NO: 139 (HE L 07F01 Kvl-9), SEQ ID NO: 141 (Sh07F01 Kvl-9 Fl), or SEQ ID NO: 149 (Sh29B06 Kv2-28).

Homology or identity may be determined in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2,
ALIGN or Megalign (DNASTAR) software. BLAST (Basic Local Alignment Search Tool) analysis using the algorithm employed by the programs blastp, blastn, blastx, tblastn and tblastx (Karlin et al., 1990) PROC. NATL. ACAD. SCI. USA 87, 2264-2268; Altschul, (1993) J. MOL. EVOL. 36, 290-300; Altschul et al., (1997) NUCLEIC ACIDS RES. 25, 3389-3402, incorporated by reference) are tailored for sequence similarity searching. The approach used by the BLAST program is to first consider similar segments between a query sequence and a database sequence, then to evaluate the statistical significance of all matches that are identified and finally to summarize only those matches which satisfy a preselected threshold of significance. For a discussion of basic issues in similarity searching of sequence databases see Altschul et al., (1994) NATURE GENETICS 6, 119-129 which is fully incorporated by reference. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. The search parameters for histogram, descriptions, alignments, expect (i.e., the statistical significance threshold for reporting matches against database sequences), cutoff, matrix and filter are at the default settings. The default scoring matrix used by blastp, blastx, tblastn, and tblastx is the BLOSUM62 matrix (Henikoff et al., 1992) PROC. NATL. ACAD. SCI. USA 89, 10915-10919, fully incorporated by reference). Four blastn parameters may be adjusted as follows: Q=10 (gap creation penalty); R=10 (gap extension penalty); wink=1 (generates word hits at every wink.sup.th position along the query); and gapw=16 (sets the window width within which gapped alignments are generated). The equivalent Blastp parameter settings may be Q=9; R=2; wink=1; and gapw=32. Searches may also be conducted using the NCBI (National Center for Biotechnology Information) BLAST Advanced Option parameter (e.g.: -G, Cost to open gap [Integer]: default = 5 for nucleotides/ 11 for proteins; -E, Cost to extend gap [Integer]: default = 2 for nucleotides/ 1 for proteins; -q, Penalty for nucleotide mismatch [Integer]: default = -3; -r, reward for nucleotide match [Integer]: default = 1; -e, expect value [Real]: default = 10; -W, wordsize [Integer]: default = 11 for nucleotides/ 28 for megablast/ 3 for proteins; -y, Dropoff (X) for blast extensions in bits: default = 20 for blastn/ 7 for others; -X, X dropoff value for gapped alignment (in bits): default = 15 for all programs, not applicable to blastn; and -Z, final X dropoff value for gapped alignment (in bits): 50 for blastn, 25 for others). ClustalW for pairwise protein alignments may also be used (default parameters may include, e.g., Blosum62 matrix and Gap Opening Penalty = 10 and Gap Extension Penalty = 0.1). A Bestfit comparison between sequences, available in the GCG
package version 10.0, uses DNA parameters GAP=50 (gap creation penalty) and LEN=3 (gap extension penalty) and the equivalent settings in protein comparisons are GAP=8 and LEN=2.

[0080] In each of the foregoing embodiments, it is contemplated herein that immunoglobulin heavy chain variable region sequences and/or light chain variable region sequences that together bind human RON may contain amino acid alterations (e.g., at least 1, 2, 3, 4, 5, or 10 amino acid substitutions, deletions, or additions) in the framework regions of the heavy and/or light chain variable regions.

[0081] In certain embodiments, the antibody binds human RON with a $K_D$ of 1 nM, 900 pM, 750 pM, 650 pM, 600 pM, 500 pM, 400 pM, 300 pM, 250 pM, 200 pM, 150 pM, 100 pM, 50 pM or lower. Unless otherwise specified, $K_D$ values are determined by surface plasmon resonance methods under the conditions described in Examples 5 and 14.

[0082] Antibody Sh29B06-78 binds human RON with a $K_D$ of 500 pM, 250 pM, 200 pM, 150 pM, 100 pM or lower as measured by surface plasmon resonance methods under the conditions described in Examples 5 and 14. In an exemplary embodiment, antibody Sh29B06-78 binds human RON with a $K_D$ of 150 pM or lower as measured by surface plasmon resonance methods at 37°C under the conditions described in Examples 5 and 14.

[0083] Antibody Sh07F01-62 binds human RON with a $K_D$ of 500 pM, 400 pM, 350 pM, 300 pM, 250 pM, 200 pM, 150 pM, 100 pM or lower as measured by surface plasmon resonance methods under the conditions described in Examples 5 and 14. In an exemplary embodiment, antibody Sh07F01-62 binds human RON with a $K_D$ of 250 pM to 350 pM or lower as measured by surface plasmon resonance methods at 37°C under the conditions described in Examples 5 and 14.

[0084] In certain embodiments, the antibodies inhibit human MSP binding to human RON. For example, the antibodies can have an IC$_{50}$ (concentration at 50% of maximum inhibition) of about 5 nM, 2 nM, 1 nM or lower, when assayed using the protocol described in Examples 8 and 15.

[0085] Although the embodiments illustrated in the Examples comprise pairs of variable regions, pairs of full length antibody chains, or pairs of CDR1, CDR2 and CDR3 regions, one from a heavy chain and one from a light chain, a skilled artisan will recognize that alternative embodiments may comprise single heavy chain variable regions or single light chain variable regions, single full length antibody chains, or CDR1, CDR2 and CDR3 regions from one
antibody chain, either heavy or light. The single variable region, full length antibody chain or CDR1, CDR2 and CDR3 region of one chain can be used to screen for corresponding domains in another chain, the two chains capable of forming an antibody that binds antigen. The screening may be accomplished by phage display screening methods using, e.g., a hierarchical dual combinatorial approach disclosed in PCT Publ. No. WO92/01047. In this approach, an individual colony containing either a heavy or light chain clone is used to infect a complete library of clones encoding the other chain (light or heavy), and the resulting two-chain specific antigen-binding domain is selected in accordance with phage display techniques as described.

II. Production of Antibodies

Methods for producing antibodies, such as those disclosed herein, are known in the art. For example, DNA molecules encoding light chain variable regions and/or heavy chain variable regions can be chemically synthesized using the sequence information provided herein. 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 antibodies. 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 polymerase chain reaction (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.

Nucleic acids encoding desired antibodies can be incorporated (ligated) into expression vectors, which can be introduced into host cells through conventional transfection or transformation techniques. Exemplary host cells are E.coli cells, Chinese hamster ovary (CHO) cells, human embryonic kidney 293 (HEK 293) 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 IgG protein. Transformed host cells can be grown under conditions that permit the host cells to express the genes that encode the immunoglobulin light and/or heavy chain variable regions.

Specific expression and purification conditions will vary depending upon the expression system employed. For example, if a gene is to be expressed in E. coli, it is first cloned into an expression vector by positioning the engineered gene downstream from a
suitable bacterial promoter, e.g., Trp or Tac, and a prokaryotic signal sequence. The expressed secreted protein accumulates in refractile or inclusion bodies, and can be harvested after disruption of the cells by French press or sonication. The refractile bodies then are solubilized, and the proteins refolded and cleaved by methods known in the art.

If the engineered gene is to be expressed in eukaryotic host cells, e.g., CHO cells, it is first inserted into an expression vector containing a suitable eukaryotic promoter, a secretion signal, IgG enhancers, and various introns. This expression vector optionally contains 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 introduced into eukaryotic host cells using conventional techniques. The host cells express \( \mathbf{V}_L \) or \( \mathbf{V}_H \) fragments, \( \mathbf{VL-VH} \) heterodimers, \( \mathbf{VH-VL} \) or \( \mathbf{VL-VH} \) single chain polypeptides, complete heavy or light immunoglobulin chains, or portions thereof, each of which may be attached to a moiety having another function (e.g., cytotoxicity). In some embodiments, a host cell is transfected with a single vector expressing a polypeptide expressing an entire, or part of, a heavy chain (e.g., a heavy chain variable region) or a light chain (e.g., a light chain variable region). In other embodiments, a host cell is transfected with a single vector encoding (a) a polypeptide comprising a heavy chain variable region and a polypeptide comprising a light chain variable region, or (b) an entire immunoglobulin heavy chain and an entire immunoglobulin light chain. In still other embodiments, a host cell is co-transfected with more than one expression vector (e.g., one expression vector expressing a polypeptide comprising an entire, or part of, a heavy chain or heavy chain variable region, and another expression vector expressing a polypeptide comprising an entire, or part of, a light chain or light chain variable region).

A polypeptide comprising an immunoglobulin heavy chain variable region or light chain variable region can be produced by growing a host cell transfected with an expression vector encoding such variable region, under conditions that permit expression of the polypeptide. Following expression, the polypeptide can be harvested and purified using techniques well known in the art, e.g., affinity tags such as glutathione-S-transferase (GST) and histidine tags.

A monoclonal antibody that binds human RON, or an antigen-binding fragment of the antibody, can be produced by growing a host cell transfected with: (a) an expression vector that encodes a complete or partial immunoglobulin heavy chain, and a separate expression vector that encodes a complete or partial immunoglobulin light chain; or (b) a single expression
vector that encodes both chains (e.g., complete or partial heavy and light chains), under conditions that permit expression of both chains. The intact antibody (or antigen-binding fragment) can be harvested and purified using techniques well known in the art, e.g., Protein A, Protein G, affinity tags such as glutathione-S-transferase (GST) and histidine tags. It is within ordinary skill in the art to express the heavy chain and the light chain from a single expression vector or from two separate expression vectors.

III. Antibody Modifications

[0092] Methods for reducing or eliminating the antigenicity of antibodies and antibody fragments are known in the art. When the antibodies are to be administered to a human, the antibodies preferably are "humanized" to reduce or eliminate antigenicity in humans. Preferably, the humanized antibodies have the same or substantially the same affinity for the antigen as the non-humanized mouse antibody from which it was derived.

[0093] In one humanization approach, chimeric proteins are created in which mouse immunoglobulin constant regions are replaced with human immunoglobulin constant regions. See, e.g., Morrison et al., 1984, PROC. NAT. ACAD. SCI. 81:685 1-6855, Neuberger et al., 1984, NATURE 312:604-608; U.S. Patent Nos. 6,893,625 (Robinson); 5,500,362 (Robinson); and 4,8 16,567 (Cabilly).

[0094] In an approach known as CDR grafting, the CDRs of the light and heavy chain variable regions are grafted into frameworks from another species. For example, murine CDRs can be grafted into human FRs. In some embodiments, the CDRs of the light and heavy chain variable regions of an anti-RON antibody are grafted into human FRs or consensus human FRs. 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 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; Rieckmann et al. (1988) NATURE 332: 323-327; Verhoeven et al. (1988) SCIENCE 239: 1534- 1536; and Winter (1998) FEBS LETT 430: 92-94.

[0095] In an approach called "SUPERHUMANIZATION™," human CDR sequences are chosen from human germline genes, based on the structural similarity of the human CDRs to
those of the mouse antibody to be humanized. See, e.g., U.S. Patent No. 6,881,557 (Foote); and Tan et al, 2002, J. IMMUNOL. 169:1119-1125.

[0096] Other methods to reduce immunogenicity include "reshaping," "hyperchimerization," and "veneering/resurfacing." 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, e.g., in U.S. Patent No. 5,639,641 (Pedersen).

[0097] Another 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 IgG 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).

[0098] Another 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.

[0099] Another approach for modifying a mouse antibody into a form suitable for medical use in humans is HUMAN ENGINEERING™ technology, which is practiced commercially by XOMA (US) LLC. See, e.g., PCT Publication No. WO 93/11794 and U.S. Patent Nos. 5,766,886 (Studnicka); 5,770,196 (Studnicka); 5,821,123 (Studnicka); and 5,869,619 (Studnicka).

[0100] Any suitable approach, including any of the above approaches, can be used to reduce or eliminate human immunogenicity of an antibody.

[0101] In addition, it is possible to create fully human antibodies in mice. Fully human mAbs lacking any non-human sequences can be prepared from human immunoglobulin transgenic mice by techniques referenced in, e.g., Lonberg et al., NATURE 368:856-859, 1994; Fishwild et al., NATURE BIOTECHNOLOGY 14:845-851, 1996; and Mendez et al, NATURE GENETICS 15:146-156, 1997. Human mAbs can also be prepared and optimized from phage

[0102] If the antibody is for use as a therapeutic, it can be conjugated to an effector agent such as a small molecule toxin or a radionuclide using standard in vitro conjugation chemistries. If the effector agent is a polypeptide, the antibody can be chemically conjugated to the effector or joined to the effector as a fusion protein. Construction of fusion proteins is within ordinary skill in the art.

IV. Use of Antibodies

[0103] The antibodies disclosed herein can be used to treat various forms of cancer, e.g., non-small cell lung cancer, breast, ovarian, prostate, cervical, colorectal, lung, pancreatic, gastric, and head and neck cancers. The cancer cells are exposed to a therapeutically effective amount of the antibody so as to inhibit or reduce proliferation of the cancer cell. In some embodiments, the antibodies inhibit cancer cell proliferation by at least 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or 100%.

[0104] In some embodiments, the antibody (e.g., 07F01, 29B06, 17F06, 18H09, 12B11, sh29B06, sh07F01) inhibits or reduces proliferation of a tumor cell by inhibiting binding of human RON to its ligand, MSP. In some embodiments, the antibody (e.g., 07F01, 29B06, 17F06, 18H09, 12B11, sh29B06, sh07F01) inhibits or reduces proliferation of a tumor cell without inhibiting RON binding to MSP. The antibody (e.g., 07F01, 29B06, 17F06, 18H09, 12B11, sh29B06, sh07F01) can also be used in therapy. The antibody (e.g., 07F01, 29B06, 17F06, 18H09, 12B11, sh29B06, sh07F01) can be used to inhibit tumor growth in a mammal (e.g., a human patient). In some embodiments, use of the antibody to inhibit tumor growth in a mammal comprises administering to the mammal a therapeutically effective amount of the antibody.

[0105] In certain embodiments, antibody Sh29B06-78 is used in therapy. For example, antibody Sh29B06-78 can be used for inhibiting or reducing proliferation of a tumor cell. Antibody Sh29B06-78 can also be used for inhibiting or reducing tumor growth in a mammal.

[0106] In other embodiments, antibody Sh07F01-62 is used in therapy. For example, antibody Sh07F01-62 can be used for inhibiting or reducing proliferation of a tumor cell. Antibody Sh07F01-62 can also be used for inhibiting or reducing tumor growth in a mammal.
Cancers associated with overexpression or inappropriate activation of RON include non-small cell lung cancer, breast cancer, ovarian cancer, prostate cancer, lung cancer, colorectal cancer, pancreatic cancer, bladder cancer, and some forms of brain cancer, melanomas, and gastrointestinal cancers.

As used herein, "treat," "treating" and "treatment" mean the treatment of a disease in a mammal, e.g., in a human. This includes: (a) inhibiting the disease, i.e., arresting its development; and (b) relieving the disease, i.e., causing regression of the disease state.

Generally, a therapeutically effective amount of active component is in the range of 0.1 mg/kg to 100 mg/kg, e.g., 1 mg/kg to 100 mg/kg, 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 of the patient, the in vivo potency of the antibody, the pharmaceutical formulation, and the route of administration. The initial dosage can be increased beyond the upper level in order to rapidly achieve the desired blood-level or tissue level. Alternatively, the initial dosage can be smaller than the optimum, and the dosage may be progressively increased during the course of treatment. 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 being treated. Exemplary dosing frequencies are once per day, once per week and once every two weeks. In some embodiments, dosing is 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, the antibody is lyophilized and reconstituted in buffered saline at the time of administration.

For therapeutic use, an antibody preferably is combined with a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" means buffers, carriers, and excipients 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 include buffers, solvents, dispersion media, coatings, isotonic and absorption delaying agents, and the like, that are compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is known in the art.
Pharmaceutical compositions containing antibodies, such as those disclosed herein, can be presented in a dosage unit form and can be prepared by any suitable method. A pharmaceutical composition should be formulated to be compatible with its intended route of administration. Examples of routes of administration are intravenous (IV), intradermal, inhalation, transdermal, topical, transmucosal, and rectal administration. A preferred route of administration for monoclonal antibodies is IV infusion. Useful formulations can be prepared by methods well known in the pharmaceutical art. For example, see Remington's Pharmaceutical Sciences, 18th ed. (Mack Publishing Company, 1990). Formulation components suitable for parenteral administration include 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 paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as EDTA; buffers such as acetates, citrates or phosphates; and agents for the adjustment of tonicity such as sodium chloride or dextrose.

For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor ELTM (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). The carrier should be stable under the conditions of manufacture and storage, and should be preserved against microorganisms. 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.

Pharmaceutical formulations preferably are sterile. Sterilization can be accomplished, for example, by filtration through sterile filtration membranes. Where the composition is lyophilized, filter sterilization can be conducted prior to or following lyophilization and reconstitution.

EXAMPLES

The following Examples are merely illustrative and are not intended to limit the scope or content of the invention in any way.

Example 1: Production of Human RON Extracellular Domain (ECD)

This Example describes the production of the antigen, hRON ECD protein. The use of the full length ECD as the immunogen, allowed for the selection of two classes of hybridomas: (a) those producing antibodies that interact with the ligand binding domain,
thereby inhibiting contact of the ligand to the receptor; and (b) those producing antibodies that
bind outside the ligand binding domain, thereby inhibiting the receptor functions through
mechanisms other than inhibition of ligand binding.

[0116] DNA encoding the extracellular domain of human RON (hRON ECD) (ref seq.
NM_002447) was amplified by PCR and subcloned using the Xmal/EcoRI restriction sites in-
frame into the pEE14.4 vector (Lonza, Basel, Switzerland) containing THXmFC
(Thrombin/His tag/ Factor Xa- AJ mouse IgG-Fc), to produce a fusion protein. The resulting
cloned was linearized using the Pvul enzyme (NEBiolabs, Cat. No. R0150), then electroporated
into CHO KISVcells (Lonza). The electroporated cells were diluted in 200 ml CD CHO media
(Gibco Cat. No. 10743-01 1). The next day, CD CHO media containing methionine sulfoximine
(MSX) for a final concentration of 50μM was added to the cells. After four weeks, positive
clones were selected by sandwich ELISA in which the immobilized antibody was commercial
monoclonal anti-hRON antibody MAB691 (R&D Systems), and the detection antibody was
commercial polyclonal anti-hRON antibody AF691 (R&D Systems). Positive clones were re-
transfected using Lipofectamine™ 2000 in a standard protocol. Cells were aliquoted into four
separate shaker flasks and selected using 50 uM, 100 uM, 200 uM, and 400 uM MSX. After
two weeks of selection, the individual flasks were checked for hRON-ECD protein expression
by ELISA. The highest selection pressure, 400 μM MSX, yielded good protein expression and
was chosen for scale-up and purification. Cells were grown for 2 weeks at 37°C in BelloCell
Bottles (Bellco Glass, Vineland, NJ) at a concentration of 2-2.5 xlO⁶ cells/ml in CD CHO
media, with a final concentration of 80 μM MSX for protein production. The resulting cells
were spun down in 500 ml conical tubes for 15 minutes. The supernatant was filtered using
vacuum filtration using a 0.45 micron filter and then a 0.22 micron filter. The protein was then
batch bound to ProSepA beads (Millipore) at 4°C overnight with rotation after adjusting the pH
to 7.5. The beads were washed with IX PBS and loaded onto disposable protein A affinity
columns (Bio-Rad Econo-Pac columns; Bio-Rad cat. No. 732-1010). The beads were washed
with 10 column volumes (CV) of glycine binding buffer (3M glycine ph 9.0, 1M NaCl). The
protein was then eluted off the column using 5-10 CV of 200 mM glycine ph 2.5 acid elution
buffer. The samples were then neutralized using 1.3 mL of 1.0 M Tris pH 8.0 neutralization
buffer concentrated using Vivaspin concentrators (Sartorius Stedim Biotech).
Example 2: Anti-RON Antibodies

This Example describes the production of anti-hRON monoclonal antibodies. Immunizations, fusions, and primary screens were conducted at Maine Biotechnology Services Inc. (Portland, ME), following the Repetitive Immunization Multiple Sites (RIMMS) protocol. Five AJ mice and five Balb/c mice were immunized with recombinant human RON extracellular domain (hRON-ECD). Two Balb/c mice with sera displaying the highest anti-RON activity by Enzyme Linked Immunosorbent Assay (ELISA) were chosen for subsequent fusion. Spleens and lymph nodes from the appropriate mice were harvested. B-cells were harvested and fused with a myeloma line. Fusion products were serially diluted onto forty 96-well plates to near clonality.

Approximately 4,000 supernatants from the cell fusions were screened by ELISA for binding to recombinant hRON-ECD. A total of 158 supernatants containing antibodies against RON were further characterized by in vitro biochemical and cell-based assays, as described below. A panel of hybridomas was selected, subcloned and expanded. Hybridoma cell lines were transferred to BioXCell (West Lebanon, NH) for antibody expression and purification by affinity chromatography on Protein G resin, under standard conditions.

Example 3: Screening Assays

A biochemical assay was carried out to identify antibodies that inhibit ligand binding. A cell-based assay was carried out to identify antibodies that inhibit MSP induced phosphoERK downstream signaling of the receptor. Antibodies that inhibited RON mediated cellular signaling were selected for further characterization regardless of whether they blocked ligand binding in the neutralization assay.

The biochemical neutralization assay measures inhibition of MSP binding to hRON by antibodies in hybridoma supernatants, using electrochemiluminescence (ECL). MA2400 96-well high binding plates (Meso Scale Discovery) were coated with 25 μg of 0.42 μg/mL hRON SEMA + PSI (an N-terminal portion of the ECD of hRON; R&D Systems) in PBS for one hour at room temperature with agitation. The plates were washed four times with PBS + 0.1% Tween-20 (PBST), and blocked with 150 μg of charcoal-stripped fetal bovine serum (FBS) (Gibco). The hybridoma supernatant were added and incubated for 45 minutes at room temperature. After incubation, 5 μg of MSP (3 μg/mL) in charcoal stripped FBS was added to each well, and incubated for 45 minutes. The plate was washed four times with PBST, and 25
µl of 1 µg/mL biotinylated anti-MSP antibody (R&D Systems) was added to the plates for one hour at room temperature with agitation. The plates were washed four times with PBST, and incubated with 25 µl of 1 µg/mL ST-streptavidin (Meso Scale Discovery) for one hour at room temperature with agitation. The plates were washed four times with PBST, and 150 µl read buffer (Meso Scale Discovery) was added to each well before the plates were analyzed on a Sector Imager 2400 (Meso Scale Discovery) instrument. Antibodies 07F01, 18H09 and 29B06 each blocked MSP binding to hRON SEMA + PSI in this neutralization assay.

[0121] In the cell-based assay, antibodies in the hybridoma supernatant were tested for inhibition of MSP-induced phosphorylation of ERK, which is a RON downstream signaling molecule. T47D cells were cultured in 96-well plates in RPMI 1640 + 10% FBS + insulin. Medium was removed, and cells were incubated in serum-free medium for 24 hours. Hybridoma supernatants containing RON antibodies were added to the cells at a dilution of 1:4 in -serum-free medium, and incubated for one hour at 37°C. MSP (5 nM) was added to the wells and incubated for 15 minutes. Medium was removed, and cells were fixed in 4% paraformaldehyde (PFA) in PBS. Total ERK and phospho-ERK were measured according to the vendor's instructions (R&D Systems, DY1018). Antibodies 07F01, 12B11, 17F06, 18H09 and 29B06 each inhibited MSP induced ERK phosphorylation in T47D cells.

[0122] As discussed herein (see Examples 8 and 9), antibodies 07F01, 12B11, 17F06, 18H09 and 29B06 each inhibited MSP induced ERK phosphorylation in T47D cells, while only antibodies 07F01, 18H09 and 29B06 each blocked MSP binding to hRON SEMA + PSI in the neutralization assay. This suggests that antibodies 12B11 and 17F06 do not neutralize binding of MSP to the hRON SEMA+PSI domain, neutralize binding of MSP to RON in the context of the full RON extracellular domain, or function by a mechanism other than blocking MSP binding to RON.

Example 4: Antibody Sequence Analysis

[0123] The light chain isotype and heavy chain isotype of each monoclonal antibody in Example 2 was determined using the IsoStrip™ Mouse Monoclonal Antibody Isotyping Kit according the kit vendor's instructions (Roche Applied Science, Indianapolis, IN). All antibodies were found to be kappa or lambda light chain and IgG1 or IgG2a heavy chain.

[0124] The heavy and light chain variable regions of the mouse monoclonal antibodies were sequenced using 5' RACE (Rapid Amplification of cDNA Ends). Total RNA was
extracted from each monoclonal hybridoma cell line using the RNeasy® Miniprep kit according to the kit vendor's instructions (Qiagen, Valencia, CA). Full-length first strand cDNA containing 5' ends was generated using either the GeneRacer™ Kit (Invitrogen, Carlsbad, California) or SMARTer™ RACE cDNA Amplification Kit (Clontech, Mountain View, CA) according to the kit vendor's instructions using random primers for 5' RACE.

The variable regions of the light (kappa or lambda) and heavy (IgGlor IgG2b) chains were amplified by PCR, using KOD Hot Start Polymerase (EMD Chemicals, Gibbstown, NJ), Expand High Fidelity PCR System (Roche Applied Science), or Advantage 2 Polymerase Mix (Clontech) according to the kit vendor's instructions. For amplification of 5' cDNA ends in conjunction with the GeneRacer™ Kit, the GeneRacer™ 5' Primer, 5' cgactggacagcaggacatga 3' (SEQ ID NO: 112) (Invitrogen) was used as a 5' primer. For amplification of 5' cDNA ends in conjunction with the SMARTer™ RACE cDNA Amplification Kit, the Universal Primer Mix A primer (Clontech), a mix of:

5' CTAAATACGACTCACTATAGGGCAAGGACAGCTATGAAGCTCTTGACAAT 3' (SEQ ID NO: 113) and
5' CTAAATACGACTCACTATAGGGCACGGGACAAACTCTTCTC 3' (SEQ ID NO: 114), was used as a 5' primer. Heavy chain variable regions were amplified using the above 5' primers and a 3' IgGl constant region specific primer, 5' TATGCAAGGCTTACAAACCACA 3' (SEQ ID NO: 115), or a 3' IgG2a constant region specific primer, 5' AGGACAGGGCTTGAAGCTCTTCTCGGAAGGACAGCTATGACC 3' (SEQ ID NO: 116). Kappa chain variable regions were amplified with the above 5' primers and a 3' kappa constant region specific primer, 5' TCTATCCTCTGGAAGCTCTTCTCGGAAGGACAGCTATGACC 3' (SEQ ID NO: 117). Lambda chain variable regions were amplified with the above 5' primers and a mix of 3' lambda constant region specific primers, 5' GCAAGGGACAACTCTTCTCAGGAGCGACAGCTATGACC 3' (SEQ ID NO: 118) and 5' CACATGTCCCCTCATGTG 3' (SEQ ID NO: 119).

Individual PCR products were isolated by agarose gel electrophoresis and purified using the Qiaquick® Gel Purification kit according to the kit vendor's instructions (Qiagen). The PCR products were subsequently cloned into the pCR®4Blunt plasmid or pCR2.1®TOPO plasmid using the Zero Blunt® TOPO® PCR Cloning Kit or the TOPO® TA Cloning Kit, respectively, according to the kit vendor's instructions (Invitrogen) and transformed into DH5-α bacteria (Invitrogen) through standard molecular biology techniques. Plasmid DNA isolated from transformed bacterial clones was sequenced using M13 Forward (5' GTAAAAACGACGGCCAGT 3') (SEQ ID NO: 120) and M13 Reverse primers (5' CAGGAAACAGCTATGACC 3') (SEQ ID NO: 121) by Beckman Genomics (Danvers, MA),
using standard dideoxy DNA sequencing methods to identify the sequence of the variable region sequences. The sequences were analyzed using Vector NTI software (Invitrogen) and the IMGT/V-Quest web server (imgt.cines.fr) to identify and confirm variable region sequences.

[0127] The nucleic acid sequences encoding and the protein sequences defining variable regions of the murine monoclonal antibodies are shown below (amino terminal signal peptide sequences are not shown). CDR sequences (Kabat definition) are indicated by bold font and underlining in the amino acid sequences.

[0128]  

**Nucleic Acid Sequence Encoding the Heavy Chain Variable Region of the 07F01 Antibody (SEQ ID NO: 1)**

1 gagggtgaagc ttctcgtgac tggaggtggc cgggtgattc cctgaacctc
tctctgctg acctcaggtt caattttagt agacactgga tgagttgggt ccggctgtgc
caggaggaag ggtcagaatt gatgcagaa attaatccag atagcagaac gataaactat
cagccatctc taagagcagaa attcatcact tccagagaca acgccaaaaa tccgcttca
tctcaaatga acagagtgaa atctgaggac acagcccttt attactgtgc aagacgggta
taggtgaagt tcctgtgcag ccctcaggat cgaatttggt cgagttgggt ctcaaatga

**Protein Sequence Defining the Heavy Chain Variable Region of the 07F01 Antibody (SEQ ID NO: 2)**

1 evkllesggg lvqpggsllk slaasgfdffs rhmswvrla pgkglewiae inpdartiny
tpske_kfi srdnaknlf lqmnrvrsed talyyccary rihyqamdo wqqgtsvtvs

**Nucleic Acid Sequence Encoding the Kappa Chain Variable Region of the 07F01 Antibody (SEQ ID NO: 3)**

1 gacattgtgt tgacccagtc tcaaaaaatc gtctccacat cagttgagac cagggtcagc
tgctcctgca aggecagtea gaattgggt tgttagtttag ttctgattca acagaaacca
tctcaatcct ctaaaactc gatttacg gcatcttcct cgtacagtt gatccctgat

cagcctcag gcagttgagc tcggagctgt tcacctcctc ccatcagcagcatc tggcagtcg
tctcaatcct ctaaaactc gatttacg gcatcttcct cgtacagtt gatccctgat
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gaattgggt
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gacattgtgt
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tgctcctgca
aggecagtea
gaattgggt

ttctgattca
acagaaacca
gacattgtgt
tgacccagtc
tcaaaaaatc
gtctccacat
cagttgagac
cagggtcagc
tgctcctgca
aggecagtea

**Protein Sequence Defining the Kappa Chain Variable Region of the 07F01 Antibody (SEQ ID NO: 4)**

1 divltqsgki vstsvgarvs vtckasqgvg ssluvwyqqkp gqspktliys asf ry sqvpd

tltisvnqs edladycgjg vmpygt_fga gtklelk

35
Nucleic Acid Sequence Encoding the Heavy Chain Variable Region of the 12B11 Antibody (SEQ ID NO: 11)

```
1  gagggtcagt  tagtggaatc  tggggaaggc  ttagtgaagc  cttgagggtc  cctgaactc
5   61  tccctgtcag  ccctcctgatt  aaccttcctg  acctatgcac  ttgctttggat  tgcocagact
12  cccgagaaga  ggcctggagtg  ggtcgcagga  atccactaag  tggtagattt  ccactactat
18  cccagacttg  tgggggaggc  ttagtgaagc  ctggagggtc  cctgaaactc  cctctcactac
24  ctggctggat  ttgggcatgt  ttcctgaaat  ttcctgaaat  cccagacttg  tgggggaggc
30  tactatggtg  ttaactttga  caccagagact  cagggccatgt  attgacttcg  aagagcaggtt
```

Protein Sequence Defining the Heavy Chain Variable Region of the 12B11 Antibody (SEQ ID NO: 12)

```
1  evqlvesggg  lvkpggslkl  scaaasgfifs  tyamswrqg  pekrl ewvag  ittn gsf  tyy
6   61  pdtvkg  rfti  srndarnily  lqmsglrsed  tamyy carcjg  yyynvfy dyw  gtttltvss
```

Nucleic Acid Sequence Encoding the Kappa Chain Variable Region of the 12B11 Antibody (SEQ ID NO: 13)

```
1  gaggtgctgta  tgaccacaacat  tccacttctcc  tctgcttgatc  gttcttggaga  tcaagactcc
5   61  atctttgca  ggtcttgatc  gaccccaagt  aacagtaacg  gaaaccattc  tccactactac
12  taccctcaaga  aaccaggccc  gtctccacag  ctccctgatct  acagggtttc  caaccgattt
18  tctggtacct  cacagacgtt  cagctgttacg  ggtacgagga  cagatttcac  acatgactcc
24  atcaagatgtg  aggctgagga  tttgggactt  tatttctgcc  tccaagttac  acatgtcccg
30  cacagcttccg  gaggggggac  caaactggaa  ttaa
```

Protein Sequence Defining the Kappa Chain Variable Region of the 12B11 Antibody (SEQ ID NO: 14)

```
1  davmtqtpls  lpvslgdqas  iscrsgqale  nesngtynlw  ylgkgpqgsq  lliyrvsnrf
6   61  sgypdrfsqs  gsgtdf tiki  irvea edigi  yfelqvtthvp  htfgggtkisel  lk
```

Nucleic Acid Sequence Encoding the Heavy Chain Variable Region of the 17F06 Antibody (SEQ ID NO: 21)

```
1  gaagtgactgc  tgggtgagtc  ggggggaggc  ttagtgaagc  cttgagggtc  tctgactactc
5   61  tctgtgcacg  ccctcctgatt  aaccttcctg  acctatgcac  ttgctttggatt  tgcocagact
12  ccagacaaga  ggtctggtgtg  ggtcgcagga  atccactaag  tggtagattt  ccactactat
18  ctgcacactg  taaaggcccc  attctcctcc  tccactgtgc  ttcctgaaat  ttcctgaaat
24  ctgcacacagt  ggtgctggaa  gttcaggaac  acggccctttg  attactttacg  aagagcaggtt
30  tggctgacacg  ctggggccct  gggactctgg  tcctcttacctg  tcagactactc  tgcactctgt
```

Protein Sequence Defining the Heavy Chain Variable Region of the 17F06 Antibody (SEQ ID NO: 22)

```
1  evklvesggg  lvkpggslkl  scaaasgfifs  sygmswvqrgt  sdkrl ewvas  issgggttyy
6   61  ldvtkg  rfti  srnaka dtly  lqmsgikased  talyy ctre_c1  whikfawyg  gttlvtsa
```
Nucleic Acid Sequence Encoding the Lambda Chain Variable Region of the 17F06 Antibody (SEQ ID NO: 23)

1 caacttggtgc tcactcagtc atttctcagcc tctttctccc tgggagcctc agcaaaactc
5 61 ac tgtgcaccc ttgagtagtca gcacactagc tacaccattg aatgtgacta gcaagtgcga
121 ctcagctcct caaatgtggtg agctggagctt aagagaatg aagccacacg cacagggttt
181 ggttggttctt ctgaagttggc cgggacttagc ctcctttggc ctggtgactt atccagactc
taccatttcc
241 aacacttacg ctcagagctc acagataatt aatctgtggtg tgggtgagac aatggagac
301 caattttcgg cgggacccacg aagtgcactc ttctctcagc

Protein Sequence Defining the Lambda Chain Variable Region of the 17F06 Antibody (SEQ ID NO: 24)

1 qlvltqsssa sfslgasaklt ctyvtgdsit sdywnwirkf pgnkleymgys isysgstyn
6 1 gipdrfsgss sgadryltis niqpedealy icvgvetied qfvyv fggt ltvsa

Nucleic Acid Sequence Encoding the Heavy Chain Variable Region of the 18H09 Antibody (SEQ ID NO: 31)

1 gaggtgcagc ttcaggagtc aggacactagc ctcgtgaac a ctcctcagac ttctgccctc
tgactcagga atctgcactc accacatcac ctcgtgaac aagttgggat ccggaaattc
tcaatcctca c caggaataa aaccttgatga catgggatat atcagactc atctgactac ttaatttccat
121 cctgccagat tctcaggctc cctgattgga gacaaggctg ccctccacct cacaggggca
181 cgggtggttctt ctgaagttggc cgggacttagc ctcctttggc ctggtgactt atccagactc
taccatttcc
241 aacacttacg ctcagagctc acagataatt aatctgtggtg tgggtgagac aatggagac
301 caattttcgg cgggacccacg aagtgcactc ttctctcagc

Protein Sequence Defining the Heavy Chain Variable Region of the 18H09 Antibody (SEQ ID NO: 32)

1 evqlqesgps lvkpsqtls1 tcyvtgdsit sdyywnwirkf pgnkleymgys isysgstyn
6 1 parfsgslig dkaaltitga qtedeaiyfc alwysnhywv fgggtkltv 1

Nucleic Acid Sequence Encoding the Lambda Chain Variable Region of the 18H09 Antibody (SEQ ID NO: 33)

1 caggctgttg tgcactcagc atctgcactc accacatcac ctcgtgaac aagttgggat ccggaaattc
tgactcagga atctgcactc accacatcac ctcgtgaac aagttgggat ccggaaattc
tcaatcctca c caggaataa aaccttgatga catgggatat atcagactc atctgactac ttaatttccat
121 cctgccagat tctcaggctc cctgattgga gacaaggctg ccctccacct cacaggggca
181 cgggtggttctt ctgaagttggc cgggacttagc ctcctttggc ctggtgactt atccagactc
taccatttcc
241 aacacttacg ctcagagctc acagataatt aatctgtggtg tgggtgagac aatggagac
301 caattttcgg cgggacccacg aagtgcactc ttctctcagc

Protein Sequence Defining the Lambda Chain Variable Region of the 18H09 Antibody (SEQ ID NO: 34)

1 qavvtqesal ttspgetvt1 tcrtssagavt tsnf anwvqe kpdhlftgli gdtninrpgv
6 1 parfsgslig dkaaltitga qtedeaiyfc alwysnhywv fgggtkltv 1
Nucleic Acid Sequence Encoding the Heavy Chain Variable Region of the 29B06 Antibody (SEQ ID NO: 41)

1 gaggtgcacg ttcaaggagtc aggaacctagc ctcgtgaaac cttctcagac tctgtccttc
d1 acctgtttctg tcactggcga ctctcatcacc agtggttact ggaactggat cccgaaattc
d21 ccagggqaata aaccttgagta catgggtgac ataaagctaca ggtgtaaaac ttactacaat
d181 ccatctctcga aaagtgcgaat ctctcatcact cggagacacat ccaagaacca ccaactcctg
d241 ccagggcata acctgttctg tcactggtcga ctccatcacc ggcacacatt actgctcagag gtctaatc
d301 gactatgctta tggactactg gggtcaagga acctcagtca ccgtctcctc

Protein Sequence Defining the Heavy Chain Variable Region of the 29B06 Antibody (SEQ ID NO: 42)

1 evqlqesgps lvkpsqlslc tcsvtgdsit stgwnwirkf pgkleyymgy isysgktyyn
6 pslksrit sit rdtksknhyl qlisvtaedt atyycar sky dyamdywggg tsvtvss

Nucleic Acid Sequence Encoding the Kappa Chain Variable Region of the 29B06 Antibody (SEQ ID NO: 43)

1 gacattgtgc tgacccaatc tccagcttct ttggctgtgt cctctaggaca gagggccacc
d1 atctcctgca gagccagcga aattgttgat aattttggca ttagttttat gaactggttc
d121 caacagaaac caggacagcc accccaaactc ctcatctatg ctcgcatcgg ccaaggaccc
d181 gggggttctg ggggtctgtg gccagtttag tggcagtggg tctgggacag acttcagcct ccaacatccat
d241 cgctgtggagg aggatgatac tgcaatgtat ttctgtcagc aaagtaagga ggttcctccg
d301 acgttcggtg gaggacccaa gctggaaatc

Protein Sequence Defining the Kappa Chain Variable Region of the 29B06 Antibody (SEQ ID NO: 44)

1 divltqspas lavslqrat i scraseivd nggisfmnnw qqkpqppk1 liyaasnpqg
6 gvpqrsqsg i sgtdfslnih pveddttamy fppqqskvepp tfggtclkeli k

The amino acid sequences defining the immunoglobulin heavy chain variable regions for the antibodies produced in Example 2 are aligned in FIG. 2. Amino terminal signal peptide sequences (for expression/secretion) are not shown. CDR₁, CDR₂, and CDR₃ (Kabat definition) are identified by boxes. FIG. 3 shows an alignment of the separate CDR₁, CDR₂, and CDR₃ sequences for each antibody.

The amino acid sequences defining the immunoglobulin light chain variable regions of the antibodies in Example 2 are aligned in FIG. 4. Amino terminal signal peptide sequences (for expression/secretion) are not shown. CDR₁, CDR₂ and CDR₃ are identified by boxes. FIG. 5 shows an alignment of the separate CDR₁, CDR₂, and CDR₃ sequences for each antibody.

Table 1 shows the SEQ ID NO. of each sequence discussed in this Example.
### Table 1

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<th>S.I.Q.ID NO.</th>
<th>Nucleic Acid or Protein</th>
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<tbody>
<tr>
<td>1</td>
<td>07FC1 Heavy Chain Variable Region — nucleic acid</td>
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Mouse monoclonal antibody heavy chain CDR sequences (Kabat, Chothia, and IMGT definitions) are shown in Table 2.

### Table 2

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To create the complete heavy or kappa chain antibody sequences, each variable sequence above is combined with its respective constant region. For example, a complete heavy chain comprises a heavy variable sequence followed by the murine IgGl or IgG2a heavy chain constant sequence, a complete kappa chain comprises a kappa variable sequence followed by the murine kappa light chain constant sequence, and a complete lambda chain comprises a lambda variable sequence followed by the murine lambda IGLCl or IGLC2 light chain constant sequence.

**Nucleic Acid Sequence Encoding the Murine IgGl Heavy Chain Constant Region**

(SEQ ID NO: 82)

```
  1 gccaaacagc caccccccac ccacagttcc cttcctatcc caactgcccttg gatctgtgctc ccaaatccac
  61 tccatgggtga cctcgagggt cctggtaaga ggtcatcatt ccetagccact gacatggacag ctctcccagac
 121 tgggaactcttg gatcctgttc cagcgttgtg caccaccttc cagctgtcc cagcgtcgg ccagcgtcgg ccagcgtcgg
 181 ctctaatctg tgcagcgttc aagtgacatg tggtggtggt gggagtgtgex gaggattgacc cagcgtcgc cagtccgac
tcgagccggg
cgagacgtc
tgagatcggttg gtagacatcag tgggtggtggt ggaagtctctcg cagcgtcgc cagtccgac
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<td>lapvcdgtttg ssvtlglvk gyfpepvtlt wnsglssgv htfpavlgd</td>
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<tr>
<td>61 lytlsssvtv</td>
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<tr>
<td>61 ggaggtgtgacttctgcacatcttcaccctcctggacacagtcctgtatgcagctggtggtcag</td>
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<tbody>
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</tr>
<tr>
<td>61 skdystmsss</td>
<td>tliltkdye rhnsyczteat hktstspivk sfnrne</td>
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<th>ID NO: 88</th>
<th>Nucleic Acid Sequence Encoding the Murine Lambda (IGLCl) Light Chain Constant Region</th>
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<tbody>
<tr>
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<td></td>
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<tr>
<td>61 actaacaagggccatctggactgttagagctgcttgctggtacagtctgcctggagttgcagtgccctggcc</td>
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</tr>
<tr>
<td>61 gsnkkymass yltltarawe rhossncqvt heghtveksl scradcs</td>
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Nucleic Acid Sequence Encoding the Murine Lambda (IGLC2) Light Chain Constant Region (SEQ ID NO: 90)

1 ggtcagcccc aagtccactcc cactctcacc tgttttccac ctctctctga ggagctcaag
5 gtcagtgaac ttcccatcat gcaccaggac tggctcaatg gcaaggagtt caaatgcagg
10 cacaoctccagtt ttacctgtca gttttcactat gaaaacaaag ggtcagccca
15 ccggccagc cctgaaactc caccgtctcc aagacgggta ttcgctgttt
20 tgttcttcatc gttgtaagcc gtggtagaca gtcacccctagt ctgcagctct
25 gaggtgaagc ccacactgtg gtgtctgatt tccaacttttt ccccgagttg tttcgcagtt
30 ccactgtca aaactgctgg gataagcc agaggtggaa tgggttgtgc taaggtgtgc
35 ccggccagc cctgaaactc caccgtctcc aagacgggta ttcgctgttt
40 tgttcttcatc gttgtaagcc gtggtagaca gtcacccctagt ctgcagctct
45 gaggtgaagc ccacactgtg gtgtctgatt tccaacttttt ccccgagttg tttcgcagtt
50 ccactgtca aaactgctgg gataagcc agaggtggaa tgggttgtgc taaggtgtgc
55 ccggccagc cctgaaactc caccgtctcc aagacgggta ttcgctgttt
60 tgttcttcatc gttgtaagcc gtggtagaca gtcacccctagt ctgcagctct
65 gaggtgaagc ccacactgtg gtgtctgatt tccaacttttt ccccgagttg tttcgcagtt
70 ccactgtca aaactgctgg gataagcc agaggtggaa tgggttgtgc taaggtgtgc
75 ccggccagc cctgaaactc caccgtctcc aagacgggta ttcgctgttt
80 tgttcttcatc gttgtaagcc gtggtagaca gtcacccctagt ctgcagctct
85 gaggtgaagc ccacactgtg gtgtctgatt tccaacttttt ccccgagttg tttcgcagtt
90 ccactgtca aaactgctgg gataagcc agaggtggaa tgggttgtgc taaggtgtgc

Protein Sequence Defining the Murine Lambda (IGLC2) Light Chain Constant Region (SEQ ID NO: 91)

1 gqpkstptlt vfpsseelk enkatlvcil snfspsgvvt awkangtpit qgvdtsnptk
5 egnkfmess hlhtsdqws hnsftcqvh egdteksls paecl
10

The following sequences represent the actual or contemplated full length heavy and light chain sequence (i.e., containing both the variable and constant regions sequences) for each antibody described in this Example. Signal sequences for proper secretion of the antibodies (e.g., signal sequences at the 5’ end of the DNA sequences or the amino terminal end of the protein sequences) are not shown in the full length heavy and light chain sequences disclosed herein and are not included in the final secreted protein. Also not shown are stop codons for termination of translation required at the 3’ end of the DNA sequences. It is within ordinary skill in the art to select a signal sequence and/or a stop codon for expression of the disclosed full length IgG heavy chain and light chain sequences. It is also contemplated that the variable region sequences can be ligated to other constant region sequences to produce active full length IgG heavy and light chains.

Nucleic Acid Sequence Encoding the Full Length Heavy Chain Sequence (Heavy Chain Variable Region and IgGl Constant Region) of 07F01 (SEQ ID NO: 92)

1 gaggtgaagc ttctcagact tggaggtgcac cttgctgacg cgggttggac ccctgaactc
5 tccctgtcag cgctattcag tggagaagac gcaactgcct cttgctgact ccctgactac
10 gacccatcttc taaagagagg atacatcgcg tggagaagac gcaactgcct cttgctgact
15 gcacccgatgc atgctccagt cagctactgc tggagaagac gcaactgcct cttgctgact
20 gcaccccctccc tggagaagac gcaactgcct cttgctgact ccctgactac
25 gcacccgatgc atgctccagt cagctactgc tggagaagac gcaactgcct cttgctgact
30 gcaccccctccc tggagaagac gcaactgcct cttgctgact ccctgactac
35 gcacccgatgc atgctccagt cagctactgc tggagaagac gcaactgcct cttgctgact
40 gcaccccctccc tggagaagac gcaactgcct cttgctgact ccctgactac
45 gcacccgatgc atgctccagt cagctactgc tggagaagac gcaactgcct cttgctgact
50 gcaccccctccc tggagaagac gcaactgcct cttgctgact ccctgactac
55 gcacccgatgc atgctccagt cagctactgc tggagaagac gcaactgcct cttgctgact
60 gcaccccctccc tggagaagac gcaactgcct cttgctgact ccctgactac
65 gcacccgatgc atgctccagt cagctactgc tggagaagac gcaactgcct cttgctgact
70 gcaccccctccc tggagaagac gcaactgcct cttgctgact ccctgactac
75 gcacccgatgc atgctccagt cagctactgc tggagaagac gcaactgcct cttgctgact
80 gcaccccctccc tggagaagac gcaactgcct cttgctgact ccctgactac
85 gcacccgatgc atgctccagt cagctactgc tggagaagac gcaactgcct cttgctgact
Protein Sequence Defining the Full Length Heavy Chain Sequence (Heavy Chain)

Variable Region and IgGl Constant Region) of 07F01 (SEQ ID NO: 93)

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<tr>
<td>61</td>
<td>tspkkekfi lsdnaknlsf lgmnrvrsed talyvccav rihyyagmdc wqggstvts</td>
</tr>
<tr>
<td>121</td>
<td>saktppsvy plagsaagq smvltiegclv kgfyfpevpt twns glivgsg vhptpplvq</td>
</tr>
<tr>
<td>181</td>
<td>dlyt1ssvut vpsswspet vtnvahple stkvkkkvip rdgckpcic tvpevssvfi</td>
</tr>
<tr>
<td>241</td>
<td>fppkkpdkvt ilntpkctcv vvdiskdpe vqfswffvdd evhataqgpr eeqfnsftrs</td>
</tr>
<tr>
<td>301</td>
<td>vselpnhqdl wlngkfkcr vnsaafpapi ektisktkkr pkapqyvttip ppkegmkdk</td>
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<tr>
<td>361</td>
<td>vsitcmtidf fpeditveqw wngppavnyk ntqpmtdtdg syfysklv nyqskweagnt</td>
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<td>421</td>
<td>ftcsvlheql hnhhteksls hspggk</td>
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Protein Sequence Defining the Full Length Light Chain Sequence (Kappa Chain)

Variable Region and Constant Region) of 07F01 (SEQ ID NO: 94)

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<tr>
<td>121</td>
<td>gttcacttaa cttaaacaact tttaatcattg gcacatatcct gtcagacttg aagcctgt</td>
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<tr>
<td>181</td>
<td>cgcttcaggc caggccagac ttggagcatt ccacctcctc aaatatatt tctcagtc</td>
</tr>
<tr>
<td>241</td>
<td>gaagactttg tgcaggtattt cctcactaca tataaataa aatcctcagtt tgtcggctt</td>
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</tr>
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<td>tcaacagtcc ggttaaccag ggttcctgcac gccctgcca catttccctc tctcagtc</td>
</tr>
<tr>
<td>421</td>
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<td>tggaccacagg acaggtattg acacataaac ggtataacc tggaggtcct ccacaaacc</td>
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Protein Sequence Defining the Full Length Light Chain Sequence (Kappa Chain)

Variable Region and Constant Region) of 07B11 (SEQ ID NO: 95)

<table>
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<th>Sequence</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>divltqsski vstsvgarvs vttcakanhv tsvldqkkp vsppktlyys asfrysvypd</td>
</tr>
<tr>
<td>61</td>
<td>riftsgqstt fttisvnvgs edladyfcqq ynynptltft ga tkelkldk aaptvlsfpp</td>
</tr>
<tr>
<td>121</td>
<td>sseqltsgga svsvflmnfb pkdkinwkki dqserqngvl nsstwqpskd stysmsstlt</td>
</tr>
<tr>
<td>181</td>
<td>ltkdeyerhn sytcathkt stspivgskfn rness</td>
</tr>
</tbody>
</table>

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

Variable Region and IgGl Constant Region) of 07B11 (SEQ ID NO: 96)

<table>
<thead>
<tr>
<th>Region</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>gaggtgacgt tagtgggatc tgggggaggc ttaattggac cttggagggtc cctgaaactc</td>
</tr>
<tr>
<td>61</td>
<td>ttcctgcag cccccatctc aatccgttcttg acctatgcaa cgttgctttgac tcgccagact</td>
</tr>
<tr>
<td>121</td>
<td>cccagagaga gccttgccagtt gcctggagac atcactaagtg gttggtagttt caccctact</td>
</tr>
<tr>
<td>181</td>
<td>cgagacactg ttgctcagac cctccctact cctagacactg ccagacactg ccagacactg</td>
</tr>
<tr>
<td>241</td>
<td>ctgcaatgga gcttgcttagc gctggcttagc cagcagacttg aatattgctac cttcactcctt</td>
</tr>
<tr>
<td>301</td>
<td>tacctctattg ttaactttga ctatcagcct tgcactggcc caagccacac cttcactcctt</td>
</tr>
<tr>
<td>361</td>
<td>aacacacact cccccatctc aatccgttcttg acctatgcaa cgttgctttgac tcgccagact</td>
</tr>
</tbody>
</table>

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

Variable Region and IgGl Constant Region) of 07B11 (SEQ ID NO: 96)

<table>
<thead>
<tr>
<th>Region</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>1</td>
<td>gaggtgacgt tagtgggatc tgggggaggc ttaattggac cttggagggtc cctgaaactc</td>
</tr>
<tr>
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<td>ttcctgcag cccccatctc aatccgttcttg acctatgcaa cgttgctttgac tcgccagact</td>
</tr>
<tr>
<td>121</td>
<td>cccagagaga gccttgccagtt gcctggagac atcactaagtg gttggtagttt caccctact</td>
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<tr>
<td>181</td>
<td>cgagacactg ttgctcagac cctccctact cctagacactg ccagacactg ccagacactg</td>
</tr>
<tr>
<td>241</td>
<td>ctgcaatgga gcttgcttagc gctggcttagc cagcagacttg aatattgctac cttcactcctt</td>
</tr>
<tr>
<td>301</td>
<td>tacctctattg ttaactttga ctatcagcct tgcactggcc caagccacac cttcactcctt</td>
</tr>
<tr>
<td>361</td>
<td>aacacacact cccccatctc aatccgttcttg acctatgcaa cgttgctttgac tcgccagact</td>
</tr>
</tbody>
</table>
Variable Region and IgGl Constant Region) of 12B11 (SEQ ID NO: 97)

Protein Sequence Defining the Full Length Heavy Chain Sequence (Heavy Chain)

[0170]

Nucleic Acid Sequence Encoding the Full Length Light Chain Sequence (Kappa Chain)

[0171]

Protein Sequence Defining the Full Length Light Chain Sequence (Kappa Chain)

[0172]
[0173] Nucleic Acid Sequence Encoding the Full Length Heavy Chain Sequence (Heavy Chain Variable Region and IgG2A Constant Region) of 17F06 (SEQ ID NO: 100)

1  gaagtaagacg ttagttgacct ggggggacat ttgatgtgaag tggaggcttc tctgaaactc
5  tctctgtgcag ctctctgatt caatttctag tccatagcgg ctcctgctgatt ctggtctgacgt
10 tcagacaaga ggtggtggat gtgcgcctcc atagtatggt ggtggtgctgact tacaatctatc
15 tggagctcag gctgcctgag cagacacttc caccacaagc gatctcgtac agcttgtgactc
20 tgtcagtctgg tggctagctg tggctagctg tggctagctg tggctagctg tggctagctg
25

[0174] Protein Sequence Defining the Full Length Heavy Chain Sequence (Heavy Chain Variable Region and IgG2A Constant Region) of 17F06 (SEQ ID NO: 101)

1  evklvesggs gtvkgssalsk ccaasqgfsy sygmswvrqt sdrkrewvas issgggttvy
5  ldtvkgfrti srenaktliy lqmsglksed talyctcrgg wlikfaywq gtltvvsaaak
10 tttaspsvpl vtlcgtgtsvg ypepvltwn gsglsqvht fpavlqsidy
15 1c015vstvs stkswpsqitv nvaikhpsatlk vdkkieprgp tipkcpccsktpnplggs
20 241vfnffnpki kvlmslspiv tcvvrvvdves dpvdqwsfvy nvnvesqvht qtqthredynst
30 301lrwsalpql hqdwmsqg thekvknkdip apiertiskpa ksgbvsqvpy lvppeemtv
35 361kkgvltcmv tdmpdmplytv ewtnqntkel nyknreoqvd slgsfysmsk lrvekknwe
40 421rnsyeswvh elghlnnhhtk sfrtsgpk

[0175] Nucleic Acid Sequence Encoding the Full Length Light Chain Sequence (Lambda Chain Variable Region and Constant Region (IGLC2)) of 17F06 (SEQ ID NO: 102)

1  caactttgtgc tcacctcgctc atcttcctcc cgcttttctcc cttgtgtctc tggagcttc acaaaactc
5  acgtcgacct tgaatgtcga gcaactcagc tacactcagtct tacttctggt ctggtgtgactc
10 tcagacaaga ggtggtggat gtgcgcctcc atagtatggt ggtggtgctgact tacaatctatc
tgabtagagc agcaaatatac atctccatgcc cagtctgaccc gagctgtgcc cacaagctgc
15 tggagctcag gctgcctgag cagacacttc caccacaagc gatctcgtac agcttgtgactc
tgcagactg gctgcctgag cagacacttc caccacaagc gatctcgtac agcttgtgactc
tgcagactg gctgcctgag cagacacttc caccacaagc gatctcgtac agcttgtgactc
tgcagactg gctgcctgag cagacacttc caccacaagc gatctcgtac agcttgtgactc
tgcagactg gctgcctgag cagacacttc caccacaagc gatctcgtac agcttgtgactc
Protein Sequence Defining the Full Length Light Chain Sequence (Lambda Chain Variable Region and Constant Region (IGLC2)) of 17F06 (SEQ ID NO: 103)

1 qvlvtqsssa sfslgasakl tctllsqght ytiewyqglp 1kpkkymvel kkdgsagatgv
61 gpdpdrfsgs sgadyltits ngqedeaivy icgygetied qfvrtyfgggt kvttljggpks
121 tpltltfpps seeeenkat lvcvisfnsfp sgvtvawkan gtpittgvdvd snptkegnkf
181 massflhtls dqwshrsnft cgvthegdtv eklspacecl

Nucleic Acid Sequence Encoding the Full Length Heavy Chain Sequence (Heavy Chain Variable Region and IgGl Constant Region) of 18H09 (SEQ ID NO: 104)

10 1 gaggtgtcgag tccagggctg aggacagctc tctctcagaga tctctcagag tctctcagcc
tcagctctgc tcaagagtaag cagagtagctg cagagtagctg cagagtagctg
cagagtagctg cagagtagctg cagagtagctg cagagtagctg cagagtagctg
cagagtagctg cagagtagctg cagagtagctg cagagtagctg cagagtagctg
cagagtagctg cagagtagctg cagagtagctg cagagtagctg cagagtagctg
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cagagtagctg cagagtagctg cagagtagctg cagagtagctg cagagtagctg
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cagagtagctg cagagtagctg cagagtagctg cagagtagctg cagagtagctg
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cagagtagctg cagagtagctg cagagtagctg cagagtagctg cagagtagctg
cagagtagctg cagagtagctg cagagtagctg cagagtagctg cagagtagctg

Protein Sequence Defining the Full Length Heavy Chain Sequence (Heavy Chain Variable Region and IgGl Constant Region) of 18H09 (SEQ ID NO: 105)

35 1 evqlqesgps lvkpsqtssl tcvytqsgist sdwnwirikf pgnkleyymg isygsstynn
61 psiksrisit rdsksnqfyl rlnsvttded atyycarchli ltiaywqggt lvtvsakat
121 ppssvpplap gsaatqsmvt lgcdvkygfpl epvttvwnsg sllsvghftp avlqsdlyt1
181 sssvtpvpsst wpvts pcmtxv msapkstikkv kdpkrypcpg khicptvptep svfvsffhpk
241 kdvtltitltp kvtecvcvds kddpevqfsw fvdvevhata qtdqreqqfn stfrsveselp
361 mitdfspedi tvwqswqgpp epnyhqtgpi mdtdgsyfvy sklnvqvksnl eaqnfctcsv
421 lhelimnht eklsnhspgk

Nucleic Acid Sequence Encoding the Full Length Light Chain Sequence (Lambda Chain Variable Region and Constant Region (IGLC1)) of 18H09 (SEQ ID NO: 106)

45 1 caggtctggt gtagctcagga atctggcactc acacatcacg ctagtgaacg agtcacacgtc
tcattcgtccac gctctcctgcc ggtggaaacg cagagtagctg cagagtagctg cagagtagctg
cagagtagctg cagagtagctg cagagtagctg cagagtagctg cagagtagctg cagagtagctg
cagagtagctg cagagtagctg cagagtagctg cagagtagctg cagagtagctg cagagtagctg
cagagtagctg cagagtagctg cagagtagctg cagagtagctg cagagtagctg cagagtagctg
cagagtagctg cagagtagctg cagagtagctg cagagtagctg cagagtagctg cagagtagctg

[0180] **Protein Sequence Defining the Full Length Light Chain Sequence (Lambda Chain)**

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>1 qavvqesal ttspgetv1 tcrssagavt tsnfanwvqe kpdhlfstgli gdtminrpgv</td>
<td></td>
</tr>
<tr>
<td>61 parfsqslig dkaalitgta qtedeaiyfc alwysnywv fgggtkltvl gqkssspstv</td>
<td></td>
</tr>
<tr>
<td>121 ifppseele nkntaivcti tdfypgvgtv dwkvdgtptv qgmcttgpkp qsnkkypas</td>
<td></td>
</tr>
<tr>
<td>181 ylctarawe rhssycqvtt heghtvekl srdac</td>
<td></td>
</tr>
</tbody>
</table>

[0181] **Nucleic Acid Sequence Defining the Full Length Heavy Chain Sequence (Heavy Chain Variable Region and IgGl Constant Region) of 29B06 (SEQ ID NO: 108)**

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>1 gagggtcagc ttcaaggactc aggacttcagc ctcctgctaa cttctcgagc tctgtgccctc</td>
<td></td>
</tr>
<tr>
<td>20 1 acctttcttg tcacttgccg cttccacatc agttggtctac ggaacttgtag cccagaattg</td>
<td></td>
</tr>
<tr>
<td>30 1 ccaggtggat gctctgctgt gctctggcag cctgtcttgg tcgctttggt gcggacttttc</td>
<td></td>
</tr>
<tr>
<td>40 1 ggcttggatgt cagctggctga cctctgggca gctctgggca gctctgggca gctctgggca</td>
<td></td>
</tr>
</tbody>
</table>

[0182] **Protein Sequence Defining the Full Length Heavy Chain Sequence (Heavy Chain Variable Region and IgGl Constant Region) of 29B06 (SEQ ID NO: 109)**

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>1 evqlqesgps lvksptqtsl tlctsvtgdst sgywnwirklf gsgnkleymg voyisyskgtyyn</td>
<td></td>
</tr>
<tr>
<td>20 1 psikrisit rdtskhynyl qlslsvenat atycgarsky dyamdwggsq tsvyvsaaakt</td>
<td></td>
</tr>
<tr>
<td>30 1 tcppsyvlplap gsaatnmsmv tgtlgvkyf gpptvtnwgs gslsgsvhtv pavlgdylty</td>
<td></td>
</tr>
<tr>
<td>40 1 lsstwvptsp twspstvtctn vahpssttkv dkkivprdcg ckmptilvpe vssvifppk</td>
<td></td>
</tr>
<tr>
<td>50 1 pkdvltitltt pkvctcvvnl skddpeqvgf qssfvdvveht atgtpqveqf nstfrsveqsl</td>
<td></td>
</tr>
<tr>
<td>60 1 mimhwdllng kefkrvrsna apfaplekti stktgrpkap qwtyispkkp qmkadkvsllt</td>
<td></td>
</tr>
<tr>
<td>70 1 cmitdmpfed itveqwqgqf paenynkntgp imdtdgsyvf ysklnvqksn weagntfctc</td>
<td></td>
</tr>
<tr>
<td>80 1 vlheglhnhh teklshtspg k</td>
<td></td>
</tr>
</tbody>
</table>
Nucleic Acid Sequence Encoding the Full Length Light Chain Sequence (Kappa Chain Variable Region and Constant Region) of 29B06 (SEQ ID NO: 110)

1 gacattgtgc tgacccaaatc tcctcgctctt ttggctgtgt ctctagggca gagggccacc
61 atctcctgca gagccacgca aatgttgtat aatatttgca ttagttttat gaactgttctc
121 caacagaaac cagagcagcc acccaaatct ctctctctat gcagttgcga ccaagatcc
181 ggggtccctgg ccaggttttag ttggcagttg acctcagcgc ctacctcctc caacatccat
241 cctgttggaqg agatgtgata tcgcaagtat ttctgtcagc aaagtaagga ggttcctccg
301 acgattcgttg gagccgacca gctggaatc aacgcggtct atgctgcacc cacagatcc
361 atcttcctcc cacattaataa ggcagtttaac ttgatggcttg ctctttcatgc gtgtgttttg
421 aaacattctc accccaaaagc cattcatact acagcagcata cagcatgcgtat ggtgcagcagc
481 aatggtgttc tgacagtttg gactgtacag gacagcagaag acagcactca cagcatgagcc
541 agcaccctca cgtggaccas ggcagtagtc gaaagcttgc acaggaatgaag aacaacttctc
601 actcacaaga ctatctcttc acccatctgc aagagcgc ccagcagaag caggaatgtg

Protein Sequence Defining the Full Length Light Chain Sequence (Kappa Chain Variable Region and Constant Region) of 29B06 (SEQ ID NO: 111)

1 divlqspas lavslgqrat iscraseivd nfgisfmmnf qqkgpqqpkl liyaasqngs
61 gvpafpsgsq sgtdfn sinh pveddtany fcqskvepp tfggktkei kradaaptvs
121 ifpssqetg sggasvvcfl nnfpydkinv kwkdgserq ngvlnsdtq dskdystms
181 stiltlkdey erhnysytceaa thktstspiv ksfnnrec

Table 4 shows the correspondence between the full-length sequences of the antibodies discussed in this Example with those presented in the Sequence Listing.

<table>
<thead>
<tr>
<th>SEQ ID NO.</th>
<th>Nucleic Acid or Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>92</td>
<td>07F01 Heavy Variable + IgG1 Constant—nucleic acid</td>
</tr>
<tr>
<td>93</td>
<td>07F01 Heavy Variable + IgG1 Constant—protein</td>
</tr>
<tr>
<td>94</td>
<td>07F01 Kappa Variable + Constant—nucleic acid</td>
</tr>
<tr>
<td>95</td>
<td>07F01 Kappa Variable + Constant—protein</td>
</tr>
<tr>
<td>96</td>
<td>12B11 Heavy Variable + IgG1 Constant—nucleic acid</td>
</tr>
<tr>
<td>97</td>
<td>12B11 Heavy Variable + IgG1 Constant—protein</td>
</tr>
<tr>
<td>98</td>
<td>12B11 Kappa Variable + Constant—nucleic acid</td>
</tr>
<tr>
<td>99</td>
<td>12B11 Kappa Variable + Constant—protein</td>
</tr>
<tr>
<td>100</td>
<td>17F06 Heavy Variable + IgG2A Constant—nucleic acid</td>
</tr>
<tr>
<td>101</td>
<td>17F06 Heavy Variable + IgG2A Constant—protein</td>
</tr>
<tr>
<td>102</td>
<td>17F06 Lambda Variable + Constant (IGLC2)—nucleic acid</td>
</tr>
<tr>
<td>103</td>
<td>17F06 Lambda Variable + Constant (IGLC2)—protein</td>
</tr>
<tr>
<td>104</td>
<td>18H09 Heavy Variable + IgG1 Constant—nucleic acid</td>
</tr>
<tr>
<td>105</td>
<td>18H09 Heavy Variable + IgG1 Constant—protein</td>
</tr>
<tr>
<td>106</td>
<td>18H09 Lambda Variable + Constant (IGL1)—nucleic acid</td>
</tr>
<tr>
<td>107</td>
<td>18H09 Lambda Variable + Constant (IGL1)—protein</td>
</tr>
<tr>
<td>108</td>
<td>29B06 Heavy Variable + IgG1 Constant—nucleic acid</td>
</tr>
<tr>
<td>109</td>
<td>29B06 Heavy Variable + IgG1 Constant—protein</td>
</tr>
<tr>
<td>110</td>
<td>29B06 Kappa Variable + Constant—nucleic acid</td>
</tr>
<tr>
<td>111</td>
<td>29B06 Kappa Variable + Constant—protein</td>
</tr>
</tbody>
</table>
Example 5: Binding Affinities

The binding affinities and kinetics of binding of antibodies 07F01, 29B06, 17F06, 18H09, and 12B11 to recombinant human RON-ECD/mFc fusion protein (rhRON ECD/mFc) and recombinant human RON SEMA and PSI domains (rhRON SEMA + PSI) (R&D Systems, Inc., Minneapolis, MN) were measured by surface plasmon resonance, using a Biacore® T100 instrument (GE Healthcare, Piscataway, NJ).

Rabbit anti-mouse IgGs (GE Healthcare) were immobilized on carboxymethylated dextran CM4 sensor chips (GE Healthcare) by amine coupling, according to a standard protocol. Analyses were performed at 25°C and 37°C, using PBS containing 0.05% surfactant P20 as running buffer. The antibodies were captured in individual flow cells at a flow rate of 10 µl/min. Injection time was varied for each antibody to yield an Rmax between 30 and 60 RU. 250 µg/mL mouse Fc were injected at 30 µl/min for 120 seconds to block non-specific binding of antibodies to Fc portion of the protein when needed. Buffer, rhRon ECD/mFc or rhRON SEMA + PSI diluted in running buffer was injected sequentially over a reference surface (no antibody captured) and the active surface (antibody to be tested) for 300 seconds at 60 µl/minute. The dissociation phase was monitored for up to 3600 seconds. The surface was then regenerated with two 60-second injections of 10 mM Glycine-HCl, pH 1.7, at a flow rate of 60 µl/min. The rhRON ECD/mFc or rhRON SEMA + PSI concentration range tested was 0.625 nM to 20 nM.

Kinetic parameters were determined using the kinetic function of the BIAevaluation software (GE Healthcare) with double reference subtraction. Kinetic parameters for each antibody, $k_a$ (association rate constant), $k_d$ (dissociation rate constant) and $K_D$ (equilibrium dissociation constant) were determined. Kinetic values of the monoclonal antibodies on rhRON ECD/mFc at 25°C and 37°C are summarized in Table 5.
Table 5
Antibody Binding to rhRON ECD/mFc

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Measurements at 25°C</th>
<th>Measurements at 37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ka (1/Ms)</td>
<td>kd (1/s)</td>
</tr>
<tr>
<td>07F01</td>
<td>AVG</td>
<td>4.0E+05</td>
</tr>
<tr>
<td></td>
<td>STDEV</td>
<td>7.1E+04</td>
</tr>
<tr>
<td>29B06</td>
<td>AVG</td>
<td>2.0E+05</td>
</tr>
<tr>
<td></td>
<td>STDEV</td>
<td>3.5E+04</td>
</tr>
<tr>
<td>17F06</td>
<td>AVG</td>
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<td>STDEV</td>
<td>4.8E+04</td>
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<tr>
<td>18H09</td>
<td>AVG</td>
<td>3.3E+05</td>
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<tr>
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<td>STDEV</td>
<td>1.5E+05</td>
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<tr>
<td>12B11</td>
<td>AVG</td>
<td>1.2E+05</td>
</tr>
<tr>
<td></td>
<td>STDEV</td>
<td>2.8E+04</td>
</tr>
</tbody>
</table>

*Outside instrument limit of detection

[0189] The data in Table 5 demonstrate that antibodies 07F01, 29B06, 17F06, 18H09, and 12B11 bind rhRON ECD/mFc with a K_D of about 1 nM or less, 750 pM or less, 650 pM or less, 600 pM or less, 500 pM or less, 400 pM or less, 300 pM or less, 250 pM or less, 200 pM or less, 150 pM or less, 100 pM or less, 75 pM or less, 50 pM or less, or 10 pM or less.

[0190] Kinetic values of the monoclonal antibodies on rhRON SEMA + PSI at 25°C and 37°C are summarized in Table 6.

Table 6
Antibody Binding to rhRON SEMA + PSI

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Measurements at 25°C</th>
<th>Measurements at 37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ka (1/Ms)</td>
<td>kd (1/s)</td>
</tr>
<tr>
<td>07F01</td>
<td>AVG</td>
<td>5.2E+06</td>
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<td></td>
<td>STDEV</td>
<td>7.0E+06</td>
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<td>29B06</td>
<td>AVG</td>
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<td>1.2E+05</td>
</tr>
<tr>
<td>17F06</td>
<td>AVG</td>
<td>1.9E+05</td>
</tr>
<tr>
<td></td>
<td>STDEV</td>
<td>3.6E+04</td>
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<tr>
<td>18H09</td>
<td>AVG</td>
<td>4.4E+05</td>
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<tr>
<td></td>
<td>STDEV</td>
<td>2.7E+04</td>
</tr>
<tr>
<td>12B11</td>
<td>AVG</td>
<td>No binding</td>
</tr>
</tbody>
</table>

[0191] The data in Table 6 demonstrate that antibodies 07F01, 29B06, 17F06 and 18H09 bind rhRON SEMA + PSI with a K_D of about 1 nM or less, 750 pM or less, 650 pM or less, 600 pM or less, 500 pM or less, 400 pM or less, 300 pM or less, 250 pM or less, 200 pM or less, 150 pM or less, 100 pM or less, 75 pM or less, 50 pM or less, or 10 pM or less. Antibody 12B11 did not bind to rhRON SEMA + PSI.
[0192] Binding to cell surface human wild-type RON and the delta 160 RON variant by antibodies 29B06 and 07F01 was measured at 4°C, using Fluorescence Activated Cell Sorting (FACS). PC3 cells expressing the human wild-type RON, and HT29 cells expressing the delta 160 variant, were harvested using cell dissociation buffer (Invitrogen), washed twice with FACS buffer (PBS with 0.5% BSA), and treated for 10 minutes with Cyto Q Antibody diluent and FC receptor block (Innovex Biosciences, Richmond, CA). Purified antibodies were diluted in FACS buffer over a concentration range from 0.02 nM to 40 nM. Cells were incubated with 100 µι of antibody for one hour, washed with FACS buffer three times, and incubated for 45 minutes with goat anti-mouse PE-conjugated antibody (Jackson ImmunoResearch Laboratories, West Grove, PA). Cells were washed three times with FACS buffer, resuspended in 300 µι of FACS buffer, and analyzed using a Beckman Coulter Cytomics FC 500 FACS instrument. Results are summarized in Table 7.

Table 7

<table>
<thead>
<tr>
<th></th>
<th>29B06</th>
<th>07F01</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human RON – K_D (nM)</td>
<td>0.133</td>
<td>0.032</td>
</tr>
<tr>
<td>Human RON - K_D range (nM)</td>
<td>0.089-0.177</td>
<td>0.025-0.039</td>
</tr>
<tr>
<td>Delta 160 RON - K_D (nM)</td>
<td>0.146</td>
<td>0.024</td>
</tr>
<tr>
<td>Delta 160 RON - K_D range (nM)</td>
<td>0.100-0.192</td>
<td>0.020-0.029</td>
</tr>
</tbody>
</table>

[0193] The results in Table 7 demonstrate that antibodies 29B06 and 07F01 bind both wild-type RON and the delta 160 RON variant on the cell surface with similar affinity.

Example 6: Cell Surface Binding

[0194] Binding to cell surface wild-type RON and delta 160 RON at 4°C was determined for antibodies 07F01, 12B11, 17F06, 18H09, and 29B06, using FACS. Cells expressing wild-type RON (PC3), and cells expressing delta 160 RON (HT-29), were harvested using cell dissociation buffer (Invitrogen), washed twice with FACS buffer (0.5% BSA PBS) and treated with CytoQ Antibody diluent and FC receptor block (Innovex). Purified antibodies were diluted at a concentration of 10 µg/ml, in FACS buffer. Cells were incubated with 100 µι of antibody mix for one hour, washed with FACS buffer three times, and incubated for 45 minutes with goat anti-mouse PE conjugated antibody (Jackson Immunoresearch Laboratories). Cells were washed three times with FACS buffer, resuspended in 300 µι of FACS buffer and
analyzed using a Beckman Coulter Cytomics FC 500 FACS instrument. Percent binding as compared to murine IgG control is shown in Table 8.

Table 8

<table>
<thead>
<tr>
<th>Antibody</th>
<th>PC3 % cell surface binding</th>
<th>HT-29 % cell surface binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>07F01</td>
<td>99.29</td>
<td>99.08</td>
</tr>
<tr>
<td>17F06</td>
<td>99.08</td>
<td>99.00</td>
</tr>
<tr>
<td>29B06</td>
<td>99.06</td>
<td>99.04</td>
</tr>
<tr>
<td>18H09</td>
<td>99.03</td>
<td>98.33</td>
</tr>
<tr>
<td>12B11</td>
<td>94.52</td>
<td>88.64</td>
</tr>
<tr>
<td>mIgG</td>
<td>5.50</td>
<td>5.62</td>
</tr>
</tbody>
</table>

The results in Table 8 demonstrate that antibodies 07F01, 29B06, 17F06, 18H09, and 12B11 bind both wild-type RON and the delta 160 RON variant expressed on the surface of cells.

Example 7: Receptor Internalization

Antibody-stimulated receptor internalization was measured using FACS. PC3 cells were used to measure antibody-stimulated internalization of the wild-type RON receptor. HT-29 cells were used for the delta 160 RON receptor variant. Antibodies were first conjugated with R-Phycocerthrin (Prozyme cat. No. PJ31K). All cells were washed with PBS and treated with CytoQ Antibody diluent and FC receptor block (Innovex). Cells were incubated with the antibodies (10 μg/ml) for 2 hours at 37°C or at 4°C. The cells were transferred to 4°C, washed with an acidic solution (0.5 M NaCl, 0.18 M Acetic Acid, 0.5% Na azide) to strip off the antibody remaining on the cell surface, and fixed using BD cytofix/cytoperm Plus kit (BD Biosciences, cat. No. 555028) to measure antibodies retained intracellularly due to internalization. At 37°C, cells can undergo antibody-mediated receptor internalization, and the process is inhibited at low temperature of 4°C, thus serving as a baseline (no internalization).

The cells were analyzed using a Beckman Coulter Cytomics FC 500 FACS instrument. A lowered anti-RON median fluorescent intensity (MFI) and a left shift of the histograms at 4°C compared to that obtained at 37°C indicate antibody-induced receptor internalization. Receptor internalization was quantified by subtracting MFI at 4°C from that at 37°C. Results are summarized in Table 9.
These results demonstrate that antibodies, 29B06, 07F01 and 12B11 induce receptor internalization in PC-3 cells expressing wild-type RON. Only 07F01 and 12B11 induce receptor internalization in HT-29 cells expressing delta 160 RON variant.

**Example 8: Inhibition of MSP-RON Binding**

Antibodies 07F01, 12B11, 17F06, 18H09, and 29B06 were tested for inhibition of MSP binding to hRON SEMA + PSI, as measured by electrochemiluminescence (ECL) assay as described in Example 3. The antibodies (concentration range: 0.006-10 µg/mL) were incubated for 45 minutes at room temperature.

The MSP-hRON binding interaction was inhibited by antibodies 07F01, 18H06, and 29B06, but not by antibodies 17F06 and 12B11 (FIG. 6). The IC50 and maximum percent inhibition values for the antibodies (IgG1) are shown in Table 10.

**Table 10**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>IC50 (nM) Average</th>
<th>Std Dev</th>
<th>Maximum Neutralization (%) Average</th>
<th>Std Dev</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>07F01</td>
<td>0.26</td>
<td>0.05</td>
<td>88.3</td>
<td>2.1</td>
<td>3</td>
</tr>
<tr>
<td>18H09</td>
<td>0.91</td>
<td>0.15</td>
<td>86.9</td>
<td>6.7</td>
<td>3</td>
</tr>
<tr>
<td>29B06</td>
<td>1.11</td>
<td>0.06</td>
<td>87.6</td>
<td>4.7</td>
<td>3</td>
</tr>
<tr>
<td>12B11</td>
<td>N/A</td>
<td>N/A</td>
<td>44.8</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>17F06</td>
<td>N/A</td>
<td>N/A</td>
<td>7.9</td>
<td>11.2</td>
<td>2</td>
</tr>
</tbody>
</table>

The results in Table 10 demonstrate that antibodies 07F01, 18H09 and 29B06 block MSP binding to hRON SEMA + PSI, while antibodies 12B11 and 17F06 do not.

**Example 9: Inhibition of downstream signaling by anti-RON antibodies**

Antibodies 07F01, 12B11, 17F06, 18H09, and 29B06 were tested for inhibition of MSP-dependent phosphorylation of ERK, a RON downstream signaling molecule using the cell-based assay described in Example 3. The antibodies (concentration range: 0.006-10 µg/mL) in RPMI were added to the cells and incubated for one hour at 37°C.
Dose-dependent inhibition of ERK phosphorylation by antibodies 07F01, 12B11, 17F06, 18H09, and 29B06 is shown in Table 11 and FIG. 7.

Table 11

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Mean IC50 (nM)</th>
<th>Std Dev</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>07F01</td>
<td>0.07</td>
<td>0.02</td>
<td>3</td>
</tr>
<tr>
<td>18H09</td>
<td>0.71</td>
<td>0.36</td>
<td>3</td>
</tr>
<tr>
<td>29B06</td>
<td>0.44</td>
<td>0.27</td>
<td>3</td>
</tr>
<tr>
<td>12B11</td>
<td>5.91</td>
<td>5.92</td>
<td>3</td>
</tr>
<tr>
<td>17F06</td>
<td>0.96</td>
<td>0.4</td>
<td>3</td>
</tr>
</tbody>
</table>

The results in Table 11 and FIG. 7 demonstrate that antibodies 07F01, 18H09, 29B06, 12B11 and 17F06 inhibit MSP-induced ERK phosphorylation in T47D breast cancer cell line, even though 12B11 and 17F06 do not effectively block MSP binding to RON (see Examples 3 and 8).

Example 10: Inhibition of MSP-Dependent Cell Migration

Antibodies 07F01, 18H09, 29B06, 12B11 and 17F06 were tested for inhibition of MSP-dependent cell migration. HPAF-II pancreatic cancer cells (ATCC) were incubated overnight under low serum conditions (1% FBS, MEM). Cells were trypsinized, counted, and placed at a concentration of 50,000/well in 45 µl of 1% FBS/MEM in the upper chamber of a BD 96-well FluoroBlok™ plate (Becton Dickinson). Antibodies were added at a concentration of 2 µg/ml, and cells were incubated for 2 hours. The bottom chamber contained 1% FBS MEM (200 µl) and 1 nM MSP, and cells were incubated for 24 hours. The number of migrated cells was determined by the addition of Calcien Dye at 4 µg/ml final concentration to the bottom chamber, followed by a one-hour incubation. Fluorescence intensity was measured using a Wallace 1420 instrument (Perkin Elmer). Baseline fluorescent measurements were done in the absence of MSP. Percent inhibition was determined by comparing antibody-treated and antibody-untreated samples to the baseline using the following formula: 100-(anti-RON antibody treated-baseline)/(control hulgG treated-baseline)*100. Results on inhibition of MSP-induced HPAFII cell migration by antibodies 07F01, 18H09, 29B06, 12B11, and 17F06 are summarized in Table 12 and FIG. 8.
The results in Table 12 demonstrate that antibodies 07F01, 18H09, 29B06, 12B11, and 17F06 inhibit MSP-dependent cell migration in HPAF-II pancreatic cancer cell lines, even though 12B11 and 17F06 do not effectively block MSP binding to RON.

**Example 11: Inhibition of Growth of Wild-Type RON-Dependent Tumor Model**

Inhibition of tumor growth was tested in a directed complementation model of wild-type RON-driven tumor growth. "Directed complementation" tumors were obtained as described in Robinson *et al.*, U.S. Patent No. 7,556,796. A cDNA encoding wild-type human RON was introduced into BH3 tumor cells by retroviral transfer. Transfected tumor cells were then implanted subcutaneously into recipient mice. Growth of the BH3 tumors was dependent on expression of an inducible HER2 gene, which was not induced. Therefore, tumors would grow only if the RON gene functionally complemented the uninduced HER2 gene. Growth of the directed complementation tumors was observed. Primary tumors were propagated *in vivo* to generate sufficient tumor material for drug efficacy studies. Quality control for the directed complemented tumors included RT-PCR for RON expression and immunohistochemistry (IHC) for protein expression. The tumors were stored as frozen archival aliquots of approximately 1.5 x 10^5 cells/vial. These tumors were thawed, washed once, resuspended in HBS+matrigel and injected subcutaneously. Tumor measurements were taken twice weekly using vernier calipers. Tumor volume was calculated using the formula: width x width x length/2. When tumors reached approximately 150 mm^3, the mice were randomized into five groups of ten mice each. Each group (ten mice each) received one of the following antibody treatments: 07F01, 29B06, 12B11, or 18H09, or murine IgG control, all at 20 mg/kg. Treatment was administered by intra-peritoneal injection, twice weekly, for two weeks.

Antibodies 29B06 and 07F01 resulted in tumor growth inhibition ("TGI") greater than 50% (p<0.001), while antibodies 18H09 and 12B11 exhibited TGI of 25% and 29%, respectively (FIG. 9). All treatments were well-tolerated with no significant loss in body weight.
Pharmacodynamic changes in RON receptor levels after 29B06 and 07F01 treatment were evaluated. Tumors were treated with 20 mg/kg of the following antibodies: mlgG (control), 29B06 or 07F01 and tumors were harvested at 24 or 48 hours. After harvest, the tumors were lysed in standard RIPA buffer (Boston Bioproducts, cat. No. BP-1 15) containing protease inhibitors (Roche, catalog No. 04693159001) and phosphatase inhibitors I and II (Sigma, cat. Nos. P2350 and P5726). Lysates were cleared and protein concentration was measured. A Western blot for total RON was done using a polyclonal anti-RON antibody (Santa Cruz, cat. No. sc-322). The Western blot analysis showed that antibody 29B06 induced receptor degradation in vivo in RON-DC xenograft at 24 hours, and to a greater extent at 48 hours.

Example 12: Inhibition of Growth of Delta 160 RON-Driven Tumor Model

Inhibition of tumor growth by the antibodies was tested in a directed complementation model of delta 160 RON-driven tumor growth. The model was obtained as described in Example 11, except that the transfected cDNA encoded human delta 160 (oncogenic) form of RON. Growth of the directed complementation tumors was observed. Primary tumors were propagated in vivo to generate sufficient tumor material for drug efficacy studies. Quality control for the directed complemented tumors included RT-PCR for RON expression and IHC for protein expression. The tumors were stored as frozen archival aliquots of approximately 1.5 x 10^5 cells/vial. These tumors were thawed, washed once, resuspended in HBS plus matrigel, and injected subcutaneously. Tumor measurements were taken twice weekly. When tumors reached approximately 150 mm^3, the mice were randomized into five groups of ten mice each. Each group (ten mice per group) received one of the following treatments: murine IgG control, 07F01, 29B06, 12B11, 17F06, and 18H09, all at 20 mg/kg. Treatment was administered by intra-peritoneal injection, twice weekly, for two weeks. Each treatment group showed similar tumor growth inhibition of greater than 60% (p<0.001) except for 18H09 (TGI 54%) as shown in FIG. 10. All treatments were well-tolerated, with no significant loss in body weight.

Example 13: Inhibition of Growth of NCI-H358 Lung Xenograft Tumor Model

Inhibition of tumor growth by the 29B06 antibody was tested in an NCI-H358 lung xenograft model. The NCI-H358 cells were grown in culture at 37°C in an atmosphere containing 5% CO_2, using RPMI medium (Invitrogen) containing 10% FBS. Cells were inoculated subcutaneously into the flank of 8-week old female CB.17 SCID mice with 5 x 10^6
cells per mouse in 50% matrigel. Tumor measurements were taken twice weekly. When tumors reached approximately 150 mm³, the mice were randomized into two groups of ten mice each. Each group received one of the following treatments: murine IgG control or 29B06 at 40 mg/kg. Treatment was administered by intra-peritoneal injection three times per week, for three weeks. Antibody 29B06 treatment resulted in tumor growth inhibition of 70% (p<0.001) (FIG. 11). Treatment was well-tolerated, with no significant loss in body weight.

Example 14: Humanization of Anti-RON Antibodies

A. Construction of Humanized and Chimeric Anti-RON Antibodies

This Example describes the humanization of two murine antibodies, designated 07F01 and 29B06, and the characterization of the resulting humanized antibodies. The humanized anti-RON antibodies were designed using the SUPERHUMANIZATION™ method (Cephalon, Inc. (Arana Therapeutics Ltd.) and Hwang, W.Y. et al. (2005) METHODS 36:35-42), the CDR grafting method with back mutations (some human framework residues were changed to murine residues) (See e.g., U.S. Patent Nos. 5,530,101; 5,693,761; 5,693,762; 5,585,089; 6,180,370; 7,022,500), or the HUMAN ENGINEERING™ method (Studnicka et al, Protein Eng. 1994 Jun;7(6):805-14; also see, e.g., PCT Publication No. WO 93/11794 and U.S. Patent Nos. 5,766,886; 5,770,196; 5,821,123; and 5,869,619). With the exception of heavy chain CDR1, the Kabat CDR definitions were used for CDR grafting onto human frameworks (SUPERHUMANIZATION™ and CDR grafting with back mutations). In some cases, a combination of Kabat and Chothia definitions were used for grafting heavy CDR1. In some cases, CDR residues (Kabat or Chothia definitions) were changed to human residues to increase humanness. Models of the murine antibodies were created using the SWISS-MODEL web server (swissmodel.expasy.org). Predicted residue contacts were determined using the Contact Map Analysis web server (ligin.weizmann.ac.il/cma/), and residue surface accessibility was determined using the Accessible Molecular Surface web server (swift.cmbi.ru.nl/servers/html/accessres.html). Residues were selected for back mutation based on predicted surface accessibility, contact with CDR residues, and involvement in the interface between heavy and light chains. Additionally, a cysteine residue present in the heavy chain CDR3 of 07F01 was changed to serine to prevent potential aggregation, and in some examples, a predicted N-linked glycosylation consensus site (N-X-S/T) in 07F01 heavy CDR2 (e.g., N58, Y59, T60) was mutated (e.g., T60A) to prevent any possible glycosylation. The designed amino acid sequences were converted to codon-optimized DNA sequences and synthesized by DNA2.0, Inc. to include (in the following order): 5' HindIII restriction site, Kozak consensus
sequence, amino terminal signal sequence, humanized variable region, human IgGl or Kappa constant region, stop codon, and a 3’ EcoRI restriction site.

[0211] The anti-RON antibody chains humanized according to the SUPERHUMANIZATION™ method, as described herein, are designated with the prefix "Sh" before the antibody chain name. The anti-RON antibody chains humanized by the CDR grafting method with back mutations, as described herein, are designated with the prefix "Hu" before the antibody chain name. The anti-RON antibody chains humanized by the HUMAN ENGINEERING™ method, as described herein, are designated with the prefix "HE" before the antibody chain name.

[0212] The anti-RON antibody heavy chain 07F01 was humanized according to the SUPERHUMANIZATION™ method. Human germline sequence IGHV3-48*01 (also referred to herein as Hv3-48) was selected as the human heavy chain framework. In some embodiments, the human Hv3-48 heavy chain framework sequence was mutated at amino acid position 28 (e.g., D28T). Amino acid numbering is based on the Kabat numbering system.

[0213] The anti-RON antibody light chain 07F01 was humanized according to the HUMAN ENGINEERING™ method. Human germline sequence IGKV1-9*01 was selected as the human light chain framework.

[0214] The anti-RON antibody heavy chain 29B06 was humanized by the CDR grafting method with back mutations. Human germline sequence IGHV4-59 *01 (also referred to herein as Hv4-59) was selected as the human framework. The human framework was back-mutated at amino acid positions 27, 30, 39, 44, 47, 48, 67, 71, and 78 to the murine sequence when the Kabat CDR definitions were used. The back-mutated human Hv4-59 framework sequence was further mutated to comprise at least one amino acid substitution at positions 27, 30, 48, 67, and 78. Amino acid substitutions in the back-mutated Hv4-59 framework sequence (e.g., amino acid substitution from a murine residue to a human residue, e.g., a human residue found in IGHV4-59) may be selected from the group consisting of D27G, T30S, M48I, I67V and Y78F. Amino acid numbering is based on the Kabat numbering system.

[0215] The anti-RON antibody light chain 29B06 was humanized according to the SUPERHUMANIZATION™ method. Human germline sequence IGKV2-28*01 was selected as the human light chain framework.
Chimeric (murine variable region and human constant region) 07F01 and 29B06
heavy (human IgGl) and light (human Kappa) chains were also constructed. The cysteine
residue present in the heavy chain CDR3 of 07F01 was changed to serine to prevent potential
aggregation. To generate chimeric antibodies, the murine variable regions were fused to the
human constant region using overlap extension PCR, including (in the following order): 5’
HindIII restriction site, Kozak consensus sequence, amino terminal signal sequence, mouse
variable region, human IgGl or Kappa constant region, stop codon, and 3’ EcoRI restriction
site.

The humanized and chimeric heavy chains were subcloned into pEE6.4 (Lonza,
Basel, Switzerland) via HindIII and EcoRI sites using In-Fusion™ PCR cloning (Clontech,
Mountain View, CA). The humanized and chimeric Kappa light chains were subcloned into
pEE14.4 (Lonza) via HindIII and EcoRI sites using In-Fusion™ PCR cloning.

Humanized antibody chains or chimeric antibody chains were transiently transfected
into 293T cells to produce antibody. Antibody was either purified or used in cell culture media
supernatant for subsequent in vitro analysis. Binding of the chimeric and humanized antibodies
to human RON was measured as described below. The results are summarized in Table 20.

Additionally, some humanized antibody heavy and light chain combinations were
stably expressed in CHOK1SV cells using the GS System™ (Lonza) in order to produce large
quantities of purified humanized antibody. A single expression vector was constructed by
combining pEE6.4 and pEE14.4 based vectors. First, pEE6.4 containing full length humanized
heavy chain cDNA was digested with NotI and SaI to isolate the hCMV-MIE promoter + full
length humanized heavy chain cDNA + SV40 poly A fragment. This fragment was inserted
into the pEE14.4 vector already containing full length humanized light chain cDNA via
NotI/SaI sites, thus creating an expression vector that simultaneously expresses heavy and
light chains. The combined heavy and light chain vector was linearized and transfected into
CHOK1SV cells. Stable clones were selected in the presence of methionine sulfoximine.

Each of the possible combinations of the humanized 07F01 immunoglobulin heavy
chain and immunoglobulin light chain variable regions are set forth below in Table 13.
Each of the possible combinations of the humanized 29B06 immunoglobulin heavy chain and immunoglobulin light chain variable regions are set forth below in Table 14.

<table>
<thead>
<tr>
<th>Light Chain Variable Region</th>
<th>Heavy Chain Variable Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>HE L 07F01 Kv1-9 Light Variable (SEQ ID NO: 139)</td>
<td>Sh07F01 Hv3-48 Heavy Variable (SEQ ID NO: 135)</td>
</tr>
<tr>
<td>HE L 07F01 Kv1-9 Light Variable (SEQ ID NO: 139)</td>
<td>Sh07F01 Hv3-48 D28T T60A L63V E65G Heavy Variable (SEQ ID NO: 137)</td>
</tr>
<tr>
<td>Sh07F01 Kv1-9 F1 Light Variable (SEQ ID NO: 141)</td>
<td>Sh07F01 Hv3-48 Heavy Variable (SEQ ID NO: 135)</td>
</tr>
<tr>
<td>Sh07F01 Kv1-9 F1 Light Variable (SEQ ID NO: 141)</td>
<td>Sh07F01 Hv3-48 D28T T60A L63V E65G Heavy Variable (SEQ ID NO: 137)</td>
</tr>
</tbody>
</table>

The nucleic acid sequences encoding and the protein sequences defining variable regions of the humanized 07F01 and 29B06 antibodies are summarized below (amino terminal signal peptide sequences are not shown). Sequences of the modified chimeric 07F01 heavy variable region in which the cysteine in CDR3 is changed to serine are also summarized below. CDR sequences (Kabat definition) are shown in bold and are underlined in the amino acid sequences.

Nucleic Acid Sequence Encoding the Chimeric 07F01 C102S Heavy Chain Variable Region (SEQ ID NO: 132)

```
1  gaggtgaagc ttctcgagtc tggaggtggc ctggtgcagc cgggtggatc cctgaaactc
61  tcctgtgcag cctcaggatt cgattttagt agacactgga tgagttgggt ccggctggct
121  ccagggaaag ggctagaatg gatcgcagaa attaatccag atagcagaac gataaactat
181  acgcacatctc taaaggagaa attatcatatc tccagagaca acgcacaaaaa ttccgttgttt
241  ctgcacatgta acagaggtag atctgagggc acagcccttt atatactgtgc aagacgggta
301  agaatctcatt actagggcgc tatggacagc tggggctcaag gaacctcagt caccgtctcc
361  tea
```
Protein Sequence Defining the Chimeric 07F01 C102S Heavy Chain Variable Region (SEQ ID NO: 133)

1 evkllesggg lvqpggs1kl scaasgfdafs rhwmswvrla pgkglewiae inpdartiny
5
6i tpkskefii srdnakns1f lgmnrvrsed talyycarrv rihyygamds wggqtsvts
121 s

Nucleic Acid Sequence Encoding the Sh07F01 Hv3-48 Heavy Chain Variable Region (SEQ ID NO: 134)

1 gaggttcagc tggtagaatc cggaggaggg ttggtccaac cttgggtgatc actcagacctt
10
6i tcatgcgcgc ccaccgcgctt tgaacttctca cgacacctaga tgagcttgggt ccggcagggctt
121 ccagcgaaggg gctctcagttgt gtttagcgag atcaatccag acacgcaac acttaactat
181 acacccagtc tgaggttccag gccctcattgata aagcctcttgata ccgcaacag ccctcagttac
241 tggcactagta actccccctgct gcctgagatc acagtctgtgt actctgctgc aacgctcgctg
301 cgaatccact attacggggc aatggattct tggggccagg gttacccctgt gactgtgaagttct
361 tct

Protein Sequence Defining the Sh07F01 Hv3-48 Heavy Chain Variable Region (SEQ ID NO: 135)

1 evqlvesggg lvqpggslrl scaasgfdafs rhwmswvrgqa pgkglewvsr inpdartiny
20
6i tpnlkefii srdnaknsly lgmnrvrsed talyycarrv rihyygamds wggqtsvts
121 s

Nucleic Acid Sequence Encoding the Sh07F01 Hv3-48 D28T T60A L63V E65G Heavy Chain Variable Region (SEQ ID NO: 136)

25 1 gaggttgcagc tggtagaatc cggaggaggg ttggtCCAac cttgggtgatc actcagacctt
30
6i tcatgcgcgc ccaccgcgctt tgaacttctca cgacacctaga tgagcttgggt ccggcagggctt
121 ccagcgaaggg gctctcagttgt gtttagcgag atcaatccag acacgcaac acttaactat
181 acacccagtc tgaggttccag gccctcattgata aagcctcttgata ccgcaacag ccctcagttac
241 tggcactagta actccccctgct gcctgagatc acagtctgtgt actctgctgc aacgctcgctg
301 cgaatccact attacggggc aatggattct tggggccagg gttacccctgt gactgtgaagttct
361 tct

Protein Sequence Defining the Sh07F01 Hv3-48 D28T T60A L63V E65G Heavy Chain Variable Region (SEQ ID NO: 137)

35 1 evqlvesggg lvqpggslrl scaasgftfs rhwmswvrgqa pgkglewvsr inpdartiny
40
6i spkvkgrffi srdnaknsly lgmnrvrsed talyycarrv rihyygamds wggqtsvts
121 s

Nucleic Acid Sequence Encoding the HE L 07F01 Kyl-9 Kappa Chain Variable Region (SEQ ID NO: 138)

1 gatatcagct tgaactcagtc tcagtctcttt tgtaqtatca cagtggccag caggggtcacc
40
6i gttacactgc cagcatcaca gaagttgqga aqctctcttg tctgtgatca gcaaaagcct
121 cggagaggg ccaaaaaact catctatctt gcctcctcttc tgtactcccg cgtaccaagt
181 agattcttctgt gtagccagtc cggagacag gttcctcacta caattcagc tgtgcagcct
Protein Sequence Defining the HE L 07F01 Kyl-9 Kappa Chain Variable Region

(SEQ ID NO: 139)

1 digltqgsqsf vstsvgdrvt vtcrasqvng ssslvwyqqkp gkskpliys asf lysgyps
61 rfsgrsggte ftiltiqvqpg efadvcpcqj ynnpit fgg gtvkeik

Nucleic Acid Sequence Encoding the sh07F01 Kyl-9 F1 Kappa Chain Variable Region

(SEQ ID NO: 140)

1 gacattcagc tgactcagtc gcgactcactg ttgtcggcgt cctgggtga cagagtgact
61 attcacagtc gcctgctcag acacctgctg tcactgtctg acagtgtacac gcagaaaccc
121 gttggaccgct catcatctca ggtcgtacttg tggatacg ggtccctcca
181 cgggacccca cggaccggag gtcacatctca gtcttcacag gttcgaaccg
241 gaagatttttg aacagtacta ctcagcggaa tacaataact accacactcag gttcggaggg
301 ggacacgaaag tagagatcag c

Protein Sequence Defining the sh07F01 Kyl-9 F1 Kappa Chain Variable Region

(SEQ ID NO: 141)

1 digltqgsqsf lsvlsvgdrvt iterasqvng ssslvwyqqkp gkapktliys asf lysgyps
61 rfsgrsggte ftiltiqvqpg efadvcpcqj ynnpit fgg gtvkeik

Nucleic Acid Sequence Encoding the Sh29B06 Hv4-59 Heavy Chain Variable Region

(SEQ ID NO: 142)

1 caagttcagc tgcaagaatc cggaccagga ttggtcaaac tcctcagagac actctctctt
61 aacgcagcagcc gtcggctggc tggatggttc acatgcaccg ctctctctct gtcgacgttaac
121 cccggtctgga agagcggqatc gaccataagc gtctgatacag taaagaaccg gttccccctt
181 cggaccgagc gttcggcagc ggtcagatgt ctctgcactt attctctctct tgcctgcacgt
241 gactacgaaag tagagacttg gggccaggtt actctctgtga ctctgtgtgc
301 gactacgaaag tagagacttg gggccaggtt actctctgtga ctctgtgtgc
t

Protein Sequence Defining the Sh29B06 Hv4-59 Heavy Chain Variable Region

(SEQ ID NO: 143)

1 qvqlqesgpg lvkpsesls fctvsggsis sgsywnwirgppgkglewyig_ isysgktyyn
61 psikrsrvtis vdetkinquqf klsstvadt avyykar sky dyamdywggg tlvtvss

Nucleic Acid Sequence Encoding the Hu29B06 Hv4-59 Heavy Chain Variable Region

(SEQ ID NO: 144)

1 caagttcagc tgcaagaatc cggaccagga ttggtcaaac tcctcagagac actctctctt
61 aacgcagcagcc gtcggctggc tggatggttc acatgcaccg ctctctctct gtcgacgttaac
121 cccggtctgga agagcggqatc gaccataagc gtctgatacag taaagaaccg gttccccctt
181 cggaccgagc gttcggcagc ggtcagatgt ctctgcactt attctctctct tgcctgcacgt
241 gactacgaaag tagagacttg gggccaggtt actctctgtga ctctgtgtgc
301 gactacgaaag tagagacttg gggccaggtt actctctgtga ctctgtgtgc
t
Protein Sequence Defining the Hu29B06 Hv4-59 Heavy Chain Variable Region
(SEQ ID NO: 145)

1 qvqlqesgpg lvkpsettsl tctvsqdsit sgywnwirkp pgkkleymg_ isysqkttyyn
61 psiksritis rdtsktnqysl klsstvaadt avyycarsky dyamdywqqg tlvtvss

Nucleic Acid Sequence Encoding the Hu29B06 D27G T30S M48I I67V Y78F
Heavy Chain Variable Region (SEQ ID NO: 146)

1 caagttcagc tgcaagaatc cggaccagga ttggtcaaac cttcagagac actcagcctg
6 acctgccacc tgagccagt cagcatatcc tccggttatt ggaactggat ccggaagcca
121 ccaggcaaga agtccgagta catgctctac atcgctata ccggaatcc ctattacaac
181 cccagctctga agagccgagt gaccataagc avyycarsky dyamdywqpg tlvtvss
241 aagtcttcct gcgtgagcgc gctgtactg attgtcagq gctcaagat
301 gactacgcaaa tggactatgg gccgccaggt actcctgtga ctgtgacctt

Protein Sequence Defining the Hu29B06 D27G T30S M48I I67V Y78F Heavy
Chain Variable Region (SEQ ID NO: 147)

1 qvqlqesgpg lvkpsettsl tctvsqdsis sgywnwirkp pgkkleyig_ isysqkttyyn
61 psiksrtvtis rdtsktnqfsl klsstvaadt avyycarsky dyamdywqqq tlvtvss

Nucleic Acid Sequence Encoding the Sh29B06 Kv2-28 Kappa Chain Variable
Region (SEQ ID NO: 148)

1 gatatcgtta tgacccagag cccacttagt ttgctctgta ctcctggcga gcctgcaagt
61 attttctgcc gtgctagcga acctttggat aacttggta tacacttcat gaattgtat
121 ctcacacaac ccggccaagg cccctgctaa ctttaccagc ccgctgctcc
cagtccagag ccaggtctccc tattgtcaac agtcccaaga ggtctcccc
241 cgggtggagc ccgagccagc ggctgtgact attgtcagq gctcaagat
301 actttccgagc gttggaacaa ggttgaggtt aag

Protein Sequence Defining the Sh29B06 Kv2-28 Kappa Chain Variable Region
(SEQ ID NO: 149)

1 divmtqspsl lpvtpgpeas iscraeivd nfgisfmwvy lqkpgpssplq liyaaasnggs
61 gvpdrfsqsg sgtdftklis rveaedvgy ycqgskveqp tfggktkveik

The amino acid sequences defining the immunoglobulin heavy chain variable
regions for the antibodies produced in Example 14 are aligned in FIGs. 12A and 12B. Amino
terminal signal peptide sequences (for proper expression/secretion) are not shown. CDR<sub>1</sub>,
CDR<sub>2</sub>, and CDR<sub>3</sub> (Kabat definition) are identified by boxes. FIGs. 13A and 13B show an
alignment of the separate CDR<sub>1</sub>, CDR<sub>2</sub>, and CDR<sub>3</sub> sequences for each of the variable region
sequences shown in FIGs. 12A and 12B, respectively.
The amino acid sequences defining the immunoglobulin light chain variable regions for the antibodies in Example 14 are aligned in FIG. 14A and 14B. Amino terminal signal peptide sequences (for proper expression/secretion) are not shown. CDR\textsubscript{1}, CDR\textsubscript{2} and CDR\textsubscript{3} are identified by boxes. FIGs. 15A and 15B show an alignment of the separate CDR\textsubscript{1}, CDR\textsubscript{2}, and CDR\textsubscript{3} sequences for each of the variable region sequences shown in FIGs. 14A and 14B, respectively.

Table 15 is a concordance chart showing the SEQ ID NO. of each sequence discussed in this Example.

<table>
<thead>
<tr>
<th>SEQ. ID NO.</th>
<th>Nucleic Acid or Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>132</td>
<td>Chimeric 07F01 C102S Heavy Chain Variable Region—nucleic acid</td>
</tr>
<tr>
<td>133</td>
<td>Chimeric 07F01 C102S Heavy Chain Variable Region—protein</td>
</tr>
<tr>
<td>5</td>
<td>Chimeric 07F01 C102S Heavy Chain CDR\textsubscript{1}</td>
</tr>
<tr>
<td>6</td>
<td>Chimeric 07F01 C102S Heavy Chain CDR\textsubscript{2}</td>
</tr>
<tr>
<td>123</td>
<td>Chimeric 07F01 C102S Heavy Chain CDR\textsubscript{3}</td>
</tr>
<tr>
<td>134</td>
<td>Sh07F01 Hv3-48 Heavy Chain Variable Region—nucleic acid</td>
</tr>
<tr>
<td>135</td>
<td>Sh07F01 Hv3-48 Heavy Chain Variable Region—protein</td>
</tr>
<tr>
<td>5</td>
<td>Sh07F01 Hv3-48 Heavy Chain CDR\textsubscript{1}</td>
</tr>
<tr>
<td>6</td>
<td>Sh07F01 Hv3-48 Heavy Chain CDR\textsubscript{2}</td>
</tr>
<tr>
<td>123</td>
<td>Sh07F01 Hv3-48 Heavy Chain CDR\textsubscript{3}</td>
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<tr>
<td>136</td>
<td>Sh07F01 Hv3-48 D28T T60A L63V E65G Heavy Chain Variable Region—nucleic acid</td>
</tr>
<tr>
<td>137</td>
<td>Sh07F01 Hv3-48 D28T T60A L63V E65G Heavy Chain Variable Region—protein</td>
</tr>
<tr>
<td>5</td>
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</tr>
<tr>
<td>122</td>
<td>Sh07F01 Hv3-48 D28T T60A L63V E65G Heavy Chain CDR\textsubscript{2}</td>
</tr>
<tr>
<td>123</td>
<td>Sh07F01 Hv3-48 D28T T60A L63V E65G Heavy Chain CDR\textsubscript{3}</td>
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<tr>
<td>138</td>
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<td>139</td>
<td>HE L 07F01 Kvl-9 Light (kappa) Chain Variable Region—protein</td>
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<td>HE L 07F01 Kvl-9 Light (kappa) Chain Variable Region—protein</td>
</tr>
<tr>
<td>141</td>
<td>HE L 07F01 Kvl-9 Light (kappa) Chain Variable Region—protein</td>
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<td>10</td>
<td>HE L 07F01 Kvl-9 Light (kappa) Chain CDR\textsubscript{3}</td>
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<tr>
<td>141</td>
<td>Sh07F01 Kvl-9 F1 Light (kappa) Chain Variable Region—protein</td>
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<tr>
<td>130</td>
<td>Sh07F01 Kvl-9 F1 Light (kappa) Chain CDR\textsubscript{1}</td>
</tr>
<tr>
<td>131</td>
<td>Sh07F01 Kvl-9 F1 Light (kappa) Chain CDR\textsubscript{2}</td>
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<tr>
<td>10</td>
<td>Sh07F01 Kvl-9 F1 Light (kappa) Chain CDR\textsubscript{3}</td>
</tr>
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<td>----------------------------------------------------------------------------------------</td>
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<tr>
<td>142</td>
<td>Sh291306 Hv4-59 Heavy Chain Variable Region—nucleic acid</td>
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<td>144</td>
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<td>Sh291306 Hv4-59 Heavy Chain CDR2</td>
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<td>Sh291306 Hv4-59 Heavy Chain CDR3</td>
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<td>Sh291306 Hv4-59 Heavy Chain CDR4</td>
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<td>149</td>
<td>Hu29 B06 Hv4-59 Heavy Chain Variable Region—protein</td>
</tr>
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<td>Hu29 B06 Hv4-59 Heavy Chain CDR1</td>
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<td>Hu29 B06 Hv4-59 Heavy Chain CDR2</td>
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<tr>
<td>152</td>
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<tr>
<td>154</td>
<td>Hu29 B06 Hv4-59 Heavy Chain CDR1</td>
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<tr>
<td>155</td>
<td>Hu29 B06 Hv4-59 Heavy Chain CDR2</td>
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<tr>
<td>156</td>
<td>Hu29 B06 Hv4-59 Heavy Chain CDR3</td>
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<tr>
<td>157</td>
<td>Hu29 B06 Hv4-59 Heavy Chain CDR4</td>
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</table>

**[0244]** Humanized monoclonal antibody heavy chain CDR sequences (Kabat, Chothia, and IMGT definitions) are shown in Table 16.
### Table 16

<table>
<thead>
<tr>
<th>Kabat</th>
<th>CDR1</th>
<th>CDR2</th>
<th>CDR3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>07F01</strong></td>
<td>RHWMS (SEQ ID NO: 5)</td>
<td>EINPDSRTINYTPSLKE (SEQ ID NO: 6)</td>
<td>RVRIHYYGAMDC (SEQ ID NO: 7)</td>
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<tr>
<td>Chimeric 07F01 C102S</td>
<td>RHWMS (SEQ ID NO: 5)</td>
<td>EINPDSRTINYTPSLKE (SEQ ID NO: 6)</td>
<td>RVRIHYYGAMDS (SEQ ID NO: 123)</td>
</tr>
<tr>
<td>Sh07F01 Hv3-48</td>
<td>RHWMS (SEQ ID NO: 5)</td>
<td>EINPDSRTINYTPSLKE (SEQ ID NO: 6)</td>
<td>RVRIHYYGAMDS (SEQ ID NO: 123)</td>
</tr>
<tr>
<td>Sh07F01 Hv3-48 D28T T60A L63V E65G</td>
<td>RHWMS (SEQ ID NO: 5)</td>
<td>EINPDSRTINYAPSVKG (SEQ ID NO: 122)</td>
<td>RVRIHYYGAMDS (SEQ ID NO: 123)</td>
</tr>
<tr>
<td><strong>29B06</strong></td>
<td>SGYWN (SEQ ID NO: 45)</td>
<td>YISYSGKTYYNPSLKS (SEQ ID NO: 46)</td>
<td>SKYDYAMDY (SEQ ID NO: 47)</td>
</tr>
<tr>
<td>Sh29B06 Hv4-59</td>
<td>SGYWN (SEQ ID NO: 45)</td>
<td>YISYSGKTYYNPSLKS (SEQ ID NO: 46)</td>
<td>SKYDYAMDY (SEQ ID NO: 47)</td>
</tr>
<tr>
<td><strong>Hu29B06 Hv4-59</strong></td>
<td>SGYWN (SEQ ID NO: 45)</td>
<td>YISYSGKTYYNPSLKS (SEQ ID NO: 46)</td>
<td>SKYDYAMDY (SEQ ID NO: 47)</td>
</tr>
<tr>
<td>Hu29B06 Hv4-59 D27G T30S M48I I67V Y78F</td>
<td>SGYWN (SEQ ID NO: 45)</td>
<td>YISYSGKTYYNPSLKS (SEQ ID NO: 46)</td>
<td>SKYDYAMDY (SEQ ID NO: 47)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chothia</th>
<th>CDR1</th>
<th>CDR2</th>
<th>CDR3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>07F01</strong></td>
<td>GFDFSRH (SEQ ID NO: 51)</td>
<td>NPDSRT (SEQ ID NO: 52)</td>
<td>RVRIHYYGAMDC (SEQ ID NO: 7)</td>
</tr>
<tr>
<td>Chimeric 07F01 C102S</td>
<td>GFDFSRH (SEQ ID NO: 51)</td>
<td>NPDSRT (SEQ ID NO: 52)</td>
<td>RVRIHYYGAMDS (SEQ ID NO: 125)</td>
</tr>
<tr>
<td>Sh07F01 Hv3-48</td>
<td>GFDFSRH (SEQ ID NO: 51)</td>
<td>NPDSRT (SEQ ID NO: 52)</td>
<td>RVRIHYYGAMDS (SEQ ID NO: 125)</td>
</tr>
<tr>
<td>Sh07F01 Hv3-48 D28T T60A L63V E65G</td>
<td>GFDFSRH (SEQ ID NO: 51)</td>
<td>NPDSRT (SEQ ID NO: 52)</td>
<td>RVRIHYYGAMDS (SEQ ID NO: 125)</td>
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<tr>
<td><strong>29B06</strong></td>
<td>GDSITSG (SEQ ID NO: 59)</td>
<td>SYSGK (SEQ ID NO: 60)</td>
<td>SKYDYAMDY (SEQ ID NO: 47)</td>
</tr>
<tr>
<td>Sh29B06 Hv4-59</td>
<td>GGSISSG (SEQ ID NO: 126)</td>
<td>SYSGK (SEQ ID NO: 60)</td>
<td>SKYDYAMDY (SEQ ID NO: 47)</td>
</tr>
<tr>
<td><strong>Hu29B06 Hv4-59</strong></td>
<td>GDSITSG (SEQ ID NO: 59)</td>
<td>SYSGK (SEQ ID NO: 60)</td>
<td>SKYDYAMDY (SEQ ID NO: 47)</td>
</tr>
<tr>
<td>Hu29B06 Hv4-59 D27G T30S M48I I67V Y78F</td>
<td>GGSISSG (SEQ ID NO: 126)</td>
<td>SYSGK (SEQ ID NO: 60)</td>
<td>SKYDYAMDY (SEQ ID NO: 47)</td>
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Table 16 Con't

<table>
<thead>
<tr>
<th></th>
<th>IMGT</th>
</tr>
</thead>
<tbody>
<tr>
<td>07F01</td>
<td>CDR1: GFDFSRHW (SEQ ID NO: 61)</td>
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<tr>
<td>Chimeric 07F01 C102S</td>
<td>CDR1: GFDFSRHW (SEQ ID NO: 61)</td>
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<td>CDR1: GFDFSRHW (SEQ ID NO: 61)</td>
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<tr>
<td>Sh07F01 Hv3-48 D28T T60A L63V E65G</td>
<td>CDR1: GFTFSRHW (SEQ ID NO: 127)</td>
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<tr>
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</tr>
<tr>
<td>Sh29B06 Hv4-59</td>
<td>CDR1: GGSISSGY (SEQ ID NO: 129)</td>
</tr>
<tr>
<td>Hu29B06 Hv4-59</td>
<td>CDR1: GDSITSGY (SEQ ID NO: 73)</td>
</tr>
<tr>
<td>Hu29B06 Hv4-59 D27G T30S M48I I67V Y78F</td>
<td>CDR1: GGSISSGY (SEQ ID NO: 129)</td>
</tr>
</tbody>
</table>

Humanized monoclonal antibody Kappa light chain CDR sequences (Kabat, Chothia, and IMGT definitions) are shown in Table 17.

Table 17

<table>
<thead>
<tr>
<th></th>
<th>Kabat/Chothia</th>
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<tbody>
<tr>
<td></td>
<td>CDR1</td>
</tr>
<tr>
<td>07F01</td>
<td>KASQNVGSSLV (SEQ ID NO: 8)</td>
</tr>
<tr>
<td>HE L 07F01 Kv1-9</td>
<td>RASNQVGSSLV (SEQ ID NO: 130)</td>
</tr>
<tr>
<td>Sh07F01 Kv1-9 F1</td>
<td>RASNQVGSSLV (SEQ ID NO: 130)</td>
</tr>
<tr>
<td>29B06</td>
<td>RASQVDFGIFS FMN (SEQ ID NO: 48)</td>
</tr>
<tr>
<td>Sh29B06 Kv2-28</td>
<td>RASQVDFGIFS FMN (SEQ ID NO: 48)</td>
</tr>
</tbody>
</table>
To create the complete chimeric and humanized heavy or kappa chain antibody sequences, each variable sequence above is combined with its respective human constant region. For example, a complete heavy chain comprises a heavy variable sequence followed by a human IgG1 heavy chain constant sequence. A complete kappa chain comprises a kappa variable sequence followed by the human kappa light chain constant sequence.

Nucleic Acid Sequence Encoding the Human IgG1 Heavy Chain Constant Region
(SEQ ID NO: 150)

<table>
<thead>
<tr>
<th>CDR1</th>
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<th>IMGT</th>
</tr>
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<tbody>
<tr>
<td>O7F01</td>
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<td>SAS</td>
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<td>HE L O7F01 Kv1-9</td>
<td>QNVGSS (SEQ ID NO: 76)</td>
<td>SAS</td>
</tr>
<tr>
<td>Sh07F01 Kv1-9 F1</td>
<td>QNVGSS (SEQ ID NO: 76)</td>
<td>SAS</td>
</tr>
<tr>
<td>29B06</td>
<td>EIVDNFGISF (SEQ ID NO: 81)</td>
<td>AAS</td>
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<tr>
<td>Sh29B06 Kv2-28</td>
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<td>AAS</td>
</tr>
</tbody>
</table>

Nucleic Acid Sequence

```
1 gcctcaacaa aaggaccaag tgtgttccca ctcgcccccta gcagcaagag tacatccggg
ggcactgcag cactcggctg cctcgtcaag gattattttc cagagccagt aaccgtgagc
tggaacagtg gagcactcac ttctggtgtc catacttttc ctgctgtcct gcaaagctct
ggcctgtact cactcagctc cgtcgtgacc gtgccatctt catctctggg cactcaagacc
tacatctgtga atgtaaaacca caaactaagtg tctctttttc aaaaagctt ccgaggtagc
tgagttgacat gtgtggagt gacgcagcct ctcggtccctg tgggagcacg tgggcttggag
tacgttgatag gttggaactgt tcataatcct aagaccaaggg ttagaggtgcg gtagtagctt
tgcgagcggcc ctcgggaggcc aagctactac accaaggctg ccgagctgtc
tgtgttgttg acagtctttc ccaggtggtcg gcaggtgagc ggacgaggttg cgggacagtc
tggtggtgttg acaggccttt ctgggaggtcg gacctgctgg ggggagagcgc
```

[0247] Nucleic Acid Sequence Encoding the Human IgG1 Heavy Chain Constant Region (SEQ ID NO: 150)
Protein Sequence Defining the Human IgGl Heavy Chain Constant Region (SEQ ID NO: 151)

1 astkgpsvfp lapsskstsg gtaalgclvk dyfpepvts wnsagltsgv htfpavglss
61 glyslsssvvt vпасs1qtq yincvnhkps tntkdvrtvep kscdkhtctp pcpapelllgg
5 121 psvflfppkp kdtldmirtp evtcwavivo hespevknw yvdygevhnk etckpregeyn
181 styrvvsvlt vlhdwlnlk eykckvsnka lpadiektis kagqgprepg vytltppsrz
241 mtknqvs1tc lvkgfypdsi aveweszgqp eynyktpppv ldsdgsfllly skltvdksrw
301 qgnvfsccsv mhealhnhyt qksls1spgk

Nucleic Acid Sequence Encoding the Human Kappa Light Chain Constant Region (used for chimeric antibodies) (SEQ ID NO: 152)

1 cgacagtctg ccgctccccct cctgttcatc tttccaccca gttatgagca actxggtctct
61 gtactgtctt ccagtctgttg tctgtcagac aaatttcacc tctgagaagc caaagtccca
121 tggagagtag acaagctgac ctaagtcccc ctaagccca aatcagttac gcagccaggt
181 tcaagagcag gtcatatcgc ccggagcagc ccttcgagccc tgtcaagagc cattacgag
241 acacacacagc ccaggggagc cagagagcagc caccagggc cggcagagc aagccacaaa
301 tcttttaacc gtcgagagtt t

Nucleic Acid Sequence Encoding the Human Kappa Light Chain Constant Region (used for humanized antibodies) (SEQ ID NO: 153)

1 cgacagtctg ccgctccccct cctgttcatc tttccaccca gttatgagca actxggtctct
61 gtactgtctt ccagtctgttg tctgtcagac aaatttcacc tctgagaagc caaagtccca
121 tggagagtag acaagctgac ctaagtcccc ctaagccca aatcagttac gcagccaggt
181 tcaagagcag gtcatatcgc ccggagcagc ccttcgagccc tgtcaagagc cattacgag
241 acacacacagc ccaggggagc cagagagcagc caccagggc cggcagagc aagccacaaa
25 301 tcttttaacc gtcgagagtt t

Protein Sequence Defining the Human Kappa Light Chain Constant Region (used for chimeric and humanized antibodies) (SEQ ID NO: 154)

1 rtvaapsvf fppsdeqlks gtasvslln ln fypreavko v wkvndalsq gnsqesvteq d
30 61 skdstyslss tltlskdye kkhvvecve vhqlsspvtk sfenrgec

The following sequences represent the actual or contemplated full length heavy and light chain sequences (i.e., containing both the variable and constant regions sequences) for each antibody described in this Example. Signal sequences for proper secretion of the antibodies (e.g., signal sequences at the 5' end of the DNA sequences or the amino terminal end of the protein sequences) are not shown in the full length heavy and light chain sequences disclosed herein and are not included in the final secreted protein. Also not shown are stop codons for termination of translation required at the 3' end of the DNA sequences. It is within ordinary skill in the art to select a signal sequence and/or a stop codon for expression of the disclosed full length IgG heavy chain and light chain sequences. It is also contemplated that the variable
region sequences can be ligated to other constant region sequences to produce active full length IgG heavy and light chains.

[Nucleic Acid Sequence Encoding the Full Length Chimeric 07F01 C102S Heavy Chain (Mouse Heavy Chain Variable Region and Human IgG1 Constant Region) (SEQ ID NO: 155)]

```
1  gaggtgagacg ttctcagtag tc gaggtgaggc ctgtgtcagc cgggtggatc cctgaaacct
gtccaggtat gcgattttact cagctgttcc gttccttctg tgcggtgct
10
tccagtgcag cctcagattg cggatttcgag acatatttag ttgattgcct gcgctgccgt
dcgagtttag gcgattttact cagctgttcc gttccttctg tgcggtgct
15
tgagaattc caagacctgg gtggacagac aagcttggc gggtcagaca gtaaaacctc
cgcgtgcttt gcctgaaacct
ggagaacct ctgctttgac cgggtggatc cctgaaacct
gtccaggtat gcgattttact cagctgttcc gttccttctg tgcggtgct
20
acaccagtcc tccccagct gggagggttt aagctatttg ccgcgtgcttt gcctgaaacct
acaccagtcc tccccagct gggagggttt aagctatttg ccgcgtgcttt gcctgaaacct
25
tgagaattc caagacctgg gtggacagac aagcttggc gggtcagaca gtaaaacctc
cgcgtgcttt gcctgaaacct
ggagaacct ctgctttgac cgggtggatc cctgaaacct
gtccaggtat gcgattttact cagctgttcc gttccttctg tgcggtgct
30
```

[Protein Sequence Defining the Full Length Chimeric 07F01 C102S Heavy Chain (Mouse Heavy Chain Variable Region and Human IgG1 Constant Region) (SEQ ID NO: 156)]

```
1  evklllesggg lvqpgggsllk scaasgfdfe rhwmswvrla pgkglewiae inpdsrtiny
tpslkekfll sdrrnaksllf lqnmmrvesd talvycarrv rihygymds wggstvs
35	sastskgspsv llapsskst ptgtaalgcUY dkyfpepvtv swnsngstg vhtfpavlqs
sglysllsvv tsvpsslgltq tyicvnvnhp sntkvdkrve pksedkthtc ppcpapellg
241  gspvflfppk ptkdtlmisrt pevtcvvvvdv shedpevknf wydvgvevhn aktpkpreeg
30
```
### Protein Sequence Defining the Full Length Chimeric 07F01 Light Chain (Mouse Kappa Chain Variable Region and Human Kappa Constant Region) (SEQ ID NO: 158)

<table>
<thead>
<tr>
<th>Position</th>
<th>Sequence</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>divltqsgk &lt;VSTSGVAR&gt;</td>
</tr>
<tr>
<td>2</td>
<td>vstsvgarv &lt;VTCKASQNVG&gt;</td>
</tr>
<tr>
<td>3</td>
<td>sstsvgarv &lt;STSTSGVNSG&gt;</td>
</tr>
<tr>
<td>4</td>
<td>vstsvgarv &lt;STSTSGVNSG&gt;</td>
</tr>
<tr>
<td>5</td>
<td>ftylspsv &lt;FTLGSPSY&gt;</td>
</tr>
</tbody>
</table>

### Nucleic Acid Sequence Encoding the Full Length Chimeric 29B06 Heavy Chain (Mouse Heavy Chain Variable Region and Human IgGl Constant Region) (SEQ ID NO: 159)

<table>
<thead>
<tr>
<th>Position</th>
<th>Sequence</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>gaggtgcagc &lt;TTCAAGGATC&gt;</td>
</tr>
<tr>
<td>2</td>
<td>ttcaaggatc &lt;TCAACTGACG&gt;</td>
</tr>
<tr>
<td>3</td>
<td>aggacctagc &lt;CTCTGAAAC&gt;</td>
</tr>
<tr>
<td>4</td>
<td>ctttcagac &lt;TCTTCCCTC&gt;</td>
</tr>
<tr>
<td>5</td>
<td>tctctgggt &lt;CTCTGCTTC&gt;</td>
</tr>
</tbody>
</table>

### Protein Sequence Defining the Full Length Chimeric 29B06 Heavy Chain (Mouse Heavy Chain Variable Region and Human IgGl Constant Region) (SEQ ID NO: 160)

<table>
<thead>
<tr>
<th>Position</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>evqlqesgps &lt;LKVPQSTSL&gt;</td>
</tr>
<tr>
<td>2</td>
<td>lkvpsqtsl1 &lt;TCSVTGSDFST&gt;</td>
</tr>
<tr>
<td>3</td>
<td>sgywnwirkf &lt;SGWYNSGWI&gt;</td>
</tr>
<tr>
<td>4</td>
<td>pgknleymg &lt;PGKNLAYMG&gt;</td>
</tr>
<tr>
<td>5</td>
<td>isysgktyn &lt;ISYSGKTYN&gt;</td>
</tr>
</tbody>
</table>

### Chimeric Constant Region (Mouse Light Chain Variable Region and Human IgGl Constant Region) (SEQ ID NO: 161)

<table>
<thead>
<tr>
<th>Position</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>divltqsgk &lt;VSTSGVAR&gt;</td>
</tr>
<tr>
<td>2</td>
<td>vstsvgarv &lt;VTCKASQNVG&gt;</td>
</tr>
<tr>
<td>3</td>
<td>sstsvgarv &lt;STSTSGVNSG&gt;</td>
</tr>
<tr>
<td>4</td>
<td>vstsvgarv &lt;STSTSGVNSG&gt;</td>
</tr>
<tr>
<td>5</td>
<td>ftylspsv &lt;FTLGSPSY&gt;</td>
</tr>
</tbody>
</table>

### Chimeric Length Region (Mouse Light Chain Variable Region and Human IgGl Constant Region) (SEQ ID NO: 162)

<table>
<thead>
<tr>
<th>Position</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>evqlqesgps &lt;LKVPQSTSL&gt;</td>
</tr>
<tr>
<td>2</td>
<td>lkvpsqtsl1 &lt;TCSVTGSDFST&gt;</td>
</tr>
<tr>
<td>3</td>
<td>sgywnwirkf &lt;SGWYNSGWI&gt;</td>
</tr>
<tr>
<td>4</td>
<td>pgknleymg &lt;PGKNLAYMG&gt;</td>
</tr>
<tr>
<td>5</td>
<td>isysgktyn &lt;ISYSGKTYN&gt;</td>
</tr>
</tbody>
</table>
Nucleic Acid Sequence Encoding the Full Length Chimeric 29B06 Light Chain (Mouse Kappa Chain Variable Region and Human Kappa Constant Region) (SEQ ID NO: 161)

1 gacatgttgtgc tggaccaaatc tcagcttctct ttggtgtgtgt ctctaggaga caagggccacctc
gatctctcgtc ttcgtcagttta tcgagtttcttc ttcagctgatc acaggtctggc
5
61 atctctgccca gcagcgccagc aatgtggttgc aattcttgcaga ttagttttagct gactgtggttctc
cagtctacaca caagcacagtgc ctgggaacctgc ctggtgcctttc cagtggtgccct
10 121 caacagaaacc cagacacgcc acacccaaac tcctacattgc ccacagccagac ccagagttgc
181 gggcttcctgac caggtgtagtta gcgtggtggcc cgggtgcacgttg acctgaggtgtctc
241 cgcttgaggg gcagatgaaaa tcgagtctgcct cagttgctggtg ctgtgctgtgc
30 361 atctctgccca caagctgatgag ccacagcttct gcgtctgtctgg cagttgctggtg ctgtgctgtgc
421 aacatatttct cccttcgagtc agcaggtgcagc gaaatcgagct gacagttgctggtg ctgtgctgtgc
15 Protein Sequence Defining the Full Length Chimeric 29B06 Light Chain (Mouse Kappa Chain Variable Region and Human Kappa Constant Region) (SEQ ID NO: 162)

1 divltqgspas lavs1ggrat iscraseivd nfegisfmwff qqkpgqppkl liyaasnqgs
gatctctcgtc ttcgtcagttta tcgagtttcttc ttcagctgatc acaggtctggc
5
61 gpvparfgsg sgtdfsslnih pveddtyt cfcqskgkpy fpfggtktle ilrtaapsvf
121 lpfpsdeqlk sgtdsvevct cmmfynprevkw qwkvmdalts gnsqsesveq dskdystsls
20 181 stiltlskady ekhkvyscev tghqgllspvts ksfnrgec

Nucleic Acid Sequence Encoding the Full Length Humanized Sh07F01 Hv3-48 Heavy Chain (Humanized Heavy Chain Variable Region and Human IgGl Constant Region) (SEQ ID NO: 163)

25 1 gaggttcagc tggtagaatc cggagggagc ttcggtcaac gactgtggttctc aacgtgacgtcttc
gatctctcgtc ttcgtcagttta tcgagtttcttc ttcagctgatc acaggtctggc
5
61 tgatgccccc cccgcggcctg tgtccttctca gcagcttctgtc cagttgctggtg ctgtgctgtgc
121 ccagacacgc gcacagagct tggagtttcttc ttcagctgatc acaggtctggc
181 gggcttcctgac caggtgtagtta gcgtggtggcc cgggtgcacgttg acctgaggtgtctc
241 cgcttgaggg gcagatgaaaa tcgagtctgcct cagttgctggtg ctgtgctgtgc
30 361 atctctgccca caagctgatgag ccacagcttct gcgtctgtctgg cagttgctggtg ctgtgctgtgc
421 aacatatttct cccttcgagtc agcaggtgcagc gaaatcgagct gacagttgctggtg ctgtgctgtgc
40 901 aagttgaag ctctctgtgc ttcgtcttct acaggtgctgc accaaactgc gctcagcgcgc
961 aacacatata gcacagcggct tcgaccaaaa gcacacagtgc ctggagtttcttc ttcagctgatc acaggtctggc
5
1021 agtagctgac aagggcagcct ctcggtacca caggtgacac ctcgggtaca ctcgggtaca ctcgggtaca ctcgggtaca
1081 gaaagagcaa aagagacgcct tgcagttgcct tggagtttcttc ttcagctgatc acaggtctggc
1141 atcagcttgc actctctcgtc ttcgtcagttta tcgagtttcttc ttcagctgatc acaggtctggc
1201 gtctgttgaga tgcggtgccg ttttttttttt ttcagctgatc acaggtctggc
1261 tggagctgcag ctctctcgtc ttcgtcagttta tcgagtttcttc ttcagctgatc acaggtctggc
1321 aaccagaagt cactctgcttc gcacgccagg aag
Protein Sequence Defining the Full Length Humanized Sh07F01 Hv3-48 Heavy Chain (Humanized Heavy Chain Variable Region and Human IgGl Constant Region) (SEQ ID NO: 164)

1  evqlvesgsg  lvgpggsrlr  scaasgfdfs  rhwmswrqra  pgkglewvse  inpsdrtiny
  5  tpslkerfti  srndknslsy  lqmnslraed  tavvycarrv  rhiygymnds  wgqgttvvts
 10  sastkgpsvf  plappsksts  gtaalclgvc  kdyfpeppty  swnsqaltsg  vhtfpavlqg
 15  sglysllsvv  tvpssllgtq  tyicvnvhkp  snktkdkrve  pkscdkthtc  ppcpapellg
 20  gpsvflfppk  pkdt1misrt  pevtcvvvdv  shedpevkh  wyvdgvevhn  aktpkpreegy
 25  nstyrsvsvl  tvlhgwdlng  keykckvsvnk  alpapiekti  skakgpsrep  qytylppsr
 30  emtknqvslt  clvkglfpsd  iavwesnqg  pennykttpp  vldsdgsfll  yskltvdksr
 35

Nucleic Acid Sequence Encoding the Full Length Humanized Sh07F01 Hv3-48 D28T T60A L63V E65G Heavy Chain (Humanized Heavy Chain Variable Region and Human IgGl Constant Region) (SEQ ID NO: 165)

1  gaggttcagc  tggtgataac  cggagggagt  ttggtccacat  ctgggtgattc  actcagactt
  5  tcagccgccc  ccacggcttt  tacctctata  cgacattgga  tgaagctttgc  ccagccgactt
 10  ccagcaaggg  gctctgatag  cgtaagcttt  ctcaacagat  gctcaaacag  accaggaatt
 15  ggtcaccgct  tctttgtcct  ccacacccac  ctttaagacta  ctgatcactca  acatcagact
 20  gtagacacact  caccctttgc  cacttctttgc  cctccatgttc  ccagaggttaa  aatgttctca
 25  gtagttctttg  atggttcttt  gataccatct  gacagtcttt  gctggctcttt  cttgcctgctt
 30  acatcactcag acctctttgtc  ctgactctgt  actacagact  gtagacagct  cctccagctt
 35  acatcactcag  acctctttgtc  ctgactctgt  actacagact  gtagacagct  cctccagctt
 40  gtagttctttg  atggttcttt  gataccatct  gacagtcttt  gctggctcttt  cttgcctgctt
 45  acatcactcag  acctctttgtc  ctgactctgt  actacagact  gtagacagct  cctccagctt
 50  acatcactcag  acctctttgtc  ctgactctgt  actacagact  gtagacagct  cctccagctt
 55  acatcactcag  acctctttgtc  ctgactctgt  actacagact  gtagacagct  cctccagctt
 60

Protein Sequence Defining the Full Length Humanized Sh07F01 Hv3-48 D28T T60A L63V E65G Heavy Chain (Humanized Heavy Chain Variable Region and Human IgGl Constant Region) (SEQ ID NO: 166)

1  evqlvesgsg  lvgpggsrlr  scaasgfdfs  rhwmswrqra  pgkglewvse  inpsdrtiny
  5  apsvkgfrtfi  srndknlsly  lqmnslraed  tavvycarrv  rhiygymnds  wgqgttvvts
 10  sastkgpsvf  plappsksts  gtaalclgvc  kdyfpeppty  swnsqaltsg  vhtfpavlqg
 15  sglysllsvv  tvpssllgtq  tyicvnvhkp  snktkdkrve  pkscdkthtc  ppcpapellg
 20  gpsvflfppk  pkdt1misrt  pevtcvvvdv  shedpevkh  wyvdgvevhn  aktpkpreegy
 25  nstyrsvsvl  tvlhgwdlng  keykckvsvnk  alpapiekti  skakgpsrep  qytylppsr
 30  emtknqvslt  clvkglfpsd  iavwesnqg  pennykttpp  vldsdgsfll  yskltvdksr
 35

...
Nucleic Acid Sequence Encoding the Full Length Humanized HE L 07F01 Kyl-9 Light Chain (Humanized Kappa Chain Variable Region and Human Constant Region) (SEQ ID NO: 167)

```
1 gtatccagt tcagctcagt tcagctccttc gttgagtcatc caggtgcgca caggtgcacc
61 gtgcacctgc gaagatcaca gaagcttggaq agctctcttg tcttgatcag gcgaaagctt
121 gggaagaccc ccacaaaccct cactcatctt cgcactccttc gtagctccgg cgtaacagt
161 agatcctctg gtacgagagt ggcggacagag ttacactctca cattacagct tgcgcaagct
241 gagcacttgagg ctgaagtgca tgcgtactct ctcagggggtc tgcctcagtaa acacagtca
261 ctggacccag cagcacaagga cagcagaagc cactacttct cactcctct cccgagctc
301 gggtctcaaggg ctgctcagtt gcagccaggg gctgtccagac tgccttcctt gccctgacc
361 agatcactcgt ctcgcagaaa cagctccttc gjttgatcag gctgacacac
10
```

Protein Sequence Defining the Full Length Humanized HE L 07F01 Kyl-9 Light Chain (Humanized Kappa Chain Variable Region and Human Constant Region) (SEQ ID NO: 168)

```
1 diqltqsgsf vstsvgdrvt vctcrasqnvsg slvwyqqkg kskptklyis asflysgvps
61 rfsgsagste ftitissvqvp edfaeycgg gnnpltfvg gtvkeikrvv aapsvflfpp
121 sdeqlkgstga ssvcllnnf y preakvqwkv dna1qsgnsq esvteqdskd styisstlit
181 lskadyekhkk vyacevthqg lssptvksfn rgc
25
```

Nucleic Acid Sequence Encoding the Full Length Humanized sh07F01 Kyl-9 F1 Light Chain (Humanized Kappa Chain Variable Region and Human Constant Region) (SEQ ID NO: 169)

```
1 gcacatccagc tgactcagtc gcgccctcagtt tttgcggcggt cctgggtgta cagaggtgact
61 atccacatgcg gccttccgca aaaagctcgg aacatcgtgg tctgtgatca gcagaaaccctc
121 ggttaaccgc ctcgatcaca gctctctcttc tgttgtagcag gctgcccttc
181 cgggtcgcctc gtcggcgggt cctcgctcgctt ccagagctcg cttggcgccct
241 gaagatttgg cagcagcctg cgtgccgcttc ggcggcggag ttcgacactca cttgtcgccgg
301 ggaagcagag gtagagctca gcgtgcaagtt gctgtcccccct acctgtcatc tcgtttcctc
361 acggatgagc acagactcgc cctcgctcttc gctctgcttc gaagtcccttc acacagttac
421 ccacagctgg ctcgagctct gcgtgaaagtt gtaatgctct tgcctctctc gcggtcttcct
481 gacgctcgaga cagacagcaag cagcagagag cactctctct cactctctct cactctctct
541 ctgggtcagag gcagagctca gaaacagagct atagctgcgg gcaggttac acacaggtt
601 tgtgcagactc gtgcagccag gtcggtccat agggcgsaaggt gcagttgc
```

Protein Sequence Defining the Full Length Humanized sh07F01 Kyl-9 F1 Light Chain (Humanized Kappa Chain Variable Region and Human Constant Region) (SEQ ID NO: 170)

```
1 diqltqsgsf lssavvdrvts itcrasqnvsg slvwyqqkg kskptklyis asflysgvps
61 rfgsagstge ftitissvqvp edfaeycgg gnnpltfvg gtvkeikrvv aapsvflfpp
121 sdeqlkgstga ssvcllnnf y preakvqwkv dna1qsgnsq esvteqdskd styisstlit
45
```
181 lskadyekhk vyacevthqg lsspvtksfn rgec

[0269] Nucleic Acid Sequence Encoding the Full Length Humanized Sh29B06 Hv4-59 Heavy Chain (Humanized Heavy Chain Variable Region and Human IgGl Constant Region)

(SEQ ID NO: 171)

```
1 caagttcagc tgcaagaatt cggaccagga ttgtctcaac cttcagagac acttcagctctg
61 acctgcacgg cgtagccggtg gctcagcttc atcgctactg gagaaggtct ccgcacgcca
121 ccagcgcaag gctctcgagtt gattggtcag atcagctactc cggagaaac gctctttaca
181 cccagtctgta agagccgagt gaccataagc gtagataacg tgaagaccca gttctcctctgt
241 aagctgagtt ccgtaacagc agctgtgtatt ctggagagta ccagggcactgc
301 gactagccaa tggtactgct ttggcgcgct gcttcggtga ctggtagttc tcgcttcaca
361 aaagacccacc gctgggttcc ctggaggtct tacagtgaac ccggcactgc
421 gcactcgagct gctctgctcag gtattatattc ccagagcagc taaccgtgag ccctggaacgtc
481 ggaagcactca cttctgggtt ccatactttt cttgagtctgt ctagaaagctt tcggcttgc
541 tcaactcatg cgtgctgacg gtcgctactc ctattgggctg gctagctggta ttggagctacgt
601 aatgtgaacaac acaagctcttg caattactag gtcgataagc ggtggtgagcc caagagctgc
661 gacaagacttc acactgtgccc cccatgcctg gccttgttac ccgtgggctg tcgggtgtgtc
tttttggttc ccacaaagcc taagatatact ctagatgataa tagaagccag ccggaggtcagt
721 tgggttggtt tagacgcttcc cccagagagc gcagagaagtg aaagcacttg gtagctgtgat
781 841 gggtagcaagc taaaagacag ccagaaagcc agctgct gagctggtgact
901 cgtgtagtga gttgcttcac gttgctgacg ctaagggtgc ccacaggtga aaagacaggtc
ttcgcttgga gggagcactcc ttggagctgac ccggaggtcagt
1001 gggcgaggctc gtcggactac gttgtggaga actgctagct cagctgactt cagctgatgat
1061 aacaaagctct gttcactcga gttggagttc ccagctcagc caccgctctg ccggaggtcagt
1121 tggagagattc acggctaggc tggaacacaat tacaaagac ccccccctgt ctggagatgt
1181 dgggtggctct ttcctccttc tcaagctgcg gagctgcggcact gcagggtgacg
1241 aacgtcttcct gctgtccgcgt gcagctaagc gccactctctg ccggaggtcagt
1301 ctagcagcttg cggcccgagga g
```

[0270] Protein Sequence Defining the Full Length Humanized Sh29B06 Hv4-59 Heavy Chain (Humanized Heavy Chain Variable Region and Human IgGl Constant Region) (SEQ ID NO: 172)

```
1 qvqlqgespg lvpksetslc tctvsgsiss gwywirqpg pkglwiegys isysgktyyn
61 psiksrtvis vdtksqfsl klsstvtaad avycarsky dyadmwgqq tltvtsaasat
121 kpgsvfplap skssstqsgta algclvkdysf pepptvswns galsgvyhfp plvqjsngly
181 slsssvvmpg slsgtqtyic nnvhpntsk vdkrpveksp dktthtcpcc apellggpsv
241 flpppkpckt lmisrtpevt cyvvdshded pevkmwyyd gvevhnaklt preegynst
301 rrsvltvltl cvdvshpyed cvvfmwyed gvevhnaklt preegynst
361 nqyslclvlk gffpsdiave wesngpgenn ykttppvlds dgsfflyslk tvdksrswwqg
421 nfvsccvmhe alhnhbytqks 1slspgk
```

[0271] Nucleic Acid Sequence Encoding the Full Length Humanized Hu29B06 Hv4-59 Heavy Chain (Humanized Heavy Chain Variable Region and Human IgGl Constant Region) (SEQ ID NO: 173)

```
1 caagttcagc tgcaagaattc cggaccagga ttgtctcaac ccagccgaaac actccctcctt
61 acctgcacgg cgtagccggtg gctcagcttc atcgctactg gagaaggtct ccgcacgcca
121 ccagcgcaag gctctcgagtt gattggtcag atcagctactc cggagaaac gctctttaca
181 cccagtctgta agagccgagt gaccataagc gtagataacg tgaagaccca gttctcctctgt
241 aagctgagtt ccgtaacagc agctgtgtatt ctggagagta ccagggcactgc
301 gactagccaa tggtactgct ttggcgcgct gcttcggtga ctggtagttc tcgcttcaca
361 aaagacccacc gctgggttcc ctggaggtct tacagtgaac ccggcactgc
421 gcactcgagct gctctgctcag gtattatattc ccagagcagc taaccgtgag ccctggaacgtc
481 ggaagcactca cttctgggtt ccatactttt cttgagtctgt ctagaaagctt tcggcttgc
541 tcaactcatg cgtgctgacg gtcgctactc ctattgggctg gctagctggta ttggagctacgt
601 aatgtgaacaac acaagctcttg caattactag gtcgataagc ggtggtgagcc caagagctgc
661 gacaagacttc acactgtgccc cccatgcctg gccttgttac ccgtgggctg tcgggtgtgtc
tttttggttc ccacaaagcc taagatatact ctagatgataa tagaagccag ccggaggtcagt
721 tgggttggtt tagacgcttcc cccagagagc gcagagaagtg aaagcacttg gtagctgtgat
781 841 gggtagcaagc taaaagacag ccagaaagcc agctgct gagctggtgact
901 cgtgtagtga gttgcttcac gttgctgacg ctaagggtgc ccacaggtga aaagacaggtc
ttcgcttgga gggagcactcc ttggagctgac ccggaggtcagt
1001 gggcgaggctc gtcggactac gttgtggaga actgctagct cagctgactt cagctgatgat
1061 aacaaagctct gttcactcga gttggagttc ccagctcagc caccgctctg ccggaggtcagt
1121 tggagagattc acggctaggc tggaacacaat tacaaagac ccccccctgt ctggagatgt
1181 dgggtggctct ttcctccttc tcaagctgcg gagctgcggcact gcagggtgacg
1241 aacgtcttcct gctgtccgcgt gcagctaagc gccactctctg ccggaggtcagt
1301 ctagcagcttg cggcccgagga g
```

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PCT/US2011/043056
301  gactacgcaa  tggactattg  gggccagggt  actctgtga  cttgtaaccg  tcgctcaaca
361  aaaggaccaa  gtggtgcccc  actcgccccct  agcagcaaga  gtacatcggg  qggcaactgca
421  gcactctgct  gcgctgcca  attgattttt  ccagagccg  taacgcgtag  ctggacagct
481  ggagacacta  cttctgttct  ccatacttttt  cctcgctgct  tggacacttc  tcggcttata
541  tcactcagct  gctgctgacg  ctgctcactc  ttgctctgct  tggacacttc  tcggcttata
601  aagttaaacca  acgacaggct  taactaaga  ggtgataacg  gggtgaaacc  caagagctgc
661  gacaagactc  acactctgcc  cccatgccct  gccccctgccc  ttgcgggctc  tccagccccg
721  tttttttctcc  ccaccaacactt  ctagatataa  ctgtaacacc  ccagagctgc
781  tgtgtggtgtg  tagacgttcc  ccacagggac  ccaggtttaga  atgtcctact  ctggcttata
841  ggagtcgaaag  tagataactg  ctaaagcaag  cctagagagg  agcaatctaa  tgtatctatc
901  cgtgtagctca  gcgttcctac  agtgcctgcac  caagagctgc  ccagagctgc
961  tcgcagctgt  cttcttgaca  gagactatcc  ctagacggag  ccagagctgc
1021  ggccgaccca  gtggtaacca  ctgctcactc  tggcacccca  ttagagagga  aatgacaaag
1081  aaccaagtct  cattgacctg  cctggtgaaa  ggcttctacc  ccagcgacat  cgccgttgag

Human
D27G

Humanized
T30S

[0272]  Protein Sequence Defining the Full Length Humanized Hu29B06 Hv4-59 Heavy Chain (Humanized Heavy Chain Variable Region and Human IgGl Constant Region) (SEQ ID NO: 174)

1  qvqlqesgpg  lvkpsetls1  tctvsgdsit  sgywnThekmp  pgkkleymyg  isysgktyyn
61  pslksrisit  rdsstnqyai  klssvtaadt  avvycarsky  dyamdyqwgg  tlvtsvssat
121  kpsvwpflap  ssksstsgta  algcvkvdvd  pepptvswms  galgsyhtf  pavlqesgyl
181  slssvqvtys  ssllqvtyic  vvlkypvsdk  vdrkvpepsc  dkhtdppcpp  apellqpsvw
241  flfpppkpdt  lmsrtpvevt  cvvvdvshed  pevkfnwyvd  gvevhnaktk  preegynst
301  rrvsvltvih  qdwlingsk  ekvnskaip  vkielqskak  ggprepqvvyt  lpsreemtka
361  nqvsstlcvk  gfypsdiave  wesngpgenn  ykttppvlds  dgsfflyskl  tvldksrwwgg
421  nffcssvmve  alnhytqks  lsrlspgk

[0273]  Nucleic Acid Sequence Encoding the Full Length Humanized Hu29B06 Hv4-59
D27G T30S M48I I67V Y78F Heavy Chain (Humanized Heavy Chain Variable Region and Human IgGl Constant Region) (SEQ ID NO: 175)

35  1  caagttcagc  tcgaagaatc  cggacacgga  tcggtaaacc  ctcagcagac  actcagctcg
61  1 ctctgacccg  tgaagcggtgg  caagatcatc  tccgtttatt  ggaactgcgt  ccgagaccca
121  1 ccaagcgaca  agttcagatg  cattgctac  atccagctaa  cgggaaaca  ctaattacca
181  1 ccctcctgta  agagccgagt  gaccataaag  agagtcaca  gtaaagacca  gttctccctg
241  1 aacctttctc  cctgtgcacc  cgctgataca  gctgtgttag  atttgcaag  ctgcacagat
301  1 gactacgaa  tggactattg  gggccagggt  actctgtga  cttgtaaccg  tcgctcaaca
361  1 aaaggaccaa  gtggtgcccc  actcgccccct  agcagcaaga  gtacatcggg  qggcaactgca
421  1 gcactctgct  gcgctgcca  attgattttt  ccagagccg  taacgcgtag  ctggacagct
481  1 ggagacacta  cttctgttct  ccatacttttt  cctcgctgct  tggacacttc  tcggcttata
541  1 tcactcagct  gctgctgacg  ctgctcactc  ttgctctgct  tggacacttc  tcggcttata
601  1 aagttaaacca  acgacaggct  taactaaga  ggtgataacg  gggtgaaacc  caagagctgc
661  1 gacaagactc  acactctgcc  cccatgccct  gccccctgccc  ttgcgggctc  tccagccccg
721  1 tttttttctcc  ccaccaacactt  ctagatataa  ctgtaacacc  ccagagctgc
781  1 tgtgtggtgtg  tagacgttcc  ccacagggac  ccaggtttaga  atgtcctact  ctggcttata
841  1 ggagtcgaaag  tagataactg  ctaaagcaag  cctagagagg  agcaatctaa  tgtatctatc
901  1 cgtgtagctca  gcgttcctac  agtgcctgcac  caagagctgc  ccagagctgc
961  1 tcgcagctgt  cttcttgaca  gagactatcc  ctagacggag  ccagagctgc
1021  1 ggccgaccca  gtggtaacca  ctgctcactc  tggcacccca  ttagagagga  aatgacaaag
1081  1 aaccaagtct  cattgacctg  cctggtgaaa  ggcttctacc  ccagcgacat  cgccgttgag
1141 tgggagagta acggtcagcc tgagaacaat tacaagacaa cccccccagt gctggatagt
1201 gacgggtctt tctttctgta cagtaagctg actgtggaca ... convenience, Table 18 provides a concordance chart showing the SEQ ID NO. of each sequence discussed in this Example.
<table>
<thead>
<tr>
<th>SEQ ID NO.</th>
<th>Nucleic Acid or Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>Human IgG1 constant—nucleic acid</td>
</tr>
<tr>
<td>151</td>
<td>Human IgG1 constant—protein</td>
</tr>
<tr>
<td>152</td>
<td>Human Kappa constant (used for chimeric antibodies)—nucleic acid</td>
</tr>
<tr>
<td>153</td>
<td>Human Kappa constant (used for humanized antibodies)—nucleic acid</td>
</tr>
<tr>
<td>154</td>
<td>Human Kappa constant (used for chimeric and humanized antibodies)—protein</td>
</tr>
<tr>
<td>155</td>
<td>Chimeric 07F01 C102S Mouse Heavy Chain Variable + Human IgG1 constant—nucleic acid</td>
</tr>
<tr>
<td>156</td>
<td>Chimeric 07F01 C102S Mouse Heavy Chain Variable + Human IgG1 constant—protein</td>
</tr>
<tr>
<td>157</td>
<td>Chimeric 07F01 Mouse Light Chain Variable + Human Kappa constant—nucleic acid</td>
</tr>
<tr>
<td>158</td>
<td>Chimeric 07F01 Mouse Light Chain Variable + Human Kappa constant—protein</td>
</tr>
<tr>
<td>159</td>
<td>Chimeric 29B06 Mouse Heavy Chain Variable + Human IgG1 constant—nucleic acid</td>
</tr>
<tr>
<td>160</td>
<td>Chimeric 29B06 Mouse Heavy Chain Variable + Human IgG1 constant—protein</td>
</tr>
<tr>
<td>161</td>
<td>Chimeric 29B06 Mouse Light Chain Variable + Human Kappa constant—nucleic acid</td>
</tr>
<tr>
<td>162</td>
<td>Chimeric 29B06 Mouse Light Chain Variable + Human Kappa constant—protein</td>
</tr>
<tr>
<td>163</td>
<td>Humanized Sh07F01 Hv3-48 Heavy Human Variable + Human IgG1 constant—nucleic acid</td>
</tr>
<tr>
<td>164</td>
<td>Humanized Sh07F01 Hv3-48 Heavy Human Variable + Human IgG1 constant—protein</td>
</tr>
<tr>
<td>165</td>
<td>Humanized Sh07F01 Hv3-48 D28T T60A L63V E65G Heavy Human Variable + Human IgG1 constant—nucleic acid</td>
</tr>
<tr>
<td>166</td>
<td>Humanized Sh07F01 Hv3-48 D28T T60A L63V E65G Heavy Human Variable + Human IgG1 constant—protein</td>
</tr>
<tr>
<td>167</td>
<td>Humanized HE L 07F01 Kv1-9 Human Variable + Human Kappa constant—nucleic acid</td>
</tr>
<tr>
<td>168</td>
<td>Humanized HE L 07F01 Kv1-9 Human Variable + Human Kappa constant—protein</td>
</tr>
<tr>
<td>169</td>
<td>Humanized sh07F01 Kv1-9 F1 Human Variable + Human Kappa constant—nucleic acid</td>
</tr>
<tr>
<td>170</td>
<td>Humanized sh07F01 Kv1-9 F1 Human Variable + Human Kappa constant—protein</td>
</tr>
<tr>
<td>171</td>
<td>Humanized Sh29B06 Hv4-59 Heavy Human Variable + Human IgG1 constant—nucleic acid</td>
</tr>
<tr>
<td>172</td>
<td>Humanized Sh29B06 Hv4-59 Heavy Human Variable + Human IgG1 constant—protein</td>
</tr>
<tr>
<td>173</td>
<td>Humanized Hu29B06 Hv4-59 Heavy Human Variable + Human IgG1 constant—nucleic acid</td>
</tr>
<tr>
<td>174</td>
<td>Humanized Hu29B06 Hv4-59 Heavy Human Variable + Human IgG1 constant—protein</td>
</tr>
<tr>
<td>175</td>
<td>Humanized Hu29B06 Hv4-59 D27G T30S M48I I67V Y78F Heavy Human Variable + Human IgG1 constant—nucleic acid</td>
</tr>
<tr>
<td>176</td>
<td>Humanized Hu29B06 Hv4-59 D27G T30S M48I I67V Y78F Heavy Human Variable + Human IgG1 constant—protein</td>
</tr>
<tr>
<td>177</td>
<td>Humanized Sh29B06 Kv2-28 Human Variable + Human Kappa constant—nucleic acid</td>
</tr>
<tr>
<td>178</td>
<td>Humanized Sh29B06 Kv2-28 Human Variable + Human Kappa constant—protein</td>
</tr>
</tbody>
</table>
Table 19 below shows antibodies containing chimeric immunoglobulin heavy and light chains and each of the possible combinations of the full-length chimeric or humanized immunoglobulin heavy and light chains.

<table>
<thead>
<tr>
<th>Antibody Name</th>
<th>Light Chain</th>
<th>Heavy Chain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sh07F01-2</td>
<td>Chimeric 07F01 Kappa (SEQ ID NO: 158)</td>
<td>Chimeric 07F01 C102S Heavy IgG1 (SEQ ID NO: 156)</td>
</tr>
<tr>
<td>Sh07F01-43</td>
<td>HE L 07F01 Kv1-9 Kappa (SEQ ID NO: 168)</td>
<td>Sh07F01 Hv3-48 IgG1 (SEQ ID NO: 164)</td>
</tr>
<tr>
<td>Sh07F01-62</td>
<td>HE L 07F01 Kv1-9 Kappa (SEQ ID NO: 168)</td>
<td>Sh07F01 Hv3-48 D28T T60A L63V E65G IgG1 (SEQ ID NO: 166)</td>
</tr>
<tr>
<td>Sh07F01-69</td>
<td>Sh07F01 Kv1-9 F1 Kappa (SEQ ID NO: 170)</td>
<td>Sh07F01 Hv3-48 IgG1 (SEQ ID NO: 164)</td>
</tr>
<tr>
<td>Sh07F01-83</td>
<td>Sh07F01 Kv1-9 F1 Kappa (SEQ ID NO: 170)</td>
<td>Sh07F01 Hv3-48 D28T T60A L63V E65G IgG1 (SEQ ID NO: 166)</td>
</tr>
<tr>
<td>Sh07F01-99</td>
<td>Chimeric 07F01 Kappa (SEQ ID NO: 158)</td>
<td>Sh07F01 Hv3-48 IgG1 (SEQ ID NO: 164)</td>
</tr>
<tr>
<td>Sh07F01-100</td>
<td>Chimeric 07F01 Kappa (SEQ ID NO: 158)</td>
<td>Sh07F01 Hv3-48 D28T T60A L63V E65G IgG1 (SEQ ID NO: 166)</td>
</tr>
<tr>
<td>Sh07F01-101</td>
<td>HE L 07F01 Kv1-9 Kappa (SEQ ID NO: 168)</td>
<td>Chimeric 07F01 C102S Heavy IgG1 (SEQ ID NO: 156)</td>
</tr>
<tr>
<td>Sh07F01-102</td>
<td>Sh07F01 Kv1-9 F1 Kappa (SEQ ID NO: 170)</td>
<td>Chimeric 07F01 C102S Heavy IgG1 (SEQ ID NO: 156)</td>
</tr>
<tr>
<td>Sh29B06-1</td>
<td>Chimeric 29B06 Kappa (SEQ ID NO: 162)</td>
<td>Chimeric 29B06 Heavy IgG1 (SEQ ID NO: 160)</td>
</tr>
<tr>
<td>Sh29B06-2</td>
<td>Chimeric 29B06 Kappa (SEQ ID NO: 162)</td>
<td>Hu29B06 Hv4-59 IgG1 (SEQ ID NO: 174)</td>
</tr>
<tr>
<td>Sh29B06-4</td>
<td>Chimeric 29B06 Kappa (SEQ ID NO: 162)</td>
<td>Sh29B06 Hv4-59 IgG1 (SEQ ID NO: 172)</td>
</tr>
<tr>
<td>Sh29B06-9</td>
<td>Sh29B06 Kv2-28 Kappa (SEQ ID NO: 178)</td>
<td>Chimeric 29B06 Heavy IgG1 (SEQ ID NO: 160)</td>
</tr>
<tr>
<td>Sh29B06-23</td>
<td>Sh29B06 Kv2-28 Kappa (SEQ ID NO: 178)</td>
<td>Hu29B06 Hv4-59 IgG1 (SEQ ID NO: 174)</td>
</tr>
<tr>
<td>Sh29B06-25</td>
<td>Sh29B06 Kv2-28 Kappa (SEQ ID NO: 178)</td>
<td>Sh29B06 Hv4-59 IgG1 (SEQ ID NO: 172)</td>
</tr>
<tr>
<td>Sh29B06-78</td>
<td>Sh29B06 Kv2-28 Kappa (SEQ ID NO: 178)</td>
<td>Hu29B06 Hv4-59 D27G T30S M48I I67V Y78F IgG1 (SEQ ID NO: 176)</td>
</tr>
<tr>
<td>Sh29B06-84</td>
<td>Chimeric 29B06 Kappa (SEQ ID NO: 162)</td>
<td>Hu29B06 Hv4-59 D27G T30S M48I I67V Y78F IgG1 (SEQ ID NO: 176)</td>
</tr>
</tbody>
</table>
The antibody constructs containing the full length chimeric heavy and light chains are designated below:

**Chimeric 07F01 C102S** = Full Length Chimeric 07F01 C102S Heavy Chain (Mouse Variable Region with C102S mutation and Human IgG1 Constant Region) (SEQ ID NO: 156) plus Full Length Chimeric 07F01 Light Chain (Mouse Variable Region and Human Kappa Constant Region) (SEQ ID NO: 158)

**Chimeric 29B06** = Full Length Chimeric 29B06 Heavy Chain (Mouse Variable Region and Human IgG1 Constant Region) (SEQ ID NO: 160) plus Full Length Chimeric 29B06 Light Chain (Mouse Variable Region and Human Kappa Constant Region) (SEQ ID NO: 162)

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

**Sh07F01-62** = Humanized Sh07F01 Hv3-48 D28T T60A L63V E65G Heavy Chain Variable Region and Human IgG1 Constant Region (SEQ ID NO: 166) plus HE L 07F01 Kvl-9 Light Chain Variable Region and Human Kappa Constant Region (SEQ ID NO: 168)

**Sh29B06-78** = Humanized Hu29B06 Hv4-59 D27G T30S M48I I67V Y78F Heavy Chain Variable Region and Human IgG1 Constant Region (SEQ ID NO: 176) plus Sh29B06 Kv2-28 Light Chain Variable Region and Human Kappa Constant Region (SEQ ID NO: 178)

**B. Binding Affinities of Humanized and Chimeric Anti-RON Monoclonal Antibodies**

The binding affinities and kinetics of interaction of monoclonal antibodies produced in Example 14 against recombinant human RON SEMA and PSI domains (rhRON SEMA + PSI) (R&D Systems, Inc., Minneapolis, MN) were measured by surface plasmon resonance using a Biacore T100 (Biacore (GE Healthcare), Piscataway, NJ) instrument.

Goat anti-human IgG Fc (Jackson ImmunoResearch, Catalog No. 109-005-098) was immobilized on carboxymethylated dextran CM4 sensor chips (Biacore) by amine coupling (Biacore) using a standard coupling protocol according to the vendor's instructions. The analyses were performed at 37°C using PBS (Invitrogen) containing 0.05% surfactant P20 (Biacore) as running buffer.
The antibodies were captured in individual flow cells at a flow rate of 60 µl/minute. Injection time was varied for each antibody to yield an R_{max} between 30 and 60 RU. Buffer or rhRON SEMA + PSI diluted in running buffer was injected sequentially over a reference surface (no antibody captured) and the active surface (antibody to be tested) for 300 seconds at 60 µl/minute. The dissociation phase was monitored for up to 1200 seconds. The surface was then regenerated with two 60 second injections of Glycine pH 2.25 (made from Glycine pH 2.0 (Biacore) and pH 2.5 (Biacore)) at 60 µl/minute. For the initial screening, only one or two concentrations of rhRON SEMA + PSI were tested, typically 10.0 and 2.5 nM (results are summarized in Table 20).

Kinetic parameters were determined using the kinetic function of the BIAevaluation software (Biacore) with double reference subtraction. Kinetic parameters for each antibody, k_a (association rate constant), k_d (dissociation rate constant) and K_D (equilibrium dissociation constant) were determined. Certain monoclonal antibodies were screened using cell culture media supernatant containing secreted antibody, and kinetic values of the monoclonal antibodies on rhRON SEMA + PSI at 37°C are summarized in Table 20.

Table 20

<table>
<thead>
<tr>
<th>Antibody</th>
<th>k_a (1/Ms)</th>
<th>k_d (1/s)</th>
<th>K_D (M)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sh07FCII-2</td>
<td>2.0E-06</td>
<td>7.3E-04</td>
<td>3.8E-10</td>
<td>3</td>
</tr>
<tr>
<td>1 h07F01-11</td>
<td>3.9E+06</td>
<td>1.4E-03</td>
<td>3.6E-10</td>
<td>2</td>
</tr>
<tr>
<td>Sh07F01-69</td>
<td>2.3E-06</td>
<td>1.2E-03</td>
<td>5.6E-10</td>
<td>2</td>
</tr>
<tr>
<td>Sh07F01-76</td>
<td>2.3E-06</td>
<td>1.3E-03</td>
<td>5.7E-10</td>
<td>2</td>
</tr>
<tr>
<td>Sh07F01-83</td>
<td>2.6E+06</td>
<td>1.4E-03</td>
<td>5.4E-10</td>
<td>2</td>
</tr>
<tr>
<td>Sh29B06-1</td>
<td>6.7E-05</td>
<td>7.6E-04</td>
<td>1.1E-09</td>
<td>3</td>
</tr>
<tr>
<td>Sh29B06-9</td>
<td>8.7E-05</td>
<td>2.2E-04</td>
<td>2.6E-10</td>
<td>1</td>
</tr>
<tr>
<td>Sh29B06-23</td>
<td>7.8E+05</td>
<td>4.8E-04</td>
<td>6.4E-10</td>
<td>4</td>
</tr>
<tr>
<td>1 h29I 11-25</td>
<td>No Binding</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The results in Table 20 demonstrate that the chimeric and each of the humanized antibodies, except Sh29B06-25, have fast association rates (k_a), very slow disassociation rates (k_d) and very high affinities (K_D). In particular, the antibodies have affinities ranging from about 260 pM to about 1.1 nM. No binding was observed for Sh29B06-25. Because Sh29B06-25 does not bind rhRON SEMA + PSI and Sh29B06-23 does, one or more of the back mutations present in the heavy chain of Sh29B06-23 appear to be required for binding with high affinity.
The binding affinities and kinetics of certain purified monoclonal antibodies were also determined. To further characterize certain antibodies, the surface plasmon resonance experiments described above were conducted using concentrations of rhRON SEMA + PSI between 0.3125 nM and 10.0 nM (a 2-fold serial dilution).

The kinetic values of certain purified monoclonal antibodies (i.e., Sh07F01-62 and Sh29B06-78) on rhRON SEMA + PSI at 25°C and 37°C are summarized in Table 21.

**Table 21**  
Antibody Binding to rhRON SEMA + PSI

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Measurements at 25°C</th>
<th>Measurements at 37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k_d$ (1/Ms)</td>
<td>$K_d$ (M)</td>
</tr>
<tr>
<td>Sh07F01-2</td>
<td>1.2E+06</td>
<td>8.2E-11</td>
</tr>
<tr>
<td>Sh07F01-43</td>
<td>1.2E+06</td>
<td>9.0E-11</td>
</tr>
<tr>
<td>Sh07F01-62</td>
<td>1.8E+06</td>
<td>8.5E-11</td>
</tr>
<tr>
<td>Sh07F01-69</td>
<td>1.1E+06</td>
<td>1.2E-10</td>
</tr>
<tr>
<td>Sh07F01-76</td>
<td>9.8E+05</td>
<td>1.3E-10</td>
</tr>
<tr>
<td>Sh07F01-83</td>
<td>1.6E+06</td>
<td>1.1E-10</td>
</tr>
<tr>
<td>Sh29B06-1</td>
<td>5.3E+05</td>
<td>3.6E-10</td>
</tr>
<tr>
<td>Sh29B06-23</td>
<td>6.7E+05</td>
<td>1.4E-10</td>
</tr>
<tr>
<td>Sh29B06-78</td>
<td>7.5E+05</td>
<td>5.2E-11</td>
</tr>
</tbody>
</table>

The results in Table 21 demonstrate the purified antibodies have affinities ranging from about 52 pM to 360 pM when tested at 25°C or about 110 pM to about 860 pM when tested at 37°C.

Binding to cell surface human wild-type RON and the delta 160 RON variant by antibodies 07F01, Sh07F01-62, 29B06, and Sh29B06-78 was measured at 4°C, using Fluorescence Activated Cell Sorting (FACS). PC3 cells expressing the human wild-type RON, and HT29 cells expressing the delta 160 variant, were harvested using cell dissociation buffer (Invitrogen), washed twice with FACS buffer (PBS with 0.5% BSA), and treated 10 minutes with Cyto Q Antibody diluent and FC receptor block (Innovex Biosciences, Richmond, CA). Purified antibodies were diluted in FACS buffer over a concentration range from 0.01 nM to 25 nM. Cells were incubated with 100 µl of antibody for one hour, washed with FACS buffer three times, and incubated for 45 minutes with goat anti-mouse PE-conjugated antibody (Jackson ImmunoResearch Laboratories, West Grove, PA) or donkey anti-human PE-conjugated antibody (Jackson ImmunoResearch Laboratories, West Grove, PA). Cells were
washed three times with FACS buffer, resuspended in 300 µι of FACS buffer, and analyzed using a Beckman Coulter Cytomics FC 500 FACS instrument. All four antibodies were compared in the same experiment. Results are summarized in Table 22.

### Table 22

<table>
<thead>
<tr>
<th></th>
<th>07F01</th>
<th>Sh07F01-62</th>
<th>29B06</th>
<th>Sh29B06-78</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human RON - K_D (nM)</td>
<td>0.053</td>
<td>0.043</td>
<td>0.136</td>
<td>0.090</td>
</tr>
<tr>
<td>Human RON - K_D range (nM)</td>
<td>0.036 to 0.069</td>
<td>0.026 to 0.060</td>
<td>0.083 to 0.190</td>
<td>0.063 to 0.117</td>
</tr>
<tr>
<td>Delta 160 RON - K_D (nM)</td>
<td>0.100</td>
<td>0.118</td>
<td>0.167</td>
<td>0.239</td>
</tr>
<tr>
<td>Delta 160 RON - K_D range (nM)</td>
<td>0.071 to 0.129</td>
<td>0.045 to 0.191</td>
<td>0.066 to 0.267</td>
<td>0.202 to 0.277</td>
</tr>
</tbody>
</table>

[0290] The results in Table 22 demonstrate that the humanized antibodies Sh07F01-62 and Sh29B06-78 retain their ability to bind both wild-type RON and the delta 160 RON variant on the cell surface with affinities equivalent to their murine antibody counterparts (i.e., 07F01 and 29B06, respectively).

#### C. Comparison of Other Anti-RON Antibodies

[0291] Three antibodies that inhibit the function of human RON were constructed and expressed using published information. One antibody, referred to as 1P3B2-BIIB Ab, was constructed based on the disclosure of Huet et al., U.S. Patent Publication No. 2009/0226442 (Biogen Idee, Inc.). Two additional antibodies, referred to as RON6 and RON8, were constructed based on the disclosure of Pereira et al., U.S. Patent Publication No. 2009/0136510 (Imclone Systems, Inc.).

[0292] Kinetic parameters for the 1P3B2-BIIB Ab, RON6, and RON8 antibodies on rhRON SEMA + PSI at 25°C and 37°C were determined by Biacore as described above (See Section B. Binding Affinities of Humanized and Chimeric Anti-RON Monoclonal Antibodies). The kinetic values for each antibody are summarized in Table 23.
Table 23

Antibody Binding to rhRON SEMA + PSI

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Measurements at 25°C</th>
<th>Measurements at 37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ka (1/Ms)</td>
<td>kd (1/s)</td>
</tr>
<tr>
<td>Sh29B06-78</td>
<td>6.8E+05</td>
<td>3.1E-05</td>
</tr>
<tr>
<td>Sh07F01-62</td>
<td>1.8E+06</td>
<td>1.6E-04</td>
</tr>
<tr>
<td>1P3B2-BIIB</td>
<td>1.5E+06</td>
<td>1.2E-03</td>
</tr>
<tr>
<td>RON6</td>
<td>2.3E+06</td>
<td>2.6E-03</td>
</tr>
<tr>
<td>RON8</td>
<td>1.2E+06</td>
<td>6.8E-04</td>
</tr>
</tbody>
</table>

[0293] The results in Table 23 demonstrate that the overall equilibrium dissociation constant ($K_D$) for Sh29B06-78 and Sh07F01-62 were smaller (i.e., higher affinity) than the $K_D$ for 1P3B2-BIIB, RON6, and RON8 at both 25°C and 37°C. The $K_D$ of 1P3B2-BIIB, RON6, and RON8 antibodies can also be compared with other humanized 29B06 or 07F01 variants by comparing Tables 21 and 23.

[0294] Therefore, the binding affinities of Sh29B06-78 and Sh07F01-62 are significantly higher than the affinities of 1P3B2-BIIB, RON6, and RON8 antibodies as disclosed herein.

Example 15: Inhibition of MSP-RON Binding

[0295] The chimeric and humanized antibodies produced in Example 14 were tested for inhibition of MSP binding to hRON SEMA + PSI, as measured by electrochemiluminescence (ECL) assay as described in Example 3. The antibodies (concentration range: 0.006-10 μg/mL) were incubated for 45 minutes at room temperature.

[0296] The MSP-hRON binding interaction was inhibited by the chimeric and humanized antibodies listed in Table 24, which were tested in this assay. The IC$_{50}$ for the antibodies (IgGl) are shown in Table 24.
The results in Table 24 demonstrate that the chimeric and humanized anti-RON antibodies listed in Table 24 (i.e., Sh29B06-1, Sh29B06-23, Sh29B06-78, Sh07F01-2, Sh07F01-43, Sh07F01-62, Sh07F01-69, Sh07F01-76, and Sh07F01-83) retain the ability to block MSP binding to hRON SEMA +PSI with high potency.

**Example 16: Inhibition of downstream signaling by anti-RON antibodies**

The chimeric and humanized anti-RON antibodies produced in Example 14 were tested for their ability to inhibit MSP-induced phosphorylation of ERK, a RON downstream signaling molecule, using the cell-based assay described in Example 3. The antibodies (concentration range: 0.006-10 μg/mL) in RPMI were added to the cells and incubated for one hour at 37°C. The IC50s of ERK phosphorylation inhibition by the chimeric and humanized anti-RON antibodies tested in this assay are shown in Table 25.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Mean IC₅₀</th>
<th>Std Dev of IC₅₀</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sh29B06-1</td>
<td>0.10</td>
<td>0.10</td>
<td>6</td>
</tr>
<tr>
<td>Sh29B06-23</td>
<td>0.11</td>
<td>0.08</td>
<td>10</td>
</tr>
<tr>
<td>Sh29B06-78</td>
<td>0.13</td>
<td>0.08</td>
<td>5</td>
</tr>
<tr>
<td>Sh07F01-2</td>
<td>0.06</td>
<td>0.06</td>
<td>7</td>
</tr>
<tr>
<td>Sh07F01-43</td>
<td>0.02</td>
<td>0.00</td>
<td>3</td>
</tr>
<tr>
<td>Sh07F01-62</td>
<td>0.03</td>
<td>0.03</td>
<td>2</td>
</tr>
<tr>
<td>Sh07F01-69</td>
<td>0.05</td>
<td>0.02</td>
<td>2</td>
</tr>
<tr>
<td>Sh07F01-76</td>
<td>0.10</td>
<td>0.03</td>
<td>2</td>
</tr>
<tr>
<td>Sh07F01-83</td>
<td>0.03</td>
<td>0.02</td>
<td>2</td>
</tr>
</tbody>
</table>
[0299] The results in Table 25 demonstrate that the chimeric and humanized anti-RON antibodies listed in Table 25 (i.e., Sh29B06-1, Sh29B06-23, Sh29B06-78, Sh07F01-2, Sh07F01-43, Sh07F01-62, Sh07F01-69, Sh07F01-76, and Sh07F01-83) inhibit MSP-induced ERK phosphorylation in T47D breast cancer cell line with high potency.

Example 17: Inhibition of MSP-Dependent Cell Migration

[0300] Humanized antibodies sh29B06-78 and sh07F01-62 as produced in Example 14 were tested for their ability to inhibit MSP-induced cell migration as described in Example 10. In this example, antibodies were added at a concentration of 1 µg/ml and serially diluted at a 1:5 dilution, and cells were incubated for 2 hours. Percent inhibition was determined by the following formula: \[ 100 - \frac{(\text{anti-RON antibody treated-baseline})}{(\text{control hulgG treated-baseline})} \times 100. \]

Results on inhibition of MSP-induced HPAF-II cell migration by anti-RON antibodies, sh29B06-78 and sh07F01-62, are summarized in Table 26 and FIG. 16.

Table 26

<table>
<thead>
<tr>
<th>AB concentration (ng/ml)</th>
<th>AVG</th>
<th>Std DEV</th>
<th>AVG</th>
<th>Std DEV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000.00</td>
<td>94.82</td>
<td>3.34</td>
<td>98.96</td>
<td>3.79</td>
</tr>
<tr>
<td>200.00</td>
<td>90.67</td>
<td>2.37</td>
<td>97.80</td>
<td>1.12</td>
</tr>
<tr>
<td>40.00</td>
<td>59.85</td>
<td>12.50</td>
<td>67.18</td>
<td>7.67</td>
</tr>
<tr>
<td>8.00</td>
<td>59.71</td>
<td>2.87</td>
<td>37.22</td>
<td>4.16</td>
</tr>
<tr>
<td>1.60</td>
<td>63.95</td>
<td>20.15</td>
<td>38.91</td>
<td>13.79</td>
</tr>
<tr>
<td>0.32</td>
<td>42.03</td>
<td>39.88</td>
<td>43.27</td>
<td>5.76</td>
</tr>
<tr>
<td>0.06</td>
<td>60.37</td>
<td>11.92</td>
<td>34.40</td>
<td>2.31</td>
</tr>
</tbody>
</table>

[0301] The results in Table 26 demonstrate that humanized anti-RON antibodies, sh29B06-78 and sh07F01-62, potently inhibit MSP-induced cell migration in HPAF-II pancreatic cancer cell lines.

Example 18: Inhibition of MSP-induced Cell Invasion

[0302] Humanized antibodies sh29B06-78 and sh07F01-62 as produced in Example 14 were tested for their ability to inhibit MSP-induced cell invasion. HPAF-II pancreatic cancer cells were trypsinized, counted, and placed at a concentration of 50,000/well in 45 µl of 10% FBS/MEM in the upper chamber of a BD 96-well BD BioCoat Matrigel invasion FluoroBlok™
plate (Becton Dickinson). Antibodies were added at a concentration of 30 µg/ml and cells were incubated for 2 hours. The bottom chamber contained 10% FBS MEM (200µl) and 1 nM MSP, and cells were incubated for 24 hours. The number of cells that underwent invasion through the membrane was determined by the addition of Calcien Dye at 4 µg/ml final concentration to the bottom chamber, followed by a one-hour incubation. Fluorescence intensity was measured using a Wallace 1420 instrument. Results on inhibition of MSP-induced HPAFII cell invasion by anti-RON antibodies are summarized in FIG. 17.

The results in Figure 17 demonstrate that humanized anti-RON antibodies sh29B06-78 and sh07F01-6 potently inhibit MSP-dependent cell invasion in HPAF-II pancreatic cancer cell line.

Example 19: Inhibition of Growth of NCI-H358 Lung Xenograft Tumor Model

Inhibition of tumor growth by the humanized anti-RON antibodies was tested in an NCI-H358 lung xenograft model. The NCI-H358 cells (ATCC) were grown in culture at 37°C in an atmosphere containing 5% CO₂, using RMPI medium (Invitrogen) containing 10% FBS. Cells were inoculated subcutaneously into the flank of 8-week old female CB.17 SCID mice (Taconic Labs) with 5 x 10⁶ cells per mouse in 50% matrigel (Becton Dickinson). Tumor measurements were taken twice weekly using vernier calipers. When tumors reached approximately 150 mm³, the mice were randomized into six groups of ten mice each. Each group received one of the following treatments: human IgG (hulgG) control, mu29B06, sh29B06-78, mu07F01, sh07F01-62 and RON8. Treatment was administered by intraperitoneal injection two times per week at 10 mg/kg for seven weeks. Treatment was well-tolerated, with no significant loss in body weight. Tumor growth inhibition is expressed as percent inhibition (baseline subtracted) to the hulgG control and statistical analysis was conducted using ANOVA. Results for tumor growth inhibition on day 41 in the NCI-H358 model are shown in Fig. 18 and Table 27.
Table 27

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TGI %</th>
<th>ANOVA (compared to hulgG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mu29B06</td>
<td>88.93</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>sh29B06-78</td>
<td>89.02</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>mu07F01</td>
<td>34.15</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>sh07F01-62</td>
<td>39.05</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>RON8</td>
<td>37.99</td>
<td>P &gt; 0.05</td>
</tr>
</tbody>
</table>

Anti-RON antibody treatments resulted in tumor growth inhibition compared to hulgG control. Specifically, mu29B06 antibody treatment resulted in tumor growth inhibition of 89% (P < 0.01); sh29B06-78 antibody treatment resulted in tumor growth inhibition of 89% (P < 0.01); mu07F01 antibody treatment resulted in tumor growth inhibition of 34% (P > 0.05); sh07F01-62 antibody treatment resulted in tumor growth inhibition of 39% (P > 0.05); and RON8 antibody treatment resulted in tumor growth inhibition of 38% (P > 0.05). These results demonstrate that sh29B06-78 and mu29B06 inhibit tumor growth in a NCI-H358 xenograft model (P < 0.01), whereas the mu07F01, sh07F01-62, and RON8 antibodies did not inhibit tumor growth in this model (P > 0.05, which is not statically significant).

**Example 20: RON Receptor Degradation**

Western blots were performed to determine total levels of RON receptor at the end of treatment. Four tumor samples from each of the treatment groups were weighed, lysed in RIPA buffer (Boston Bioproducts), ImM EDTA(Boston Bioproducts), 1 mM Sodium OrthoVandate (Sigma), IX protease inhibitor (Sigma) and IX Phosphatase Inhibitor I and II (Sigma). The samples were homogenized using a hand-held electric homogenizer and incubated for 10 minutes on ice. Samples are spun down at 11,000 RPM for 30 minutes at 4°C. Supernatants were collected and protein concentrations were determined using Pierce BCA assay kit according to the manufacturers protocol. The C-20 (Santa Cruz) antibody was used to detect total RON protein, β-tubulin (Cell Signaling Technologies) was blotted as loading control. The Western blots were blocked for one hour in 5% Milk in IX TBST (TBS- 0.1% TWEEN) (Sigma), followed by primary antibody incubation over night at 4°C in 5% BSA IX TBST at 1:1000 for both antibodies. Western blots were washed three times with IX TBST, incubated with anti-rabbit HRP conjugated secondary antibody (Cell Signaling Technologies),
for one hour at room temperature. Western blots were washed three times with I X TBST and then developed using Dura Signal (Pierce).

[0308] The results in Fig. 19 demonstrate RON receptor degradation in the mu29B06 and sh29B06-78 treated samples and to a lesser extent in the mu07F01 and sh07F01-62 treated samples. RON receptor degradation was not observed in the RON8 treated samples.

INCORPORATION BY REFERENCE

[0309] The entire disclosure of each of the patent documents and scientific articles referred to herein is incorporated by reference for all purposes.

EQUIVALENTS

[0310] 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 the range of equivalency of the claims are intended to be embraced therein.

[0311] WHAT IS CLAIMED IS:
1. An isolated antibody that binds human RON, comprising an immunoglobulin heavy chain variable region and an immunoglobulin light chain variable region selected from the group consisting of:

   (a) (i) an immunoglobulin heavy chain variable region comprising a CDR$_{H1}$ comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 45 (29B06), SEQ ID NO: 59 (29B06), and SEQ ID NO: 126 (Hu29B06 Hv4-59 D27G T30S M48I I67V Y78F), a CDR$_{H2}$ comprising the amino acid sequence of SEQ ID NO: 46 (29B06), and a CDR$_{H3}$ comprising the amino acid sequence of SEQ ID NO: 47 (29B06);

   (ii) an immunoglobulin light chain variable region comprising a CDR$_{L1}$ comprising the amino acid sequence of SEQ ID NO: 48 (29B06), a CDR$_{L2}$ comprising the amino acid sequence of SEQ ID NO: 49 (29B06), and a CDR$_{L3}$ comprising the amino acid sequence of SEQ ID NO: 50 (29B06);

   (b) (i) an immunoglobulin heavy chain variable region comprising a CDR$_{H1}$ comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 51 (07F01), SEQ ID NO: 51 (07F01) and SEQ ID NO: 124 (Sh07F01 Hv3-48 D28T T60A L63V E65G), a CDR$_{H2}$ comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 6 (07F01) and SEQ ID NO: 122 (Sh07F01 Hv3-48 D28T T60A L63V E65G), and a CDR$_{H3}$ comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 7 (07F01) and SEQ ID NO: 123 (Chimeric 07F01 C102S, Sh07F01 Hv3-48, Sh07F01 Hv3-48 D28T T60A L63V E65G); and

   (ii) an immunoglobulin light chain variable region comprising a CDR$_{L1}$ comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 8 (07F01) and SEQ ID NO: 130 (HE L 07F01 Kvl-9, Sh07F01 Kvl-9 Fl), a CDR$_{L2}$ comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 9 (07F01) and SEQ ID NO: 131 (HE L 07F01 Kvl-9, Sh07F01 Kvl-9 Fl), and a CDR$_{L3}$ comprising the amino acid sequence of SEQ ID NO: 10 (07F01);

   (c) (i) an immunoglobulin heavy chain variable region comprising a CDR$_{H1}$ comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 15 (12B11) and SEQ ID NO: 53 (12B11), a CDR$_{H2}$ comprising the amino acid sequence of SEQ ID NO: 16 (12B11), and a CDR$_{H3}$ comprising the amino acid sequence of SEQ ID NO: 17 (12B11); and
(ii) an immunoglobulin light chain variable region comprising a CDR_{L,i} comprising
the amino acid sequence of SEQ ID NO: 18 (12B11), a CDR_{L,2} comprising the amino acid
sequence of SEQ ID NO: 19 (12B11), and a CDR_{L,3} comprising the amino acid sequence of
SEQ ID NO: 20 (12B11);

d (i) an immunoglobulin heavy chain variable region comprising a CDR_{H,i} comprising
an amino acid sequence selected from the group consisting of SEQ ID NO: 25 (17F06) and
SEQ ID NO: 55 (17F06), a CDR_{H,2} comprising the amino acid sequence of SEQ ID NO: 26
(17F06), and a CDR_{H,3} comprising the amino acid sequence of SEQ ID NO: 27 (17F06); and

(ii) an immunoglobulin light chain variable region comprising a CDR_{L,1} comprising
the amino acid sequence of SEQ ID NO: 28 (17F06), a CDR_{L,2} comprising the amino acid
sequence of SEQ ID NO: 29 (17F06), and a CDR_{L,3} comprising the amino acid sequence of
SEQ ID NO:30 (17F06); and

e (i) an immunoglobulin heavy chain variable region comprising a CDR_{H,i} comprising
an amino acid sequence selected from the group consisting of SEQ ID NO: 35 (18H09) and
SEQ ID NO: 57 (18H09), a CDR_{H,2} comprising the amino acid sequence of SEQ ID NO: 36
(18H09), and a CDR_{H,3} comprising the amino acid sequence of SEQ ID NO: 37 (18H09); and

(ii) an immunoglobulin light chain variable region comprising a CDR_{L,1} comprising
the amino acid sequence of SEQ ID NO: 38 (18H09), a CDR_{L,2} comprising the amino acid
sequence of SEQ ID NO: 39 (18H09), and a CDR_{L,3} comprising the amino acid sequence of
SEQ ID NO: 40 (18H09).

2. The antibody of claim 1, wherein the immunoglobulin heavy chain variable region
comprises a CDR_{H,i} comprising an amino acid sequence selected from the group consisting of
SEQ ID NO: 45 (29B06) and SEQ ID NO: 126 (Sh29B06 Hv4-59, Hu29B06 Hv4-59 D27G
T30S M48I I67V Y78F), a CDR_{H,2} comprising the amino acid sequence of SEQ ID NO: 46
(29B06), and a CDR_{H,3} comprising the amino acid sequence of SEQ ID NO: 47 (29B06); and
the immunoglobulin light chain variable region comprises a CDR_{L,1} comprising the amino acid
sequence of SEQ ID NO: 48 (29B06), a CDR_{L,2} comprising the amino acid sequence of SEQ ID
NO: 49 (29B06), and a CDR_{L,3} comprising the amino acid sequence of SEQ ID NO: 50
(29B06).

3. The antibody of claim 1, wherein the immunoglobulin heavy chain variable region
comprises a CDR_{H,i} comprising an amino acid sequence selected from the group consisting of
SEQ ID NO: 5 (07F01) and SEQ ID NO: 124 (Sh07F01 Hv3-48 D28T T60A L63V E65G), a
CDR H2 comprising the amino acid sequence of SEQ ID NO: 122 (Sh07F01 Hv3-48 D28T
T60A L63V E65G), and a CDR H3 comprising the amino acid sequence of SEQ ID NO: 123
(Chimeric 07F01 C102S, Sh07F01 Hv3-48, Sh07F01 Hv3-48 D28T T60A L63V E65G); and
the immunoglobulin light chain variable region comprises a CDR L1 comprising the
amino acid sequence of SEQ ID NO: 130 (HE L 07F01 Kvl-9, Sh07F01 Kvl-9 Fl), a CDR L2
comprising the amino acid sequence of SEQ ID NO: 131 (HE L 07F01 Kvl-9, Sh07F01 Kvl-
9 Fl), and a CDR L3 comprising the amino acid sequence of SEQ ID NO: 10 (07F01).

4. The antibody of anyone of claims 1-3, wherein the CDR sequences are interposed
between human and humanized framework sequences.

5. The antibody of claim 2, further comprising a human germline framework sequence.

6. The antibody of claim 5, wherein the human germline framework sequence is IGHV4-
59*01.

7. The antibody of claim 6, wherein the framework sequence comprises at least one
substitution at amino acid position 27, 30, 48, 67 or 78, where in the amino acid numbering is
based on Kabat.

8. The antibody of claim 7, wherein the at least one substitution is selected from the group
consisting of D27G, T30S, M48I, I67V, and Y78F.

9. The antibody of anyone of claims 1-8, wherein the antibody is an antigen-binding
fragment.

10. An isolated nucleic acid comprising a nucleotide sequence encoding an
immunoglobulin heavy chain variable region of any one of claims 1-3.

11. An isolated nucleic acid comprising a nucleotide sequence encoding an
immunoglobulin light chain variable region of any one of claims 1-3.

12. An expression vector comprising the nucleic acid of claim 10.

13. An expression vector comprising the nucleic acid of claim 11.

14. The expression vector of claim 13, further comprising the nucleic acid of claim 10.

15. A host cell comprising the expression vector of claim 12.

16. A host cell comprising the expression vector of claim 13.
17. A host cell comprising the expression vector of claim 14.

18. The host cell comprising of claim 16, further comprising the expression vector of claim 12.

19. A method of producing a polypeptide comprising an immunoglobulin heavy chain variable region or an immunoglobulin light chain variable region, the method comprising:
(a) growing the host cell of claim 15 or 16 under conditions so that the host cell expresses the polypeptide comprising the immunoglobulin heavy chain variable region or the immunoglobulin light chain variable region; and
(b) purifying the polypeptide comprising the immunoglobulin heavy chain variable region or the immunoglobulin light chain variable region.

20. A method of producing an antibody that binds human RON or an antigen binding fragment of the antibody, the method comprising:
(a) growing the host cell of claim 17 or 18 under conditions so that the host cell expresses a polypeptide comprising the immunoglobulin heavy chain variable region and the immunoglobulin light chain variable region, thereby producing the antibody or the antigen-binding fragment of the antibody; and
(b) purifying the antibody or the antigen-binding fragment of the antibody.

21. An isolated antibody that binds human RON, comprising an immunoglobulin heavy chain variable region and an immunoglobulin light chain variable region selected from the group consisting of:
(a) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 42 (29B06), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 44 (29B06);
(b) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 147 (Hu29B06 Hv4-59 D27G T30S M48I I67V Y78F), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 149 (Sh29B06 Kv2-28)
(c) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 2 (07F01), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 4 (07F01);
(d) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 137 (Sh07F01 Hv3-48 D28T T60A L63V E65G), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 139 (HE L 07F01 Kvl-9);

(e) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 12 (12B11), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 14 (12B11);

(f) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 22 (17F06), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 24 (17F06); and

(g) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 32 (18H09), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 34 (18H09).

22. The antibody of claim 21, wherein the immunoglobulin heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 42 (29B06), and the immunoglobulin light chain variable region comprises the amino acid sequence of SEQ ID NO: 44 (29B06).

23. The antibody of claim 21, wherein the immunoglobulin heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 147 (Hu29B06 Hv4-59 D27G T30S M48I I67V Y78F), and the immunoglobulin light chain variable region comprises the amino acid sequence of SEQ ID NO: 149 (Sh29B06 Kv2-28).

24. The antibody of claim 21, wherein the immunoglobulin heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 2 (07F01), and the immunoglobulin light chain variable region comprises the amino acid sequence of SEQ ID NO: 4 (07F01).

25. The antibody of claim 21, wherein the immunoglobulin heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 137 (Sh07F01 Hv3-48 D28T T60A L63V E65G), and the immunoglobulin light chain variable region comprises the amino acid sequence of SEQ ID NO: 139 (HE L 07F01 Kvl-9).

26. An isolated nucleic acid comprising a nucleotide sequence encoding an immunoglobulin heavy chain variable region of claim 21.
27. An isolated nucleic acid comprising a nucleotide sequence encoding an
   immunoglobulin light chain variable region of claim 21.
28. An expression vector comprising the nucleic acid of claim 36.
29. An expression vector comprising the nucleic acid of claim 37.
30. The expression vector of claim 29, further comprising the nucleic acid of claim 36.
31. A host cell comprising the expression vector of claim 28.
32. A host cell comprising the expression vector of claim 29.
33. A host cell comprising the expression vector of claim 30.
34. The host cell of claim 32, further comprising the expression vector of claim 28.
35. A method of producing a polypeptide comprising an immunoglobulin heavy chain
   variable region or an immunoglobulin light chain variable region, the method comprising:
   (a) growing the host cell of claim 31 or 32 under conditions so that the host cell
   expresses the polypeptide comprising the immunoglobulin heavy chain variable region or the
   immunoglobulin light chain variable region; and
   (b) purifying the polypeptide comprising the immunoglobulin heavy chain variable
   region or the immunoglobulin light chain variable region.
36. A method of producing an antibody that binds human RON or an antigen binding
   fragment of the antibody, the method comprising:
   (a) growing the host cell of claim 33 or 34 under conditions so that the host cell
   expresses a polypeptide comprising the immunoglobulin heavy chain variable region and the
   immunoglobulin light chain variable region, thereby producing the antibody or the antigen-
   binding fragment of the antibody; and
   (b) purifying the antibody or the antigen-binding fragment of the antibody.
37. An isolated antibody that binds human RON comprising an immunoglobulin heavy
   chain and an immunoglobulin light chain selected from the group consisting of:
   (a) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID
   NO: 109 (29B06), and an immunoglobulin light chain comprising the amino acid sequence of
   SEQ ID NO: 111 (29B06);
(b) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 176 (Hu29B06 Hv4-59 D27G T30S M48I I67V Y78F IgGl), and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 178 (Sh29B06 Kv2-28 Kappa);

c) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 93 (07F01), and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 95 (07F01);

d) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 166 (Sh07F01 Hv3-48 D28T T60A L63V E65G IgGl), and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 168 (HE L 07F01 Kv1-9 Kappa);

e) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 97 (12B11), and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 99 (12B11);

f) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 101 (17F06), and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 103 (17F06); and

g) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 105 (18H09), and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 107 (18H09).

38. The antibody of claim 37, wherein the immunoglobulin heavy chain comprises the amino acid sequence of SEQ ID NO: 109 (29B06), and the immunoglobulin light chain comprises the amino acid sequence of SEQ ID NO: 111 (29B06).

39. The antibody of claim 37, wherein the immunoglobulin heavy chain comprises the amino acid sequence of SEQ ID NO: 93 (07F01), and the immunoglobulin light chain comprises the amino acid sequence of SEQ ID NO: 95 (07F01).

40. The antibody of claim 37, wherein the immunoglobulin heavy chain comprises the amino acid sequence of SEQ ID NO: 176 (Hu29B06 Hv4-59 D27G T30S M48I I67V Y78F IgGl), and the immunoglobulin light chain comprises the amino acid sequence of SEQ ID NO: 178 (Sh29B06 Kv2-28 Kappa).
41. The antibody of claim 37, wherein the immunoglobulin heavy chain comprises the amino acid sequence of SEQ ID NO: 166 (Sh07F01 Hv3-48 D28T T60A L63V E65G IgGl), and the immunoglobulin light chain comprises the amino acid sequence of SEQ ID NO: 168 (HE L 07F01 Kvl-9 Kappa).

42. The antibody of any one of claims 21 or 37, wherein the antibody is an antigen-binding fragment.

43. An isolated nucleic acid comprising a nucleotide sequence encoding an immunoglobulin heavy chain of claim 37.

44. An isolated nucleic acid comprising a nucleotide sequence encoding an immunoglobulin light chain of claim 37.

45. An expression vector comprising the nucleic acid of claim 43.

46. An expression vector comprising the nucleic acid of claim 44.

47. The expression vector of claim 46, further comprising the nucleic acid of claim 43.

48. A host cell comprising the expression vector of claim 45.

49. A host cell comprising the expression vector of claim 46.

50. A host cell comprising the expression vector of claim 47.

51. The host cell of claim 49, further comprising the expression vector of claim 45.

52. A method of producing a polypeptide comprising an immunoglobulin heavy chain variable region or an immunoglobulin light chain variable region, the method comprising:

(a) growing the host cell of claim 48 or 49 under conditions so that the host cell expresses the polypeptide comprising the immunoglobulin heavy chain variable region or the immunoglobulin light chain variable region; and

(b) purifying the polypeptide comprising the immunoglobulin heavy chain variable region or the immunoglobulin light chain variable region.

53. A method of producing an antibody that binds human RON or an antigen binding fragment of the antibody, the method comprising:

(a) growing the host cell of claim 50 or 51 under conditions so that the host cell expresses a polypeptide comprising the immunoglobulin heavy chain variable region and the
immunoglobulin light chain variable region, thereby producing the antibody or the antigen-binding fragment of the antibody; and

(b) purifying the antibody or the antigen-binding fragment of the antibody.

54. The antibody of any one of claims 1-9, 21-25, or 37-42, wherein the antibody binds human RON with a $K_D$ of 900 pM or lower as measured by surface plasmon resonance.

55. The antibody of claim 54, wherein the antibody binds human RON with a $K_D$ of 500 pM or lower as measured by surface plasmon resonance.

56. The antibody of claim 55, wherein the antibody binds human RON with a $K_D$ of 250 pM or lower as measured by surface plasmon resonance.

57. An isolated antibody that inhibits the biological activity of human RON without inhibiting binding of MSP to human RON.

58. A method of inhibiting or reducing proliferation of a tumor cell comprising exposing the cell to an effective amount of the antibody of any one of claims 1-9, 21-25, 37-42 or 54-57 to inhibit or reduce proliferation of the tumor cell.

59. A method of inhibiting or reducing tumor growth in a mammal, the method comprising exposing the mammal to an effective amount of the antibody of any one of claims 1-9, 21-25, 37-42 or 54-57 to inhibit or reduce proliferation of the tumor.

60. A method of treating cancer in a human patient, the method comprising administering an effective amount of the antibody of any one of claims 1-9, 21-25, 37-42 or 54-57 to a mammal in need thereof.

61. The method of claim 60, wherein the cancer is selected from the group consisting of breast, ovarian, prostate, cervical, colorectal, lung, pancreatic, gastric, and head and neck cancers.

62. The antibody of any one of claims 1-9, 21-25, 37-42 or 54-57 for use in therapy.

63. The antibody of any one of claims 1-9, 21-25, 37-42 or 54-57 for use in inhibiting or reducing proliferation of a tumor cell.

64. The antibody of any one of claims 1-9, 21-25, 37-42 or 54-57 for use inhibiting or reducing tumor growth in a mammal.
<table>
<thead>
<tr>
<th>Antibody</th>
<th>CDR1</th>
<th>CDR2</th>
<th>CDR3</th>
</tr>
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<tbody>
<tr>
<td>07F01</td>
<td>EVKL</td>
<td>EVKL</td>
<td>SRDN</td>
</tr>
<tr>
<td>12B11</td>
<td>VLESGGGLVQPGGLKLSLCAASGFDFSRHWS</td>
<td>VLESGGGLVQPGGLKLSLCAASGFDFSRHWS</td>
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</tr>
<tr>
<td>17F06</td>
<td>VLESGGGLVQPGGLKLSLCAASGFDFSRHWS</td>
<td>VLESGGGLVQPGGLKLSLCAASGFDFSRHWS</td>
<td>VLESGGGLVQPGGLKLSLCAASGFDFSRHWS</td>
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<tr>
<td>18H09</td>
<td>VLESGGGLVQPGGLKLSLCAASGFDFSRHWS</td>
<td>VLESGGGLVQPGGLKLSLCAASGFDFSRHWS</td>
<td>VLESGGGLVQPGGLKLSLCAASGFDFSRHWS</td>
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<td>VLESGGGLVQPGGLKLSLCAASGFDFSRHWS</td>
<td>VLESGGGLVQPGGLKLSLCAASGFDFSRHWS</td>
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**Fig. 2**
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<th>CDR2</th>
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</tr>
</thead>
<tbody>
<tr>
<td>07F01</td>
<td>RHWMS</td>
<td>EINPDSRTINYPSSLKE (SEQ ID NO: 5)</td>
<td>RVRHINYGAMDC (SEQ ID NO: 7)</td>
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<tr>
<td>12B11</td>
<td>TYAMS</td>
<td>GITNGGCSFTYYPDTVKG (SEQ ID NO: 15)</td>
<td>QGYYGYNF--DY (SEQ ID NO: 17)</td>
</tr>
<tr>
<td>17F06</td>
<td>SYGMS</td>
<td>SISSGGGTTYLDTVKG (SEQ ID NO: 25)</td>
<td>GQWLLKF---AY (SEQ ID NO: 27)</td>
</tr>
<tr>
<td>18H09</td>
<td>SDYWN</td>
<td>YIS--GSTYNNPSLKS (SEQ ID NO: 35)</td>
<td>THILTI----AY (SEQ ID NO: 37)</td>
</tr>
<tr>
<td>29B06</td>
<td>SGYWN</td>
<td>YIS--YSGKTYNPSLKS (SEQ ID NO: 45)</td>
<td>SKYDYAM----DY (SEQ ID NO: 47)</td>
</tr>
</tbody>
</table>

**Fig.3**
Complete Light (Kappa or Lambda) Chain Variable Region Amino Acid Alignments

<table>
<thead>
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<th>CDR1</th>
<th>CDR2</th>
</tr>
</thead>
<tbody>
<tr>
<td>07F01</td>
<td>(1) DIVLTSOKIVSTSVGARVSTCKASQ----NVGSSLWYQQKPGQSPKTLYIASFR----YSVPDR</td>
<td></td>
</tr>
<tr>
<td>12B11</td>
<td>(1) DAVMTQTPLSLPVLGDQASISOGRSSQSLENSIGNYLNWLVKQPQSPQLINYTVSNR----FSGVDR</td>
<td></td>
</tr>
<tr>
<td>17F06</td>
<td>(1) QLVLTQSSS-ASFSLAGAKLTCQTLSSLQ----HTTYTIEWQQLPLKPKYVMELKKGSHSTGVGIPDR</td>
<td></td>
</tr>
<tr>
<td>18H09</td>
<td>(1) QAATQQESA-LITSPGETVILCTPSSAGAV----TTSNFANWQQEKPDHLFTGLIDNIR----AFGVPAR</td>
<td></td>
</tr>
<tr>
<td>29B06</td>
<td>(1) DIVLTQSPASLAVSLGQRATISCRASETVDNFGISFNNWFQQKPGQPKLIIYASNNQ----GSVPAR</td>
<td></td>
</tr>
</tbody>
</table>

CDR

<table>
<thead>
<tr>
<th>Antibody</th>
<th>CDR1</th>
<th>CDR2</th>
</tr>
</thead>
<tbody>
<tr>
<td>07F01</td>
<td>(62) FTGSNGTDSLTLTISNVQSEDLADYFCQQYNNYP----LTFAGGTKLELK (SEQ ID NO: 4)</td>
<td></td>
</tr>
<tr>
<td>12B11</td>
<td>(67) FSGSNGTDSLTLKIRVEAEDGLYLFGCLQVTHV----HTGGGTKLELK (SEQ ID NO: 14)</td>
<td></td>
</tr>
<tr>
<td>17F06</td>
<td>(66) FSGSNGADRYLTISNIQPEDAEAIYGVGETIEDQFYVYFGGGTKVTWL (SEQ ID NO: 24)</td>
<td></td>
</tr>
<tr>
<td>18H09</td>
<td>(64) FSGSNGDKALTITGAQTEDEAIYFGCALWYSNHY---WYFGGGTKLTVL (SEQ ID NO: 34)</td>
<td></td>
</tr>
<tr>
<td>29B06</td>
<td>(66) FSGSNGTDSLSINHVPEDDAMTAMFCQQSKEVP----PFNAGGGGTKLEIK (SEQ ID NO: 44)</td>
<td></td>
</tr>
</tbody>
</table>

Fig.4
**Light (Kappa or Lambda) 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>07F01</td>
<td>KAS----NVGSSLV (SEQ ID NO: 8)</td>
<td>SASFR----YS (SEQ ID NO: 9)</td>
<td>QQYNNYP-----LT (SEQ ID NO: 10)</td>
</tr>
<tr>
<td>12B11</td>
<td>RSSQLENSSNLYLN (SEQ ID NO: 18)</td>
<td>RVSNR----FS (SEQ ID NO: 19)</td>
<td>LQVTHVP-----HT (SEQ ID NO: 20)</td>
</tr>
<tr>
<td>17F06</td>
<td>TLSQ----HTYTIIE (SEQ ID NO: 28)</td>
<td>LKKGSHSTGV (SEQ ID NO: 29)</td>
<td>GVGETIEDQFVVV (SEQ ID NO: 30)</td>
</tr>
<tr>
<td>18H09</td>
<td>RSSAGAV----TTSNFAN (SEQ ID NO: 38)</td>
<td>DTNR----AP (SEQ ID NO: 39)</td>
<td>ALWYSNHY----WV (SEQ ID NO: 40)</td>
</tr>
<tr>
<td>29B06</td>
<td>RASEIVDN-FGISFMN (SEQ ID NO: 48)</td>
<td>AASNQ-----GS (SEQ ID NO: 49)</td>
<td>QQSKEVP-----PT (SEQ ID NO: 50)</td>
</tr>
</tbody>
</table>

**Fig.5**
FIG. 6

Neutralization of ligand binding

% Neutralization

log nM AB

-3  -2  -1  0  1  2

07F01
29B06
17F06
12B11
18H09
FIG. 7

p-ERK Inhibition

% Inhibition

log nM AB

29B06
17F06
07F01
18H09
12B11
FIG. 8
FIG. 9

Tumor Volume, mm³

Day

- 29066
- 07F01
- 12B11
- 18H09
- IgG
Complete Heavy Chain Variable Region Amino Acid Alignments

**Antibody**

**CDR1**

- 07F01 EVKLLESGGGLVQPGGLKL2CAASGGFDSRNYMHWGVRAPGKLEWIEHNPDSRTYNFTPSLKERFTI
- Chimeric 07F01 C102S EVKLLESGGGLVQPGGLKL2CAASGGFDSRNYMHWGVRAPGKLEWIEHNPDSRTYNFTPSLKERFTI
- Sh07F01 Hv3-48 EVQLVESGGGLVQPGGLKL2CAASGGFDSRNYMHWGVRAPGKLEWIEHNPDSRTYNFTPSLKERFTI

**CDR2**

- Sh07F01 Hv3-48 D28T T60A L63V E65G EVQLVESGGGLVQPGGLKL2CAASGGFDSRNYMHWGVRAPGKLEWIEHNPDSRTYNFTPSLKERFTI

**CDR3**

- 07F01 SRDNKNSLFLQMNRVRSEDATLYYYCARGVRTHIYYGAMDDMQQGTSVTSS (SEQ ID NO: 2)
- Chimeric 07F01 C102S SRDNKNSLFLQMNRVRSEDATLYYYCARGVRTHIYYGAMDDMQQGTSVTSS (SEQ ID NO: 133)
- Sh07F01 Hv3-48 SRDNKNSLYLQMNLSRAEDTAVYCYCARGVRTHIYYGAMDDMQQGTTVTSS (SEQ ID NO: 135)
- Sh07F01 Hv3-48 D28T T60A L63V E65G SRDNKNSLYLQMNLSRAEDTAVYCYCARGVRTHIYYGAMDDMQQGTTVTSS (SEQ ID NO: 137)

**Fig. 12A**

**Antibody**

**CDR1**

- 29B06 EVQLQESGPQLVKPSQTLSLCTCSVTGSITSGYWNIRKFGPGKLEYMTQYSKTTYNPSLKRERVIT
- Sh29B06 Hv4-59 QVQLQESGPQLVKPSQTLSLCTCSVTGSITSGYWNIRKFGPGKLEYMTQYSKTTYNPSLKRERVIT
- Hu29B06 Hv4-59 QVQLQESGPQLVKPSQTLSLCTCSVTGSITSGYWNIRKFGPGKLEYMTQYSKTTYNPSLKRERVIT

**CDR2**

- Hu29B06 Hv4-59 D27G T30S M48I I67V Y78F QVQLQESGPQLVKPSQTLSLCTCSVTGSITSGYWNIRKFGPGKLEYMTQYSKTTYNPSLKRERVIT

**CDR3**

- 29B06 RDISKNHYLQSLISVTADTTAYCYCARGSKYDAMYAMNGQGTSVTSS (SEQ ID NO: 42)
- Sh29B06 Hv4-59 VTDISKNQFSLKLSVTAADTTAVYCYCARGSKYDAMYAMNGQGTLVTSS (SEQ ID NO: 143)
- Hu29B06 Hv4-59 RDISKNQFSLKLSVTAADTTAVYCYCARGSKYDAMYAMNGQGTLVTSS (SEQ ID NO: 145)

- Hu29B06 Hv4-59 D27G T30S M48I I67V Y78F RDISKNQFSLKLSVTAADTTAVYCYCARGSKYDAMYAMNGQGTLVTSS (SEQ ID NO: 147)

**Fig. 12B**
### Heavy Chain CDR Amino Acid Alignments

<table>
<thead>
<tr>
<th>Antibody</th>
<th>CDR1</th>
<th>CDR2</th>
<th>CDR3</th>
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<tbody>
<tr>
<td>07F01</td>
<td>RHWMS (SEQ ID NO: 5)</td>
<td>EINPDGRTNITYTPSLKE (SEQ ID NO: 6)</td>
<td>RVRHHYGAMDS (SEQ ID NO: 7)</td>
</tr>
<tr>
<td>Chimeric 07F01 C102S</td>
<td>RHWMS (SEQ ID NO: 5) EINPDGRTNITYTPSLKE (SEQ ID NO: 6)</td>
<td>RVRHHYGAMDS (SEQ ID NO: 123)</td>
<td></td>
</tr>
<tr>
<td>Sh07F01 Hv3-48</td>
<td>RHWMS (SEQ ID NO: 5) EINPDGRTNITYTPSLKE (SEQ ID NO: 6)</td>
<td>RVRHHYGAMDS (SEQ ID NO: 123)</td>
<td></td>
</tr>
<tr>
<td>Sh07F01 Hv3-48 D28T T60A L63V E65G</td>
<td>RHWMS (SEQ ID NO: 5) EINPDGRTNITYAPSVKG (SEQ ID NO: 122)</td>
<td>RVRHHYGAMDS (SEQ ID NO: 123)</td>
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</tbody>
</table>

**Fig. 13A**

<table>
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<tr>
<th>Antibody</th>
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<th>CDR2</th>
<th>CDR3</th>
</tr>
</thead>
<tbody>
<tr>
<td>29B06</td>
<td>SGYWN (SEQ ID NO: 45)</td>
<td>YISYSGKTYNPSLKS (SEQ ID NO: 46)</td>
<td>SKYDYAMDY (SEQ ID NO: 47)</td>
</tr>
<tr>
<td>Sh29B06 Hv4-59</td>
<td>SGYWN (SEQ ID NO: 45) YISYSGKTYNPSLKS (SEQ ID NO: 46)</td>
<td>SKYDYAMDY (SEQ ID NO: 47)</td>
<td></td>
</tr>
<tr>
<td>Hu29B06 Hv4-59</td>
<td>SGYWN (SEQ ID NO: 45) YISYSGKTYNPSLKS (SEQ ID NO: 46)</td>
<td>SKYDYAMDY (SEQ ID NO: 47)</td>
<td></td>
</tr>
<tr>
<td>Hu29B06 Hv4-59 D27G T30S M48I I67V Y78F</td>
<td>SGYWN (SEQ ID NO: 45) YISYSGKTYNPSLKS (SEQ ID NO: 46)</td>
<td>SKYDYAMDY (SEQ ID NO: 47)</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 13B**
Complete Light (Kappa) Chain Variable Region Amino Acid Alignments

**Antibody**

07F01 DIVLTQSQKIVSTSGVARSVTCASHGNVGLASSLYFKQGQPKPTKLIYSGSRFSGVPDRFSGSGTD
HE L 07F01 Kv1-9 DIQLTQSQSFVSTSGVDRVTAVCRASGNVGLASSLYFKGKSPKTLYSGSFLYSVPFRSFGSGSGTE
Sh07F01 Kv1-9 F1 DIQLTQPSFSALSASGDRVTTACRASHGNVGLASSLYFKGKAPKTLYSGSFLYSVPFRSFGSGSGTE

**CDR1**

07F01 FTTISNQEDLADYFYQNNYPLTGFAGTKLEIK (SEQ ID NO: 4)
HE L 07F01 Kv1-9 FTTISVQPEDFADYFYQNNYPLTFGGGTKVEIK (SEQ ID NO: 139)
Sh07F01 Kv1-9 F1 FTTISLQPEDFATYFYQNNYPLTFGGGTKVEIK (SEQ ID NO: 141)

**Fig. 14A**

**Antibody**

29B06 DIVLTQSPASLSLGLQORATASCRASEIVDNFGISFMNYFGQQPKQMLIYASAOGSGVIPARFSGSG
Sh29B06 Kv2-28 DIVMTQSPSLPVTGEPASCRASEIVDNFGISFMNYLQKQQSPQLLIYASAOGSGVIPDRFSGSG

**CDR1**

29B06 SGTDFOALNHPVEEDDTAMYFYQQSKEVPPTFGGGTKLEIK (SEQ ID NO: 44)
Sh29B06 Kv2-28 SGTDFLTISKREVAEDVGVYQQSKEVPPTFGGGTKVEIK (SEQ ID NO: 149)

**Fig. 14B**
### 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>07F01</td>
<td>RASQNVGSSLV (SEQ ID NO: 8)</td>
<td>SASFRLYS (SEQ ID NO: 9)</td>
<td>QQYNYNPLT (SEQ ID NO: 10)</td>
</tr>
<tr>
<td>HE L 07F01 Kv1-9</td>
<td>RASQNVGSSLV (SEQ ID NO: 130)</td>
<td>SASFRLYS (SEQ ID NO: 131)</td>
<td>QQYNYNPLT (SEQ ID NO: 10)</td>
</tr>
<tr>
<td>Sh07F01 Kv1-9 F1</td>
<td>RASQNVGSSLV (SEQ ID NO: 130)</td>
<td>SASFRLYS (SEQ ID NO: 131)</td>
<td>QQYNYNPLT (SEQ ID NO: 10)</td>
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**Fig. 15A**

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</tr>
</thead>
<tbody>
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<td>RASEIVDNGISFMN (SEQ ID NO: 48)</td>
<td>AASNQGS (SEQ ID NO: 49)</td>
<td>QQSKEVPPT (SEQ ID NO: 50)</td>
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<td>Sh29B06 Kv2-28</td>
<td>RASEIVDNGISFMN (SEQ ID NO: 48)</td>
<td>AASNQGS (SEQ ID NO: 49)</td>
<td>QQSKEVPPT (SEQ ID NO: 50)</td>
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</table>

**Fig. 15B**
Fig. 16
Fig. 17
Fig. 18