c-Met Antibodies

The present invention relates to antibodies that bind c-Met and their use in treating conditions and disorders in which pathogenesis is mediated by this receptor.

c-Met, a member of the tyrosine kinase superfamily, is the receptor for Hepatocyte Growth Factor (HGF). Binding of HGF to c-Met leads to receptor dimerization or multimerization, phosphorylation of multiple tyrosine residues in the intracellular region, catalytic activation, and downstream signaling. c-Met is also activated via ligand-independent mechanisms, including receptor over-expression, amplification, and mutation. c-Met activation enhances cellular proliferation, migration, morphogenesis, survival (including protection from apoptosis), and protease synthesis, characteristics that are associated with invasive cell phenotype and poor clinical outcomes and drug resistance in cancer patients. The c-Met signaling pathway is one of the most frequently dysregulated pathways in human cancers, and occurs in virtually all types of solid tumors.

PCT International Publication WO 09/007427 discloses murine and CDR-grafted, humanized c-Met antibodies. Murine antibody 224G11 disclosed therein did not bind the c-Met Sema domain. Further functional properties of the humanized IgG1 derivative of this murine antibody, denoted h224G11 Mab, are reported in Abstracts nos. 835 (*in vitro* data) and 2792 (*in vivo* data) and their accompanying posters presented at the meeting of the American Association for Cancer Research (Denver, CO) in April, 2009. These abstracts and posters disclose that bivalent h224G11 Mab is devoid of intrinsic agonistic properties, behaves as a full antagonist of c-Met, and potently decreases c-Met dimerization. Murine 224G11 is reported to down-regulate c-Met and block c-Met phosphorylation *in vivo*. In the case of other receptors, dimerization is a prerequisite for receptor internalization and degradation. These abstracts and posters disclose no data relating to c-Met internalization. Furthermore, the epitope to which the humanized antibody binds within c-Met is not identified.

PCT International Publication WO 05/016382 also discloses c-Met antibodies but does not identify the epitope(s) to which the antibodies bind. An epitope mapping example is provided, however, the reported results merely indicate that six c-Met antibodies bind to a common epitope while a seventh c-Met antibody binds a distinct epitope. The particular epitopes to which these c-Met antibodies bind is not provided.

There exists a need for antagonist antibodies to human c-Met, binding of which to the α-chain of human c-Met facilitates internalization of the receptor from the cell surface, in the presence and/or absence of HGF. There is also a need for antagonist antibodies to human c-Met, which binding to the α-chain of human c-Met facilitates internalization of the receptor from the cell surface in cells comprising c-Met variants containing gain of function mutations. There is also a need for antagonist antibodies to human c-Met which induce c-Met degradation and reduction of phosphorylated c-Met. Such antagonist activities could decrease the number of available binding sites for HGF on tumor cell surfaces, and terminate the pathway activation caused by overexpression, amplification, or mutation of c-Met. At the same time, such antagonist antibodies should inhibit HGF binding to c-Met and HGF-induced c-Met activation, and induce little or no agonist activity themselves.

Antibody compounds of the present invention meet these needs. They bind to epitopes in the α-chain of the human c-Met Sema domain, inhibiting HGF-binding to c-Met and receptor activation, while inducing little or no agonist activity. The antibodies of the present invention also induce internalization of the receptor in the presence or absence of HGF and also in cells comprising c-Met variants containing gain of function mutations. They induce degradation of c-Met and induce reduction of phosphorylated human c-Met, and inhibit HGF-dependent and HGF-independent proliferation of tumor cells that express this receptor. In view of these properties, these antibody compounds should be therapeutically useful in treating cancers mediated by c-Met via a variety of different mechanisms.

In addition, the present antibody compounds possess a number of other desirable properties. They exhibit high affinity (K_D) to c-Met, block HGF-mediated c-Met phosphorylation and downstream signaling, cellular proliferation, and cellular migration; and induce only weak phosphorylation of c-Met while inducing little or no HGF-like biological agonist activities such as induction of tumor cell proliferation, motility, invasion, tubulogenesis, angiogenesis, or anti-apoptotic effects. They inhibit both ligand (HGF)-dependent and ligand-independent c-Met pathway activation. Additionally, antibody compounds of the present invention preferentially bind human c-Met extracellular domain (ECD) compared to the ECDs of the closely related receptors RON and PlexinA2, and do not cause "shedding" of the c-Met ECD.

Accordingly, the present invention provides:

A monoclonal antibody, or antigen-binding fragment thereof, that:

a) binds an epitope within the α-chain of human c-Met, and

b) induces internalization of cell surface human c-Met.

Any one of the foregoing antibodies or antigen-binding fragment thereof wherein the antibody or antigen-binding fragment thereof induces hepatocyte-growth factor-independent internalization of cell surface human c-Met. In a preferred embodiment, the monoclonal antibody or antigen-binding fragment thereof comprises a light chain and a heavy chain, wherein the light chain comprises the amino acid sequence of SEQ ID NO: 28 and the heavy chain comprises the amino acid sequence of SEQ ID NO: 40. In another preferred embodiment, the monoclonal antibody or antigen-binding fragment thereof comprises a light chain and a heavy chain, wherein the light chain comprises the amino acid sequence of SEQ ID NO: 29 and the heavy chain comprises the amino acid sequence of SEQ ID NO: 41.

Any one of the foregoing monoclonal antibodies or antigen-binding fragments thereof that induces internalization of human c-Met in cells comprising a human c-Met variant containing a gain of function mutation. The gain of function mutation can be c-Met kinase domain mutation M1149T or juxtamembrane domain mutation R988C.

Any of the foregoing antibodies or antigen-binding fragments thereof wherein the antibody or antigen-binding fragment thereof induces at least 40% internalization of cell surface human c-Met in cells. Any of the foregoing antibodies or antigen-binding fragments thereof wherein the antibody or antigen-binding fragment thereof induces at least 45% internalization of cell surface human c-Met in cells. Any of the foregoing antibodies or antigen-binding fragments thereof wherein the antibody or antigen-binding fragment thereof induces at least 50% internalization of cell surface human c-Met in cells. Any of the foregoing antibodies or antigen-binding fragments thereof wherein the antibody or antigen-binding fragment thereof induces at least 55% internalization of cell surface human c-Met in cells. Any of the foregoing antibodies or antigen-binding fragments thereof wherein the antibody or antigen-binding fragment thereof induces at least 60% internalization of cell surface human c-Met in cells. Any of the foregoing antibodies or antigen-binding fragments thereof wherein the antibody or antigen-binding fragment thereof induces at least 65% internalization of human c-Met in cells. Any of

the foregoing antibodies or antigen-binding fragments thereof wherein the antibody or antigen-binding fragment thereof induces at least 70% internalization of cell surface human c-Met in cells.

Any one of the foregoing monoclonal antibodies or antigen-binding fragments thereof, which induces reduction of total c-Met in hepatocyte growth factor-independent tumor cells. In a preferred embodiment, the monoclonal antibody or antigen-binding fragment thereof which induces reduction of total c-Met in hepatocyte growth factor-independent tumor cells comprises a light chain and a heavy chain, wherein the light chain comprises the amino acid sequence of SEQ ID NO: 28 and the heavy chain comprises the amino acid sequence of SEQ ID NO: 40. In another preferred embodiment, the monoclonal antibody or antigen-binding fragment thereof which induces reduction of total c-Met in hepatocyte growth factor-independent tumor cells comprises a light chain and a heavy chain, wherein the light chain comprises the amino acid sequence of SEQ ID NO: 29 and the heavy chain comprises the amino acid sequence of SEQ ID NO: 41.

Any one of the foregoing monoclonal antibodies or antigen-binding fragments thereof, which induces reduction of phosphorylated c-Met in hepatocyte growth factor-independent tumor cells.

Any one of the foregoing monoclonal antibodies or antigen-binding fragments thereof, which binds the α-chain of human c-Met at substantially the same epitope as an antibody comprising a light chain having the amino acid sequence shown in SEQ ID NO:28 and a heavy chain having the amino sequence shown in SEQ ID NO:40, or which binds the α-chain of human c-Met at substantially the same epitope as an antibody comprising a light chain having the amino acid sequence shown in SEQ ID NO:29 and a heavy chain having the amino sequence shown in SEQ ID NO:41.

Any one of the foregoing monoclonal antibodies or antigen-binding fragments thereof, excluding those comprising a light chain having the amino acid sequence shown in SEQ ID NO:26 and a heavy chain having the amino sequence shown in SEQ ID NO:38, wherein the epitope comprises one or more amino acid residues within $_{144}$HVFPHNHTADIQS$_{156}$ (SEQ ID NO: 82) inclusive.

Any one of the foregoing antibodies or antigen-binding fragments, wherein the epitope further comprises one or more amino acid residues within $_{123}$DTYYDD$_{128}$ (SEQ ID NO:81) inclusive.

Any one of the foregoing antibodies or antigen-binding fragments, wherein the epitope further comprises one or more amino acid residues within $_{192}$FINF$_{195}$ (SEQ ID NO:83) inclusive.

Any one of the foregoing antibodies or antigen-binding fragments, wherein the epitope further comprises one or more amino acid residues within $_{220}$KETKDGFM$_{227}$ (SEQ ID NO:84) inclusive.

Any one of the foregoing antibodies or antigen-binding fragments thereof, wherein the antibody binds within an amino acid sequence selected from the group consisting of:

a) $_{121}$VVDTYYDDQL$_{130}$ (SEQ ID NO:77),

b) $_{131}$ISCGSVNRGTCQRHVFPHNHTADIQS$_{156}$ (SEQ ID NO:78),

c) $_{179}$ALGAKVLSSVKDRFINF$_{195}$ (SEQ ID NO:79), and

d) $_{216}$VRRLKETKDGFM$_{227}$ (SEQ ID NO:80),

inclusive.

Any one of the foregoing antibodies or antigen-binding fragments thereof, wherein the antibody binds within an amino acid sequence selected from the group consisting of:

a) $_{123}$DTYYDD$_{128}$ (SEQ ID NO:81),

b) $_{144}$HVFPHNHTADIQS$_{156}$ (SEQ ID NO: 82),

c) $_{192}$FINF$_{195}$ (SEQ ID NO:83), and

d) $_{220}$KETKDGFM$_{227}$ (SEQ ID NO:84),

inclusive.

Any one of the foregoing monoclonal antibodies or antigen-binding fragments thereof which bind to an amino acid sequence within the epitope characterized by $_{121}$VVDTYYDDQL$_{130}$ (SEQ ID NO:77), $_{131}$ISCGSVNRGTCQRHVFPHNHTADIQS$_{156}$ (SEQ ID NO:78), $_{179}$ALGAKVLSSVKDRFINF$_{195}$ (SEQ ID NO:79), and $_{216}$VRRLKETKDGFM$_{227}$ (SEQ ID NO:80), inclusive.

Any one of the foregoing monoclonal antibodies or antigen-binding fragments thereof which bind to an amino acid sequence within the epitope characterized by $_{123}$DTYYDD$_{128}$ (SEQ ID NO:81), $_{144}$HVFPHNHTADIQS$_{156}$ (SEQ ID NO: 82), $_{192}$FINF$_{195}$ (SEQ ID NO:83), and $_{220}$KETKDGFM$_{227}$ (SEQ ID NO:84) inclusive.

Any one of the foregoing antibodies or antigen-binding fragments thereof, wherein the antibody binds within an amino acid sequence of $_{95}$CFPCQDCSSKA$_{105}$ (SEQ ID NO: 86) inclusive.

Any one of the foregoing monoclonal antibodies or antigen-binding fragments thereof, excluding those comprising a light chain having the amino acid sequence shown in SEQ ID NO:29 and a heavy chain having the amino sequence shown in SEQ ID NO:41, wherein the epitope comprises one or more amino acid residues within $_{95}$CFPCQDCSSKA$_{105}$ (SEQ ID NO: 86) inclusive.

Any one of the foregoing monoclonal antibodies, or antigen-binding fragments thereof, that binds human c-Met, which comprises three light chain complementarity determining regions (LCDRs) and three heavy chain complementarity determining regions (HCDRs),

wherein said three LCDRs and said three HCDRs are selected from the group consisting of:

a) LCDR1 comprising the amino acid sequence SVSSSISSTNLH (SEQ ID NO:49);

LCDR2 comprising the amino acid sequence GTSX$_1$LX$_2$S (SEQ ID NO:87), wherein X$_1$ is Y or R, and X$_2$ is A or R;

LCDR3 comprising the amino acid sequence QQWSSYPYS (SEQ ID NO:51);

HCDR1 comprising the amino acid sequence GYTFTSRYIH (SEQ ID NO:59);

HCDR2 comprising the amino acid sequence WIYPVTGDTYYX$_7$EX$_8$FKG (SEQ ID NO:90), wherein X$_7$ is N, I, or R, and X$_8$ is K or P; and

HCDR3 comprising the amino acid sequence GYGAFX$_9$Y (SEQ ID NO:91), wherein X$_9$ is Y or F; and

b) LCDR1 comprising the amino acid sequence SVSSSVX$_3$SIYLH (SEQ ID NO:88), wherein X$_3$ is S or R;

LCDR2 comprising the amino acid sequence STSNLAS (SEQ ID NO:54);

LCDR3 comprising the amino acid sequence X$_4$X$_5$YX$_6$GYPLT (SEQ ID NO:89), wherein X$_4$ is I or Q, X$_5$ is Q or V, and X$_6$ is S or R;

HCDR1 comprising the amino acid sequence GYTFTDYYMH (SEQ ID NO:65);

HCDR2 comprising the amino acid sequence RVNPX$_{10}$RX$_{11}$X$_{12}$TTYNQKFEG (SEQ ID NO:92), wherein X$_{10}$ is N or Y, X$_{11}$ is G or R, and X$_{12}$ is G or S; and

HCDR3 comprising the amino acid sequence X$_{13}$NX$_{14}$LDY (SEQ ID NO:93), wherein X$_{13}$ is T or A, and X$_{14}$ is W or I;

wherein said monoclonal antibody or antigen-binding fragment thereof binds an epitope within the α-chain of said human c-Met and induces internalization of cell surface human c-Met.

Any one of the foregoing monoclonal antibodies, or antigen-binding fragments thereof, that binds human c-Met, which comprises three light chain complementarity determining regions (LCDRs) and three heavy chain complementarity determining regions (HCDRs), wherein the antibody comprises three light chain complementarity determining regions (LCDRs) and three heavy chain complementarity determining regions (HCDRs), wherein

LCDR1 comprises the amino acid sequence SVSSSISSTNLH (SEQ ID NO:49);

LCDR2 comprises the amino acid sequence GTSX$_1$LX$_2$S (SEQ ID NO:87), wherein X$_1$ is Y or R, and X$_2$ is A or R;

LCDR3 comprises the amino acid sequence QQWSSYPYS (SEQ ID NO:51);

HCDR1 comprises the amino acid sequence GYTFTSRYIH (SEQ ID NO:59);

HCDR2 comprises the amino acid sequence WIYPVTGDTYYX$_7$EX$_8$FKG (SEQ ID NO:90), wherein X$_7$ is N, I, or R, and X$_8$ is K or P; and

HCDR3 comprises the amino acid sequence GYGAFX$_9$Y (SEQ ID NO:91), wherein X$_9$ is Y or F.

Any one of the foregoing monoclonal antibodies, or antigen-binding fragments thereof, that binds human c-Met, which comprises three light chain complementarity determining regions (LCDRs) and three heavy chain complementarity determining regions (HCDRs), wherein

LCDR1 comprises the amino acid sequence SVSSSVX$_3$SIYLH (SEQ ID NO:88); wherein X$_3$ is S or R;

LCDR2 comprises the amino acid sequence STSNLAS (SEQ ID NO:54);

LCDR3 comprises the amino acid sequence X$_4$X$_5$YX$_6$GYPLT (SEQ ID NO:89), wherein X$_4$ is I or Q, X$_5$ is Q or V, and X$_6$ is S or R;

HCDR1 comprises the amino acid sequence GYTFTDYYMH (SEQ ID NO:65);

HCDR2 comprises the amino acid sequence RVNPX$_{10}$RX$_{11}$X$_{12}$TTYNQKFEG (SEQ ID NO:92), wherein X$_{10}$ is N or Y, X$_{11}$ is G or R, and X$_{12}$ is G or S; and

HCDR3 comprises the amino acid sequence X$_{13}$NX$_{14}$LDY (SEQ ID NO:93), wherein X$_{13}$ is T or A, and X$_{14}$ is W or I.

Any one of the foregoing monoclonal antibodies, or antigen-binding fragments thereof, that binds human c-Met, which comprises three light chain complementarity determining regions (LCDRs) and three heavy chain complementarity determining regions (HCDRs), and

wherein said three LCDRs and said three HCDRs are selected from the group consisting of:

a) LCDR1 comprising the amino acid sequence SVSSSISSTNLH (SEQ ID NO:49);

LCDR2 comprising the amino acid sequence GTSYLAS (SEQ ID NO:50);

LCDR3 comprising the amino acid sequence QQWSSYPYS (SEQ ID NO:51);

HCDR1 comprising the amino acid sequence GYTFTSRYIH (SEQ ID NO:59);

HCDR2 comprising the amino acid sequence WIYPVTGDTYYNEKFKG (SEQ ID NO:60); and

HCDR3 comprising the amino acid sequence GYGAFYY (SEQ ID NO:61);

b) LCDR1 comprising the amino acid sequence SVSSSISSTNLH (SEQ ID NO:49);

LCDR2 comprising the amino acid sequence GTSYLAS (SEQ ID NO:50);

LCDR3 comprising the amino acid sequence QQWSSYPYS (SEQ ID NO:51);

HCDR1 comprising the amino acid sequence GYTFTSRYIH (SEQ ID NO:59);

HCDR2 comprising the amino acid sequence WIYPVTGDTYYIEKFKG (SEQ ID NO:62); and

HCDR3 comprising the amino acid sequence GYGAFFY (SEQ ID NO:63);

c) LCDR1 comprising the amino acid sequence SVSSSISSTNLH (SEQ ID NO:49);

LCDR2 comprising the amino acid sequence GTSRLRS (SEQ ID NO:52);

LCDR3 comprising the amino acid sequence QQWSSYPYS (SEQ ID NO:51);

HCDR1 comprising the amino acid sequence GYTFTSRYIH (SEQ ID NO:59);

HCDR2 comprising the amino acid sequence WIYPVTGDTYYREPFKG (SEQ ID NO:64), and

HCDR3 comprising the amino acid sequence GYGAFYY (SEQ ID NO:61);

d) LCDR1 comprising the amino acid sequence SVSSSVSSIYLH (SEQ ID NO:53);

LCDR2 comprising the amino acid sequence STSNLAS (SEQ ID NO:54);

LCDR3 comprising the amino acid sequence IQYSGYPLT (SEQ ID NO:55);

HCDR1 comprising the amino acid sequence GYTFTDYYMH (SEQ ID NO:65);

HCDR2 comprising the amino acid sequence RVNPNRGGTTYNQKFEG (SEQ ID NO:66), and

HCDR3 comprising the amino acid sequence TNWLDY (SEQ ID NO:67);

e) LCDR1 comprising the amino acid sequence SVSSSVSSIYLH (SEQ ID NO:53);

LCDR2 comprising the amino acid sequence STSNLAS (SEQ ID NO:54);

LCDR3 comprising the amino acid sequence QVYSGYPLT (SEQ ID NO:56);

HCDR1 comprising the amino acid sequence GYTFTDYYMH (SEQ ID NO:65);

HCDR2 comprising the amino acid sequence RVNPNRRGTTYNQKFEG (SEQ ID NO:68); and

HCDR3 comprising the amino acid sequence ANWLDY (SEQ ID NO:69); and

f) LCDR1 comprising the amino acid sequence SVSSSVRSIYLH (SEQ ID NO:57);

LCDR2 comprising the amino acid sequence STSNLAS (SEQ ID NO:54);

LCDR3 comprising the amino acid sequence QVYRGYPLT (SEQ ID NO:58);

HCDR1 comprising the amino acid sequence GYTFTDYYMH (SEQ ID NO:65);

HCDR2 comprising the amino acid sequence RVNPYRGSTTYNQKFEG (SEQ ID NO:70); and

HCDR3 comprising the amino acid sequence ANILDY (SEQ ID NO:71); and

wherein said monoclonal antibody or antigen-binding fragment thereof binds an epitope within the α-chain of said human c-Met and induces internalization of cell surface human c-Met.

Any one of the foregoing monoclonal antibodies or antigen-binding fragments thereof comprising three light chain complementarity determining regions (LCDRs) and three heavy chain complementarity determining regions (HCDRs), wherein said three LCDRs and said three HCDRs are selected from the group consisting of:

a) LCDR1 comprising the amino acid sequence SVSSSISSTNLH (SEQ ID NO:49);

LCDR2 comprising the amino acid sequence GTSYLAS (SEQ ID NO:50);

LCDR3 comprising the amino acid sequence QQWSSYPYS (SEQ ID NO:51);

HCDR1 comprising the amino acid sequence GYTFTSRYIH (SEQ ID NO:59);

HCDR2 comprising the amino acid sequence WIYPVTGDTYYNEKFKG (SEQ ID NO:60); and

HCDR3 comprising the amino acid sequence GYGAFYY (SEQ ID NO:61);

b) LCDR1 comprising the amino acid sequence SVSSSISSTNLH (SEQ ID NO:49);

LCDR2 comprising the amino acid sequence GTSYLAS (SEQ ID NO:50);

LCDR3 comprising the amino acid sequence QQWSSYPYS (SEQ ID NO:51);

HCDR1 comprising the amino acid sequence GYTFTSRYIH (SEQ ID NO:59;

HCDR2 comprising the amino acid sequence WIYPVTGDTYYIEKFKG (SEQ ID NO:62); and

HCDR3 comprising the amino acid sequence GYGAFFY (SEQ ID NO:63);

c) LCDR1 comprising the amino acid sequence SVSSSISSTNLH (SEQ ID NO:49);

LCDR2 comprising the amino acid sequence GTSRLRS (SEQ ID NO:52);

LCDR3 comprising the amino acid sequence QQWSSYPYS (SEQ ID NO:51);

HCDR1 comprising the amino acid sequence GYTFTSRYIH (SEQ ID NO:59);

HCDR2 comprising the amino acid sequence WIYPVTGDTYYREPFKG (SEQ ID NO:64); and

HCDR3 comprising the amino acid sequence GYGAFYY (SEQ ID NO:61).

Any one of the foregoing monoclonal antibodies or antigen-binding fragments thereof comprising three light chain complementarity determining regions (LCDRs) and three heavy chain complementarity determining regions (HCDRs), wherein said three LCDRs and said three HCDRs are selected from the group consisting of:

a) LCDR1 comprising the amino acid sequence SVSSSVSSIYLH (SEQ ID NO:53);

LCDR2 comprising the amino acid sequence STSNLAS (SEQ ID NO:54);

LCDR3 comprising the amino acid sequence IQYSGYPLT (SEQ ID NO:55);

HCDR1 comprising the amino acid sequence GYTFTDYYMH (SEQ ID NO:65);

HCDR2 comprising the amino acid sequence RVNPNRGGTTYNQKFEG (SEQ ID NO:66); and

HCDR3 comprising the amino acid sequence TNWLDY (SEQ ID NO:67);

b) LCDR1 comprising the amino acid sequence SVSSSVSSIYLH (SEQ ID NO:53);

LCDR2 comprising the amino acid sequence STSNLAS (SEQ ID NO:54);

LCDR3 comprising the amino acid sequence QVYSGYPLT (SEQ ID NO:56);

HCDR1 comprising the amino acid sequence GYTFTDYYMH (SEQ ID NO:65);

HCDR2 comprising the amino acid sequence RVNPNRRGTTYNQKFEG (SEQ ID NO:68); and

HCDR3 comprising the amino acid sequence ANWLDY (SEQ ID NO:69); and

c) LCDR1 comprising the amino acid sequence SVSSSVRSIYLH (SEQ ID NO:57);

LCDR2 comprising the amino acid sequence STSNLAS (SEQ ID NO:54);

LCDR3 comprising the amino acid sequence QVYRGYPLT (SEQ ID NO:58);

HCDR1 comprising the amino acid sequence GYTFTDYYMH (SEQ ID NO:65);

HCDR2 comprising the amino acid sequence RVNPYRGSTTYNQKFEG (SEQ ID NO:70); and

HCDR3 comprising the amino acid sequence ANILDY (SEQ ID NO:71).

Any one of the foregoing monoclonal antibodies, or antigen-binding fragments thereof comprising three light chain complementarity determining regions (LCDRs) and three heavy chain complementarity determining regions (HCDRs), wherein:

LCDR1 comprising the amino acid sequence SVSSSVSSIYLH (SEQ ID NO:53);

LCDR2 comprising the amino acid sequence STSNLAS (SEQ ID NO:54);

LCDR3 comprising the amino acid sequence IQYSGYPLT (SEQ ID NO:55);

HCDR1 comprising the amino acid sequence GYTFTDYYMH (SEQ ID NO:65);

HCDR2 comprising the amino acid sequence RVNPNRGGTTYNQKFEG (SEQ ID NO:66); and

HCDR3 comprising the amino acid sequence TNWLDY (SEQ ID NO:67);

Any one of the foregoing monoclonal antibodies, or antigen-binding fragments thereof comprising three light chain complementarity determining regions (LCDRs) and three heavy chain complementarity determining regions (HCDRs), wherein:

LCDR1 comprises the amino acid sequence SVSSSVSSIYLH (SEQ ID NO:53);

LCDR2 comprises the amino acid sequence STSNLAS (SEQ ID NO:54);

LCDR3 comprises the amino acid sequence QVYSGYPLT (SEQ ID NO:56);

HCDR1 comprises the amino acid sequence GYTFTDYYMH (SEQ ID NO:65);

HCDR2 comprises the amino acid sequence RVNPNRRGTTYNQKFEG (SEQ ID NO:68); and

HCDR3 comprises the amino acid sequence ANWLDY (SEQ ID NO:69).

Any one of the foregoing monoclonal antibodies, or antigen-binding fragments thereof, comprising a light chain variable region (LCVR) and a heavy chain variable region (HCVR), wherein said LCVR and said HCVR, respectively, comprise amino acid sequences selected from the group consisting of:

a) SEQ ID NO:94 and SEQ ID NO:96; and

b) SEQ ID NO:95 and SEQ ID NO:97,

wherein said monoclonal antibody or antigen-binding fragment thereof binds an epitope within the α-chain of said human c-Met and induces internalization of cell surface human c-Met.

Any one of the foregoing monoclonal antibodies, or antigen-binding fragments thereof comprising a light chain variable region (LCVR) and a heavy chain variable

region (HCVR), wherein said LCVR comprises SEQ ID NO:94 and said HCVR comprises SEQ ID NO:96.

Any one of the foregoing monoclonal antibodies, or antigen-binding fragments thereof comprising a light chain variable region (LCVR) and a heavy chain variable region (HCVR), wherein said LCVR comprises SEQ ID NO:95 and said HCVR comprises SEQ ID NO:97.

Any one of the foregoing monoclonal antibodies or antigen-binding fragments thereof, wherein said LCVR and said HCVR comprise amino acid sequences which are selected from the group consisting of:

a) LCVR is SEQ ID NO:1 and HCVR is SEQ ID NO:13;

b) LCVR is SEQ ID NO:2 and HCVR is SEQ ID NO:14;

c) LCVR is SEQ ID NO:3 and HCVR is SEQ ID NO:15;

d) LCVR is SEQ ID NO:4 and HCVR is SEQ ID NO:16;

e) LCVR is SEQ ID NO:5 and HCVR is SEQ ID NO:17; and

f) LCVR is SEQ ID NO:6 and HCVR is SEQ ID NO:18.

Any one of the foregoing monoclonal antibodies or antigen-binding fragments thereof, wherein said LCVR and said HCVR, respectively, comprise amino acid sequences selected from the group consisting of:

a) LCVR is SEQ ID NO:1 and HCVR is SEQ ID NO:13;

b) LCVR is SEQ ID NO:2 and HCVR is SEQ ID NO:14; and

c) LCVR is SEQ ID NO:3 and HCVR is SEQ ID NO:15.

Any one of the foregoing monoclonal antibodies or antigen-binding fragments thereof, wherein said LCVR and said HCVR, respectively, comprise amino acid sequences selected from the group consisting of:

a) LCVR is SEQ ID NO:4 and HCVR is SEQ ID NO:16;

b) LCVR is SEQ ID NO:5 and HCVR is SEQ ID NO:17; and

c) LCVR is SEQ ID NO:6 and HCVR is SEQ ID NO:18.

Any one of the foregoing monoclonal antibodies or antigen-binding fragments thereof, wherein said LCVR comprises the amino acid sequence of SEQ ID NO: 4 and said HCVR comprises the amino acid sequence of SEQ ID NO: 16.

Any one of the foregoing monoclonal antibodies or antigen-binding fragments thereof, wherein said LCVR comprises the amino acid sequence of SEQ ID NO: 5 and said HCVR comprises the amino acid sequence of SEQ ID NO: 17.

Any one of the foregoing monoclonal antibodies wherein said antibody comprises a light chain and a heavy chain wherein the light chain and the heavy chain comprise amino acid sequences which are selected from the group consisting of:

a) light chain is SEQ ID NO:25 and heavy chain is SEQ ID NO:37;

b) light chain is SEQ ID NO:26 and heavy chain is SEQ ID NO:38;

c) light chain is SEQ ID NO:27 and heavy chain is SEQ ID NO:39;

d) light chain is SEQ ID NO:28 and heavy chain is SEQ ID NO:40;

e) light chain is SEQ ID NO:29 and heavy chain is SEQ ID NO:41; and

f) light chain is SEQ ID NO:30 and heavy chain is SEQ ID NO:42.

Any one of the foregoing monoclonal antibodies wherein said antibody comprises a light chain and a heavy chain wherein the light chain and the heavy chain comprise amino acid sequences which are selected from the group consisting of

a) light chain is SEQ ID NO:25 and heavy chain is SEQ ID NO:37;

b) light chain is SEQ ID NO:26 and heavy chain is SEQ ID NO:38; and

c) light chain is SEQ ID NO:27 and heavy chain is SEQ ID NO:39.

Any one of the foregoing monoclonal antibodies wherein said antibody comprises a light chain and a heavy chain wherein the light chain and the heavy chain comprise amino acid sequences which are selected from the group consisting of:

a) light chain is SEQ ID NO:28 and heavy chain is SEQ ID NO:40;

b) light chain is SEQ ID NO:29 and heavy chain is SEQ ID NO:41; and

c) light chain is SEQ ID NO:30 and heavy chain is SEQ ID NO:42.

Any one of the foregoing monoclonal antibodies wherein said light chain comprises the amino acid sequence of SEQ ID NO: 28 and said heavy chain comprises the amino acid sequence of SEQ ID NO: 40.

Any one of the foregoing monoclonal antibodies wherein said light chain comprises the amino acid sequence of SEQ ID NO: 29 and said heavy chain comprises the amino acid sequence of SEQ ID NO: 41.

Any one of the foregoing monoclonal antibodies wherein said antibody comprises two light chains and two heavy chains, wherein each light chain comprises the amino acid

sequence of SEQ ID NO: 28 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 40.

Any one of the foregoing monoclonal antibodies wherein said antibody comprises two light chains and two heavy chains, wherein each light chain comprises the amino acid sequence of SEQ ID NO: 29 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 41.

A monoclonal antibody or antigen-binding fragment thereof that competes with any of the foregoing c-Met monoclonal antibodies or antigen-binding fragments thereof for binding to c-Met. Such competing monoclonal antibody or antigen-binding fragment thereof can bind to the same epitope of c-Met as any one of the foregoing c-Met monoclonal antibodies or antigen-binding fragments thereof. In a preferred embodiment, the monoclonal antibody or antigen-binding fragment thereof competes with an antibody which comprises a light chain and a heavy chain, wherein the light chain comprises the amino acid sequence of SEQ ID NO: 28 and the heavy chain comprises the amino acid sequence of SEQ ID NO: 40. In another preferred embodiment, the monoclonal antibody or antigen-binding fragment thereof competes with an antibody which comprises a light chain and a heavy chain, wherein the light chain comprises the amino acid sequence of SEQ ID NO: 29 and the heavy chain comprises the amino acid sequence of SEQ ID NO: 41.

Any one of the foregoing monoclonal antibodies or antigen-binding fragments thereof, which induces reduction of total human c-Met and phosphorylated human c-Met in hepatocyte growth factor-independent tumor cells that constitutively overexpress said human c-Met. In a preferred embodiment, the monoclonal antibody or antigen-binding fragment thereof which induces reduction of total human c-Met and phosphorylated human c-Met in hepatocyte growth factor-independent tumor cells that constitutively overexpress said human c-Met, comprises a light chain and a heavy chain, wherein the light chain comprises the amino acid sequence of SEQ ID NO: 28 and the heavy chain comprises the amino acid sequence of SEQ ID NO: 40. In another preferred embodiment, the monoclonal antibody or antigen-binding fragment thereof which induces reduction of total human c-Met and phosphorylated human c-Met in hepatocyte growth factor-independent tumor cells that constitutively overexpress said human c-Met, comprises a light chain and a heavy chain, wherein the light chain comprises the amino acid sequence

of SEQ ID NO: 29 and the heavy chain comprises the amino acid sequence of SEQ ID NO: 41.

Any one of the foregoing monoclonal antibodies or antigen-binding fragments thereof, which induces reduction of total human c-Met and phosphorylated human c-Met in hepatocyte growth factor-independent tumor cells that constitutively phosphorylate said human c-Met. In a preferred embodiment, the monoclonal antibody or antigen-binding fragment thereof which induces reduction of total human c-Met and phosphorylated human c-Met in hepatocyte growth factor-independent tumor cells that constitutively phosphorylate said human c-Met, comprises a light chain and a heavy chain, wherein the light chain comprises the amino acid sequence of SEQ ID NO: 28 and the heavy chain comprises the amino acid sequence of SEQ ID NO: 40. In another preferred embodiment, the monoclonal antibody or antigen-binding fragment thereof which induces reduction of total human c-Met and phosphorylated human c-Met in hepatocyte growth factor-independent tumor cells that constitutively phosphorylate said human c-Met, comprises a light chain and a heavy chain, wherein the light chain comprises the amino acid sequence of SEQ ID NO: 29 and the heavy chain comprises the amino acid sequence of SEQ ID NO: 41.

Any one of the foregoing monoclonal antibodies or antigen-binding fragments thereof, which induces reduction of total human c-Met and phosphorylated human c-Met in hepatocyte growth factor-independent tumor cells that are hepatocyte growth factor-responsive.

Any one of the foregoing monoclonal antibodies or antigen-binding fragments thereof, which preferentially binds human c-Met extracellular domain compared to human RON extracellular domain or human PlexinA2 extracellular domain.

Any one of the foregoing monoclonal antibodies or antigen-binding fragments thereof, which does not induce shedding of human c-Met extracellular domain. In a preferred embodiment, the monoclonal antibody or antigen-binding fragment thereof which does not induce shedding of human c-Met extracellular domain, comprises a light chain and a heavy chain, wherein the light chain comprises the amino acid sequence of SEQ ID NO: 28 and the heavy chain comprises the amino acid sequence of SEQ ID NO: 40. In another preferred embodiment, the monoclonal antibody or antigen-binding fragment thereof which does not induce shedding of human c-Met extracellular domain,

comprises a light chain and a heavy chain, wherein the light chain comprises the amino acid sequence of SEQ ID NO: 29 and the heavy chain comprises the amino acid sequence of SEQ ID NO: 41.

Any one of the foregoing monoclonal antibodies or antigen-binding fragments thereof, which does not protect tumor cells expressing human c-Met from staurosporine-induced apoptosis. In a preferred embodiment, the monoclonal antibody or antigen-binding fragment thereof which does not protect tumor cells expressing human c-Met from staurosporine-induced apoptosis, comprises a light chain and a heavy chain, wherein the light chain comprises the amino acid sequence of SEQ ID NO: 28 and the heavy chain comprises the amino acid sequence of SEQ ID NO: 40. In another preferred embodiment, the monoclonal antibody or antigen-binding fragment thereof which does not protect tumor cells expressing human c-Met from staurosporine-induced apoptosis, comprises a light chain and a heavy chain, wherein the light chain comprises the amino acid sequence of SEQ ID NO: 29 and the heavy chain comprises the amino acid sequence of SEQ ID NO: 41.

Any one of the foregoing monoclonal antibodies or antigen-binding fragments thereof, which inhibits hepatocyte growth factor-dependent and hepatocyte growth factor-independent proliferation of tumor cells that express human c-Met.

Any one of the foregoing monoclonal antibodies or antigen-binding fragments thereof, which inhibits binding of human hepatocyte growth factor to human c-Met.

Any one of the foregoing monoclonal antibodies or antigen-binding fragments thereof which does not induce HGF-like biological agonist activities. HGF-like biological agonist activities include tumor cell proliferation, tumor cell motility, tumor cell invasion, tubulogenesis, angiogenesis, and anti-apoptotic effects. In a preferred embodiment, the monoclonal antibody or antigen-binding fragment thereof which does not induce HGF-like biological agonist activities, comprises a light chain and a heavy chain, wherein the light chain comprises the amino acid sequence of SEQ ID NO: 29 and the heavy chain comprises the amino acid sequence of SEQ ID NO: 41.

A pharmaceutical composition, comprising any one of the foregoing monoclonal antibodies or antigen-binding fragments thereof, and a pharmaceutically acceptable carrier, diluent, or excipient.

Any one of the foregoing monoclonal antibodies or antigen-binding fragments thereof for use in therapy. In a preferred embodiment, the monoclonal antibody or antigen-binding fragment thereof for use in therapy comprises a light chain and a heavy chain, wherein the light chain comprises the amino acid sequence of SEQ ID NO: 28 and the heavy chain comprises the amino acid sequence of SEQ ID NO: 40. In another preferred embodiment, the monoclonal antibody or antigen-binding fragment thereof for use in therapy comprises a light chain and a heavy chain, wherein the light chain comprises the amino acid sequence of SEQ ID NO: 29 and the heavy chain comprises the amino acid sequence of SEQ ID NO: 41.

Any one of the foregoing monoclonal antibodies or antigen-binding fragments thereof for use in treating a cancer in a human. In a preferred embodiment, the monoclonal antibody or antigen-binding fragment thereof for use in treating a cancer in a human comprises a light chain and a heavy chain, wherein the light chain comprises the amino acid sequence of SEQ ID NO: 28 and the heavy chain comprises the amino acid sequence of SEQ ID NO: 40. In another preferred embodiment, the monoclonal antibody or antigen-binding fragment thereof for use in treating a cancer in a human comprises a light chain and a heavy chain, wherein the light chain comprises the amino acid sequence of SEQ ID NO: 29 and the heavy chain comprises the amino acid sequence of SEQ ID NO: 41.

Any one of the foregoing monoclonal antibodies or antigen-binding fragments thereof for use in treating a cancer in a human in combination with another therapeutic agent. In a preferred embodiment, the monoclonal antibody or antigen-binding fragment thereof for use in treating a cancer in a human in combination with another therapeutic comprises a light chain and a heavy chain, wherein the light chain comprises the amino acid sequence of SEQ ID NO: 28 and the heavy chain comprises the amino acid sequence of SEQ ID NO: 40. In another preferred embodiment, the monoclonal antibody or antigen-binding fragment thereof for use in treating a cancer in a human in combination with another therapeutic comprises a light chain and a heavy chain, wherein the light

chain comprises the amino acid sequence of SEQ ID NO: 29 and the heavy chain comprises the amino acid sequence of SEQ ID NO: 41.

A pharmaceutical composition comprising any one of the foregoing monoclonal antibodies or antigen-binding fragments thereof and a pharmaceutically acceptable carrier, diluent, or excipient.

Use of any one of the foregoing monoclonal antibodies or antigen-binding fragments thereof for the manufacture of a medicament for treating a cancer in a human.

A method of treating a cancer, comprising administering to a human patient in need thereof an effective amount of any one of the foregoing monoclonal antibodies or antigen-binding fragments thereof.

Definitions

A full-length antibody as it exists naturally is an immunoglobulin molecule comprising 2 heavy (H) chains and 2 light (L) chains interconnected by disulfide bonds. The amino terminal portion of each chain includes a variable region of about 100-110 or more amino acids primarily responsible for antigen recognition via the complementarity determining regions (CDRs) contained therein. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function.

The CDRs are interspersed with regions that are more conserved, termed framework regions ("FR"). Each light chain variable region (LCVR) and heavy chain variable region (HCVR) is composed of 3 CDRs and 4 FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The 3 CDRs of the light chain are referred to as "LCDR1, LCDR2, and LCDR3" and the 3 CDRs of the heavy chain are referred to as "HCDR1, HCDR2, and HCDR3." The CDRs contain most of the residues which form specific interactions with the antigen. The numbering and positioning of CDR amino acid residues within the LCVR and HCVR regions are in accordance with the well-known Kabat numbering convention.

Light chains are classified as kappa or lambda, and are characterized by a particular constant region as known in the art. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, and define the isotope of an antibody as IgG, IgM, IgA, IgD, or IgE, respectively. IgG antibodies can be further divided into subclasses, e.g., IgG1, IgG2,

IgG3, IgG4. Each heavy chain type is characterized by a particular constant region with a sequence well known in the art.

As used herein, the term "monoclonal antibody" (Mab) as applied to the present antibody compounds refers to an antibody that is derived from a single copy or clone including, for example, any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. Mabs of the present invention preferably exist in a homogeneous or substantially homogeneous population. Complete Mabs contain 2 heavy chains and 2 light chains. "Antigen-binding fragments" of such monoclonal antibodies include, for example, Fab fragments, Fab' fragments, $F(ab')_2$ fragments, single chain Fv fragments, and one-armed antibodies comprising a light chain and a heavy chain. Monoclonal antibodies and antigen-binding fragments thereof of the present invention can be produced, for example, by recombinant technologies, phage display technologies, synthetic technologies, e.g., CDR-grafting, or combinations of such technologies, or other technologies known in the art.

"Antibody compounds" refers to Mabs and Fabs disclosed herein. Additional antibody compounds exhibiting similar functional properties according to the present invention can be generated by conventional methods. For example, mice can be immunized with human c-Met or fragments thereof, the resulting antibodies can be recovered and purified, and determination of whether they possess binding and functional properties similar to or the same as the antibody compounds disclosed herein can be assessed by the methods disclosed in Examples 2-19, below. Antigen-binding fragments can also be prepared by conventional methods. Methods for producing and purifying antibodies and antigen-binding fragments are well known in the art and can be found, for example, in Harlow and Lane (1988) *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, chapters 5-8 and 15, ISBN 0-87969-314-2.

The phrase "human engineered antibodies" refers to monoclonal antibodies and antigen-binding fragments in addition to the antibody compounds disclosed herein that have binding and functional properties according to the invention similar to those disclosed herein, and that have framework regions that are substantially human or fully human surrounding CDRs derived from a non-human antibody. "Framework region" or "framework sequence" refers to any one of framework regions 1 to 4. Human engineered

antibodies and antigen-binding fragments encompassed by the present invention include molecules wherein any one or more of framework regions 1 to 4 is substantially or fully human, i.e., wherein any of the possible combinations of individual substantially or fully human framework regions 1 to 4, is present. For example, this includes molecules in which framework region 1 and framework region 2, framework region 1 and framework region 3, framework region 1, 2, and 3, etc., are substantially or fully human. Substantially human frameworks are those that have at least about 80% sequence identity to a known human germline framework sequence. Preferably, the substantially human frameworks have at least about 85%, about 90%, about 95%, or about 99% sequence identity to a known human germline framework sequence.

Fully human frameworks are those that are identical to a known human germline framework sequence. Human framework germline sequences can be obtained from ImMunoGeneTics (IMGT) via their website http://imgt.cines.fr, or from *The Immunoglobulin FactsBook* by Marie-Paule Lefranc and Gerard Lefranc, Academic Press, 2001, ISBN 012441351. For example, germline light chain frameworks can be selected from the group consisting of: A11, A17, A18, A19, A20, A27, A30, LI, L1I, L12, L2, L5, L15, L6, L8, O12, O2, and O8, and germline heavy chain framework regions can be selected from the group consisting of: VH2-5, VH2-26, VH2-70, VH3-20, VH3-72, VHI-46, VH3-9, VH3-66, VH3-74, VH4-31, VHI-18, VHI-69, VI-13-7, VH3-11, VH3-15, VH3-21, VH3-23, VH3-30, VH3-48, VH4-39, VH4-59, and VH5-5I.

Human engineered antibodies in addition to those disclosed herein exhibiting similar functional properties according to the present invention can be generated using several different methods. In one approach, the parent antibody compound CDRs are grafted into a human framework that has a high sequence identity with the parent antibody compound framework. The sequence identity of the new framework will generally be at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% identical to the sequence of the corresponding framework in the parent antibody compound. In the case of frameworks having fewer than 100 amino acid residues, one, two, or three amino acid residues can be changed. This grafting may result in a reduction in binding affinity compared to that of the parent antibody. If this is the case, the framework can be back-mutated to the parent framework at certain positions based on specific criteria disclosed by Queen et al. (1991) *Proc. Natl. Acad. Sci. USA*

88:2869. Additional references describing methods useful in humanizing mouse antibodies include U.S. Patent Nos. 4,816,397; 5,225,539, and 5,693,761; computer programs ABMOD and ENCAD as described in Levitt (1983) *J. Mol. Biol.* 168:595-620; and the method of Winter and co-workers (Jones et al. (1986) *Nature* 321:522-525; Riechmann et al. (1988) *Nature* 332:323-327; and Verhoeyen et al. (1988) *Science* 239:1534-1536.

The identification of residues to consider for back-mutation can be carried out as follows.

When an amino acid falls under the following category, the framework amino acid of the human germ-line sequence that is being used (the "acceptor framework") is replaced by a framework amino acid from a framework of the parent antibody compound (the "donor framework"):

(a) the amino acid in the human framework region of the acceptor framework is unusual for human frameworks at that position, whereas the corresponding amino acid in the donor immunoglobulin is typical for human frameworks at that position;

(b) the position of the amino acid is immediately adjacent to one of the CDRs; or

(c) any side chain atom of a framework amino acid is within about 5-6 angstroms (center-to-center) of any atom of a CDR amino acid in a three dimensional immunoglobulin model.

When each of the amino acids in the human framework region of the acceptor framework and a corresponding amino acid in the donor framework is generally unusual for human frameworks at that position, such amino acid can be replaced by an amino acid typical for human frameworks at that position. This back-mutation criterion enables one to recover the activity of the parent antibody compound.

Another approach to generating human engineered antibodies exhibiting similar functional properties to the antibody compounds disclosed herein involves randomly mutating amino acids within the grafted CDRs without changing the framework, and screening the resultant molecules for binding affinity and other functional properties that are as good as or better than those of the parent antibody compounds. Single mutations can also be introduced at each amino acid position within each CDR, followed by

assessing the effects of such mutations on binding affinity and other functional properties. Single mutations producing improved properties can be combined to assess their effects in combination with one another.

Further, a combination of both of the foregoing approaches is possible. After CDR grafting, one can back-mutate specific framework regions in addition to introducing amino acid changes in the CDRs. This methodology is described in Wu et al. (1999) *J. Mol. Biol.* 294:151-162.

Applying the teachings of the present invention, a person skilled in the art can use common techniques, e.g., site-directed mutagenesis, to substitute amino acids within the presently disclosed CDR and framework sequences and thereby generate further variable region amino acid sequences derived from the present sequences. Up to all naturally occurring amino acids can be introduced at a specific substitution site. The methods disclosed herein can then be used to screen these additional variable region amino acid sequences to identify sequences having the indicated *in vivo* functions. In this way, further sequences suitable for preparing human engineered antibodies and antigen-binding portions thereof in accordance with the present invention can be identified. Preferably, amino acid substitution within the frameworks is restricted to one, two, or three positions within any one or more of the 4 light chain and/or heavy chain framework regions disclosed herein. Preferably, amino acid substitution within the CDRs is restricted to one, two, or three positions within any one or more of the 3 light chain and/or heavy chain CDRs. Combinations of the various changes within these framework regions and CDRs described above are also possible.

That the functional properties of the antibody compounds generated by introducing the amino acid modifications discussed above conform to those exhibited by the specific molecules disclosed herein can be confirmed by the methods disclosed below in Examples 2-19.

The term “epitope” refers to a specific arrangement of amino acids located on a peptide or protein to which an antibody or antibody fragment binds. Epitopes often consist of a chemically active surface grouping of molecules such as amino acids or sugar side chains, and have specific three dimensional structural characteristics as well as specific charge characteristics. Epitopes can be linear, i.e., involving binding to a single sequence of amino acids, or conformational, i.e., involving binding to two or more

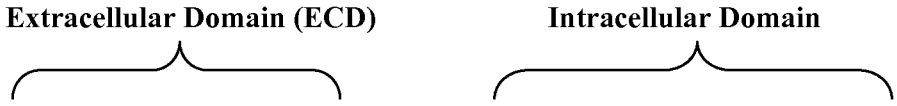
sequences of amino acids in various regions of the antigen that may not necessarily be contiguous. The epitopes disclosed herein can consist of, consist essentially of, or comprise the amino acid sequences disclosed in Example 3.

Monoclonal antibodies or antigen-binding fragments thereof that "compete" with the molecules disclosed herein are those that bind human c-Met at site(s) that are identical to, or overlapping with, the site(s) at which the present molecules bind. Competing monoclonal antibodies or antigen-binding fragments thereof can be identified, for example, via an antibody competition assay. For example, a sample of purified or partially purified human c-Met can be bound to a solid support. Then, an antibody compound or antigen binding fragment thereof of the present invention and a monoclonal antibody or antigen-binding fragment thereof suspected of being able to compete with such invention antibody compound are added. One of the two molecules is labeled. If the labeled compound and the unlabeled compound bind to separate and discrete sites on c-Met, the labeled compound will bind to the same level whether or not the suspected competing compound is present. However, if the sites of interaction are identical or overlapping, the unlabeled compound will compete, and the amount of labeled compound bound to the antigen will be lowered. If the unlabeled compound is present in excess, very little, if any, labeled compound will bind. For purposes of the present invention, competing monoclonal antibodies or antigen-binding fragments thereof are those that decrease the binding of the present antibody compounds to c-Met by about 50%, about 60%, about 70%, about 80%, about 85%, about 90%, about 95%, or about 99%. Details of procedures for carrying out such competition assays are well known in the art and can be found, for example, in Harlow and Lane (1988) *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pages 567-569, ISBN 0-87969-314-2. Such assays can be made quantitative by using purified antibodies. A standard curve is established by titrating one antibody against itself, i.e., the same antibody is used for both the label and the competitor. The capacity of an unlabeled competing monoclonal antibody or antigen-binding fragment thereof to inhibit the binding of the labeled molecule to the plate is titrated. The results are plotted, and the concentrations necessary to achieve the desired degree of binding inhibition are compared. Whether monoclonal antibodies or antigen-binding fragments thereof that compete with antibody compounds of the present invention in such competition assays

possess the same or similar functional properties of the present antibody compounds can be determined via the methods disclosed in Examples 2-19 herein.

Monoclonal antibodies or antigen-binding fragments thereof that bind substantially the same epitope(s) of c-Met as the monoclonal antibodies or antigen-binding fragments disclosed herein are those that bind human c-Met at site(s) that are overlapping with the site(s) at which the present molecules bind. Methods that facilitate identification of monoclonal antibodies or antigen-binding fragments thereof that bind substantially the same epitope of c-Met as the c-Met monoclonal antibodies or antigen-binding fragments disclosed herein are well known in the art and are described, for example, in PCT International Publication WO 00/64946. Whether such monoclonal antibodies or antigen-binding fragments thereof that bind substantially the same c-Met epitope(s) as those disclosed herein possess the same or similar functional properties of the present antibody compounds can be determined via the methods disclosed in Examples 2-19 herein.

"c-Met" or "human c-Met" refers to any human c-Met, as well as functionally active, mutated forms thereof. The structure of the c-Met is depicted schematically as:



SEMA: Sema domain

PSI: Plexin, Semaphorins, and Integrins domain

IPT: 4 Immunoglobulins, Plexins, and Transcription factor domains

TM: Transmembrane region

JM: Juxtamembrane domain

KD: Kinase domain

In the human c-Met ECD (SEQ ID NO:75), amino acids 1-24 comprise the signal sequence. The mature protein begins at amino acid 25 (E). The Sema domain consists of approximately 500 amino acid residues at the N-terminus of c-Met, and contains the α-chain (amino acid residues 25-307) and part of the β-chain (amino acid residues 308-519).

The term "inhibit" means the ability to substantially antagonize, prohibit, prevent, restrain, slow, disrupt, eliminate, stop, reduce, or reverse the biological effects of c-Met.

The term "treating" (or "treat" or "treatment") means slowing, interrupting, arresting, controlling, stopping, reducing, or reversing the progression or severity of a symptom, disorder, condition, or disease, but does not necessarily involve a total elimination of all disease-related symptoms, conditions, or disorders.

Acute events and chronic conditions may be treated. In an acute event, an antibody or antigen-binding fragment thereof is administered at the onset of a symptom, disorder, condition, or disease, and is discontinued when the acute event ends. In contrast, a chronic symptom, disorder, condition, or disease is treated over a more protracted time frame.

The term "effective amount" refers to the amount or dose of an antibody compound of the present invention which, upon single or multiple dose administration to a patient, provides the desired treatment or prevention. Therapeutically effective amounts of the present antibody compounds can comprise an amount in the range of from about 0.1 mg/kg to about 20 mg/kg per single dose. A therapeutically effective amount for any individual patient can be determined by the health care provider by monitoring the effect of the antibody compounds on a biomarker, such as cell surface c-Met in tumor or non-tumor tissues, tumor regression, etc. Analysis of the data obtained by these methods permits modification of the treatment regimen during therapy so that optimal amounts of antibody compounds, whether employed alone or in combination with one another therapeutic agent, are administered, and so that the duration of treatment can be determined as well. In this way, the dosing/treatment regimen can be modified over the course of therapy so that the lowest amounts of antibody compounds used alone or in combination that exhibit satisfactory tumor reducing effectiveness are administered, and so that administration of such compounds is continued only so long as is necessary to successfully treat the patient.

The antibody compounds of the present invention can be used as medicaments in human medicine, administered by a variety of routes. Most preferably, such compositions are for parenteral administration. Such pharmaceutical compositions can be prepared by methods well known in the art. *See*, e.g., *Remington: The Science and Practice of Pharmacy*, 19th ed. (1995), A. Gennaro et al., Mack Publishing Co., and

comprise one or more antibody compounds disclosed herein, and a pharmaceutically acceptable carrier, diluent, or excipient.

The term "tumor" refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues. The terms "cancer", "cancerous", and "tumor" are not mutually exclusive as used herein.

The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by aberrant cell growth/proliferation. Examples of cancers include, but are not limited to, carcinomas, lymphomas, blastomas, sarcomas, and leukemias.

c-Met and Cancer

Deregulated c-Met pathways can be induced by transcriptional up regulation, c-Met gene amplification, specific genetic alterations, or ligand-dependent autocrine or paracrine mechanisms. The most frequent cause of constitutive c-Met activation in human tumors is increased protein expression as a consequence of transcriptional upregulation, in the absence of gene amplification. In addition, amplification of the *MET* gene, with consequent protein overexpression and constitutive kinase activation, has been reported in a number of human primary tumors, including gastric and oesophageal carcinomas, non-small-cell lung (NSCL) carcinomas, and medulloblastomas. Tumors of mesenchymal origin, such as osteosarcomas and rhabdomyosarcomas, often utilize autocrine mechanisms by producing HGF. Elevated HGF levels and overexpression of c-Met are often associated with poor clinical outcomes that include more aggressive disease, increased tumor metastasis, and shortened patient survival. Further, high levels of HGF and/or c-Met proteins in tumors confer resistance to chemotherapy and radiotherapy. In addition to abnormal HGF and c-Met expression, the c-Met pathway can be activated through genetic alternations such as c-Met mutations, gene amplification, and gene rearrangement. Missense *c-MET* mutations are found in all individuals with well-characterized hereditary papillary renal cell carcinomas (PRCC) and in a small subset (13%) of sporadic PRCC samples. Some of the mutations possess oncogenic potential due to increased kinase activity. Trisomy of chromosome 7, where both *HGF* and *c-MET* genes reside, occurs frequently in PRCC, and results in non-random duplication of the mutant *c-MET* allele. In addition, somatic *c-MET* mutations have been

identified in other human cancers, including gastric, head and neck, liver, ovarian, non-small cell lung and thyroid cancers, as well as in metastases of some of these cancers. Unlike PRCC, where mutations are typically confined to the kinase domain, these mutations are often located in other regions of the receptor, for example, the juxtamembrane domain. In addition to mutation, the *c-MET* gene is often amplified in breast, liver, brain, colorectal, gastric, lung and stomach cancers, which is correlated to disease progression in some patients.

Therapeutic indications

Aberrant HGF/c-MET signaling has been documented in a wide range of human malignancies, including bladder, breast, cervical, colorectal, endometrial, esophageal, gastric, head and neck, kidney, liver, lung, nasopharyngeal, ovarian, pancreatic, prostate and thyroid cancers, as well as cholangiocarcinoma, osteosarcoma, rhabdomyosarcoma, synovial sarcoma, Kaposi's sarcoma, leiomyosarcomas, and MFH/fibrosarcoma. In addition, abnormal HGF and/or c-Met expression has also been reported in hematological malignancies such as acute myelogenous leukemia, adult T-cell leukemia, chronic myeloid leukemia, lymphomas and multiple myeloma, as well as other tumors such as melanoma, mesothelioma, Wilms' tumor, glioblastomata, and astrocytomas (summarized in Liu et al. (2008) *Expert Opin. Investig. Drugs* 17(7):997-1011). The c-Met antibodies of the present invention can inhibit both HGF-dependent and HGF-independent tumors.

The following non-limiting examples illustrate various aspects of the present invention.

In the examples below, the humanized IgG2 and IgG4 and murine IgG (also sometimes referred to as mIgG1) control antibodies are isotype control antibodies unrelated to the present c-Met antibodies. Antibodies C8, D11, and optD11 are murine antibodies. In all cases, human HGF is obtained from R&D Systems (#294).

Example 1

c-Met Antibodies

The amino acid sequences of the light chain and heavy chain variable regions, the complete light and heavy chains, and the respective encoding nucleotide sequences of the foregoing, of the present human engineered antibodies are listed below in the section entitled "Amino Acid and Nucleotide Sequences." The light chain and heavy chain CDR amino acid sequences are shown in Tables 1 and 2, respectively.

Table 1. Light Chain CDRs

Antibody	CDR 1	CDR 2	CDR 3
D11-S17Y	SVSSSISSTNLH (SEQ ID NO:49)	GTSYLAS (SEQ ID NO:50)	QQWSSYPYS (SEQ ID NO:51)
D11-8B8	SVSSSISSTNLH (SEQ ID NO:49)	GTSYLAS (SEQ ID NO:50)	QQWSSYPYS (SEQ ID NO:51)
D11-C27G3	SVSSSISSTNLH (SEQ ID NO:49)	GTSRLRS (SEQ ID NO:52)	QQWSSYPYS (SEQ ID NO:51)
D11 Consensus Sequence	--	GTSX_1LX_2S (SEQ ID NO:87)	--
C8-6	SVSSSVSSIYLH (SEQ ID NO:53)	STSNLAS (SEQ ID NO:54)	IQYSGYPLT (SEQ ID NO:55)
C8-H241	SVSSSVSSIYLH (SEQ ID NO:53)	STSNLAS (SEQ ID NO:54)	QVYSGYPLT (SEQ ID NO:56)
C8-co-16	SVSSSVRSIYLH (SEQ ID NO:57)	STSNLAS (SEQ ID NO:54)	QVYRGYPLT (SEQ ID NO:58)
C8 Consensus Sequence	SVSSSVX_3SIYLH (SEQ ID NO:88)	--	X_4X_5YX_6GYPLT (SEQ ID NO:89)

X_1 is Y or R, and X_2 is A or R;

X_3 is S or R;

X_4 is I or Q, X_5 is Q or V, and X_6 is S or R;

Table 2. Heavy Chain CDRs

Antibody	CDR 1	CDR 2	CDR 3
D11-S17Y	GYTFTSRYIH (SEQ ID NO:59)	WIYPVTGDTYYNEKFKG (SEQ ID NO:60)	GYGAFYY (SEQ ID NO:61)
D11-8B8	GYTFTSRYIH (SEQ ID NO:59)	WIYPVTGDTYYIEKFKG (SEQ ID NO:62)	GYGAFFY (SEQ ID NO:63)
D11-C27G3	GYTFTSRYIH (SEQ ID NO:59)	WIYPVTGDTYYREPFKG (SEQ ID NO:64)	GYGAFYY (SEQ ID NO:61)
D11 Consensus Sequence	--	WIYPVTGDTYYX_7EX_8FKG (SEQ ID NO:90)	GYGAFX_9Y (SEQ ID NO:91)
C8-6	GYTFTDYYMH (SEQ ID NO:65)	RVNPNRGGTTYNQKFEG (SEQ ID NO:66)	TNWLDY (SEQ ID NO:67)
C8-H241	GYTFTDYYMH (SEQ ID NO:65)	RVNPNRRGTTYNQKFEG (SEQ ID NO:68)	ANWLDY (SEQ ID NO:69)
C8-co-16	GYTFTDYYMH (SEQ ID NO:65)	RVNPYRGSTTYNQKFEG (SEQ ID NO:70)	ANILDY (SEQ ID NO:71)
C8 Consensus Sequence	--	RVNPX_{10}R$X_{11}X_{12}$TTYNQKFEG (SEQ ID NO:92)	X_{13}NX_{14}LDY (SEQ ID NO:93)

X_7 is N, I, or R, and X_8 is K or P;

X_9 is Y or F;

X_{10} is N or Y, X_{11} is G or R, and X_{12} is G or S;

X_{13} is T or A, and X_{14} is W or I;

Consensus sequences for D11- and C8- antibody light and heavy chain variable regions are:

D11- Antibody Light ChainVariable Region Consensus Sequence (SEQ ID NO:94)

EIVLTQSPGTLSLSPGERATLSCSVSSSISSTNLHWYQQKPGQAPRLLIY GTSX_1LX_2SGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQWSSYPYSFG QGTKLEIK

wherein X_1 is Y or R, and X_2 is A or R;

C8- Antibody Light Chain Variable Region Consensus Sequence (SEQ ID NO:95)

DIQMTQSPSSLSASVGDRVTITCSVSSSVX$_3$SIYLHWYQQKPGKAPKLLIY
STSNLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCX$_4$X$_5$YX$_6$GYPLTFG
GGTKVEIK

wherein X$_3$ is S or R, X$_4$ is I or Q, X$_5$ is Q, or V, and X$_6$ is S or R;

D11- Antibody Heavy Chain Variable Region Consensus Sequence (SEQ ID NO:96)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSRYIHWVRQAPGQGLEWMGW
IYPVTGDTYYX$_7$EX$_8$FKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARGY
GAFX$_9$YWGQGTLVTVS

wherein X$_7$ is N, I, or R, X$_8$ is K or P, and X$_9$ is Y or F;

C8- Antibody Heavy Chain Variable Region Consensus Sequence (SEQ ID NO:97)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYMHWVRQAPGQGLEWMGR
VNPX$_{10}$RX$_{11}$X$_{12}$TTYNQKFEGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARX$_{13}$
NX$_{14}$LDYWGQGTTVTVS

wherein X$_{10}$ is Y or N, X$_{11}$ is G or R, X$_{12}$ is S or G, X$_{13}$ is A or T, and X$_{14}$ is I or W.

Antibodies are transiently expressed in HEK293 EBNA cells (Edge BioSystems, #90500130) using standard transfection procedures. Transfected cells are cultured in standard serum-free medium containing geneticin (G418) and tobramycin for 48 to 120 hours at 37°C after transfection. Antibodies are purified on a 60 mL rProtein A Sepharose column (Amersham Biosciences, #17-1279-04) by following the manufacturer's instructions, and further concentrated and purified by size exclusion chromatography (XK50/60 Superdex200, Pharmacia) with phosphate buffered saline (PBS), pH 7.4, as the mobile phase. Antibodies are then filtered using Millev-GV, PVDF membranes, 0.22 μm, 33mm, (Millipore, # SLGV033RS), and stored at 4 to 8°C.

Murine IgG1 c-Met antibody 5D5 (U.S. Patent No. 5,686,292), discussed in many of the examples below, is isolated and purified from hybridoma HB-11895 obtained from the American Type Culture Collection, Manassas, VA, as described above.

Example 2

Binding Kinetics of c-Met Antibodies to Various c-Met Extracellular Domains

The extracellular domains (ECDs) of human, cynomolgus monkey, and rat c-Met sequences are expressed as Fc fusion proteins with a flag- and His-tag (Flis-tag) at the C-terminus of the Fc (SEQ ID NOs:72-74). These c-Met ECD Fc fusion proteins are separately transiently expressed in HEK293 EBNA cells and purified as described in Example 1.

A Biacore® 2000 instrument is used to measure the binding kinetics of c-Met antibodies to human, cynomolgus monkey, and rat c-Met ECDs. Measurements are performed at 25°C. Samples are dissolved in HBS-EP buffer (150 mM sodium chloride, 3 mM EDTA, 0.005% (w/v) surfactant P-20, and 10 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) at pH 7.4; #BR-1001-88). $F(ab')_2$ fragment of goat anti human IgG, $F(ab')_2$ fragment specific (Jackson Immunoresearch Inc, #109-006-097) is immobilized on flow cells 1 to 4 of a CM5 sensor chip at a level of 4000 response units (RUs) using amine coupling chemistry to capture anti-c-Met antibodies.

Binding is evaluated using multiple cycles. Each cycle is performed at a flow rate of 50 µL/min., and consists of the following steps: injection of about 10 µL of a c-Met antibody at 10 µg/mL aiming at a capture of 40-100 RUs, injection of 250 µL of human, cynomolgus, or rat c-Met-Flis-Fc ECD (starting at 100 nM and using two-fold serial dilutions for each cycle) followed by 20 min. for dissociation, and regeneration using about 30 µL of 10 mM glycine hydrochloride, pH 1.5. Association and dissociation rates for each cycle are evaluated using a "1:1 (Langmuir) binding" model in the BIAevaluation software, version 4.1. For binding of antibody D11-S17Y to rat c-Met-Flis-Fc ECD, a heterogeneous ligand model is used to fit the data adequately; therefore, two binding affinities are obtained.

The results are shown in Tables 3-5 below.

Table 3

Binding Kinetics and Affinity of c-Met Antibodies to Human c-Met-Flis-Fc ECD

Antibody	k_{on} (1/Ms)	k_{off} (1/s)	K_D (nM)
D11-8B8	$1.0 \pm 0.1 \times 10^{5}$	$0.5 \pm 0.2 \times 10^{-4}$	0.5 ± 0.2
D11-C27G3	$6.4 \pm 0.2 \times 10^{4}$	$0.9 \pm 0.2 \times 10^{-4}$	1.4 ± 0.3
D11-S17Y	$0.7 \pm 0.1 \times 10^{5}$	$2.8 \pm 0.1 \times 10^{-4}$	4.2 ± 0.9
C8-H241	$1.1 \pm 0.3 \times 10^{5}$	$<10^{-5}$	<0.1
C8-6	$1.6 \pm 0.4 \times 10^{5}$	$3 \pm 2 \times 10^{-4}$	4 ± 1
C8-co16	$1.1 \pm 0.2 \times 10^{5}$	$0.3 \pm 0.2 \times 10^{-4}$	0.3 ± 0.1

Table 4

Binding Kinetics and Affinity of c-Met Antibodies

to Cynomolgus Monkey c-Met-Flis-Fc ECD

Antibody	k_{on} (1/Ms)	k_{off} (1/s)	K_D (nM)
D11-8B8	$0.78 \pm 0.02 \times 10^{5}$	$2.23 \pm 0.07 \times 10^{-4}$	2.9 ± 0.2
D11-C27G3	$0.5 \pm 0.1 \times 10^{5}$	$3.2 \pm 0.4 \times 10^{-4}$	6.5 ± 0.7
D11-S17Y	$0.70 \pm 0.08 \times 10^{5}$	$3.6 \pm 0.5 \times 10^{-4}$	5.1 ± 0.2
C8-H241	$0.80 \pm 0.06 \times 10^{5}$	$<10^{-5}$	<0.2
C8-6	$1.4 \pm 0.5 \times 10^{5}$	$4.5 \pm 0.2 \times 10^{-4}$	3.6 ± 1.1
C8-co16	$1.03 \pm 0.02 \times 10^{5}$	$<10^{-5}$	<0.1

Table 5

Binding Kinetics and Affinity of c-Met Antibodies to Rat c-Met-Flis-Fc ECD

Antibody	k_{on} (1/Ms)	k_{off} (1/s)	K_D (pM)	k_{on} (1/Ms)	k_{off} (1/s)	K_D (nM)
D11-8B8	$2.1 \pm 0.2 \times 10^{5}$	$1.9 \pm 0.3 \times 10^{-4}$	0.89 ± 0.04			
D11-C27G3	$1.3 \pm 0.1 \times 10^{5}$	$3.4 \pm 0.5 \times 10^{-4}$	2.7 ± 0.2			
D11-	0.66×10^{5}	189×10^{-4}	286	2.5×10^{5}	3.0×10^{-4}	1.2

S17Y						

These data demonstrate that antibodies C8-H241, C8-6, and C8-co16 bind both human and cynomolgus monkey c-Met ECDs with similar affinity, but not rat c-Met ECD. Additional data (not shown) indicate that these antibodies do not bind mouse c-Met ECD at up to 100 nM of antibody with 1 μg/mL ECD coated on ELISA plates. Antibodies D11-8B8, D11-C27G3, and D11-S17Y, however, bind human, cynomolgus monkey, and rat c-Met ECDs.

Example 3

Epitope Mapping

The epitopes of the present c-Met antibodies are mapped by a combination of hydrogen-deuterium exchange mass spectrometry (HDXMS) (Yamada et al. (2002) *Rapid Commun. Mass Spectrom.* 16(4):293-299) and diethyl pyrocarbonate (DEPC) labeling (Mendoza et al. (2008) *Analy. Chem.* 80(8):2895-2904). Hydrogen-deuterium exchange reaction of human c-Met Sema domain is carried out in the presence or absence of c-Met antibodies. Sema domain regions that gain less deuterium in the presence of an antibody than in its absence are identified as the epitope(s) for the antibody. DEPC can react with amino groups of surface-exposed lysine or histidine residues in the Sema domain, forming ethyl carbamate lysine or histidine. If these amino acids are located in the epitope region, they will be protected, and will not react with DEPC upon antibody binding. This helps to further localize and/or confirm the epitope regions determined by HDXMS.

Expression and Purification of c-Met Sema Domain. The Sema domain of human c-Met is expressed with a Flis tag at the C-terminus (SEQ ID NO:76) in HEK293 EBNA cells and purified as described in Examples 1 and 2. The purified protein is then stored at 4°C in PBS at pH 7.4. This domain binds to the c-Met antibodies of the present invention with affinity similar to that of full length human c-Met ECD, indicating that the epitopes for these antibodies are located in this region of human c-Met.

Deglycosylation and Desialylation of the c-Met Sema Domain. 100 μL of 1.2 mg/mL human c-Met Sema domain solution are treated with 1 μL of PNGase F solution (Prozyme, GKE-5006B) at 37°C overnight for de-N-glycosylation. LC/MS analysis

demonstrates that most of the protein is deglycosylated after this treatment. Separately, 100 μL of 1.2 mg/mL human c-Met Sema domain solution are treated with 2 μL of 10 U/mL neuraminidase solution (Roche, Cat. # 10 269 611 001) at 37°C for 1 hour to desialylate the Sema domain.

Formation of c-Met Sema Domain/Antibody Complexes. 10 μL of deglycosylated c-Met Sema domain solution (1.2 mg/mL) are mixed with an aliquot containing 29 μg protein of antibody solution (2.07 μL of C8-H241, 2.01 μL of D11-8B8, or 3.87 μL of an unrelated control Mab), and then diluted with 1X PBS solution to a final volume of 40 μL. Separately, 5 μL of desialylated human c-Met Sema domain solution (1.2 mg/mL) are mixed with an aliquot containing 14 μg protein of antibody solution (1.04 μL of C8-H241, 1.01 μL of D11-8B8, or 1.94 μL of the unrelated control Mab), and then diluted with 1X PBS solution to a final volume of 15 μl. Each of the mixed solutions of each antibody is then subjected to HDXMS analysis.

HDXMS Assays. 4 μL of deglycosylated or desialylated c-Met Sema domain/antibody mixture are mixed with 16 μL of 100% D_2O (Acros, Code 166310500; 80% D during exchange), and incubated at ambient temperature for 90 seconds. The exchange is then quenched with 50 μL of 0.5% (v/v) formic acid in water at 0°C. The quenched solution is immediately treated with 2 μL of 5 mg/mL (v/v) pepsin solution (Sigma, Cat. # P6887) at 0°C for 3.5 or 4 min. The digested solution is immediately manually injected onto an RP-HPLC column (Polymer Laboratories, Part #1912-1802; 2.1×50mm, 1000 Å pore size, 8 μM particle size). The HPLC buffer stream from the HPLC pump (Waters, 2795 HPLC) passes through a metal tube (approximately 1 mL) to a manual injector. The column eluate is then passed to a Micromass LCT Premier or SYNAPT mass spectrometer. The metal tube, injector loop, and column are submerged in an ice water bath.

The column is equilibrated with 99% A (0.05% (v/v) aqueous TFA (trifluoroacetic acid) and 1% B (0.04% (v/v) TFA in acetonitrile) at a flow rate of 0.2 mL/min. An isocratic gradient elution is performed for 1 min., from 1 to 10% B over 1 min., to 40% B over 12 min., to 90% B over 4 min. with a 3 min. hold, and then rapidly returned to the initial conditions. The mass spectrometry is performed on a Micromass LCT Premier Mass Spectrometer with a positive spray, W mode, and the following settings: a capillary

voltage of 1.5 kV, a cone voltage of 100 V, Aperture 1 of 25 V, a mass range of 200 to 2000, a desolvation temperature of 150°C, and a desolvation gas flow of 500 L/h. The mass spectrum of each peptic peptide of c-Met is obtained after D/H exchange with or without c-Met antibody. The average mass of each peptide is calculated according to the isotopic distribution of the most intense ion peak.

DEPC Labeling of c-Met/Antibody Complexes. 8.3 μL of 1.2 mg/mL human c-Met Sema domain solution are mixed with an antibody solution (24 μg protein: 1.71 μL of C8-H241, 1.67 μL of D11-8B8, or 3.2 μL of unrelated control Mab), and 1X PBS solution to a final volume of 76 μL. Each c-Met/antibody mixture is treated with 4 μL of 10 mg/mL (w/v) DEPC in isopropanol at ambient temperature for 5 min. and then quenched with 10 μL of 20 mg/mL histidine in 0.1 M Tris-HCl buffer, pH 8, and 10 μL of 0.2 mg/mL lysine in 0.1 M Tris-HCl buffer, pH 8. Each solution is mixed with 5 μL of 0.2 mg/mL (w/v) porcine trypsin solution (Promega, Cat. # V528A), and half of the mixture is mixed with 0.5 μL of 50 mg/mL (w/v) dithiothreitol solution. Each sample solution is incubated at 37°C for 5 hours and then treated with 0.5 μL of PNGase F solution for an additional hour. Each digested solution is acidified with 2 μL of 10% (v/v) acetic acid solution. 2 μL of 100 mg/mL TCEP (tris(2-carboxyethyl) phosphine hydrochloride (Sigma, Cat. # C4702-2G) solution are added to the unreduced digest without added dithiothreitol. Each of the solutions is subjected to LC/MS analysis using a Waters Acquity UPLC and Waters SYNAPT Mass spectrometer. The HPLC uses a Waters Acquity UPLC BEH C8 column (2.1×50mm, 1.7μm, Waters, part #186002877) at 60 °C, and peptides are eluted with an acetonitrile gradient in a water/acetonitrile/TFA HPLC mobile phase system. A 45 min. run time is used for the digests. The column is equilibrated with 99% A (0.05% (v/v) TFA aqueous solution) and 1% B (0.04% (v/v) TFA in acetonitrile) at a flow rate of 0.2 mL/min. A gradient elution is performed in isocratic state for 2 min., from 1 to 25% B over 25 min., to 45% B over 10 min., to 90% B over 1 min., with 1.5 min. hold (at the same period of time, flow rate of 0.3 mL/min.), and then rapidly returned to 1% B.

Results. Mab C8-H241 appears to bind a conformational epitope comprising four regions of the c-Met Sema domain, located in the α-chain of the human c-Met extracellular domain (amino acid residues 25-307 of SEQ ID NO:75):

$_{121}$VVDTYYDDQL$_{130}$ (SEQ ID NO:77),

$_{131}$ISCGSVNRGTCQRHVFPHNHTADIQS$_{156}$ (SEQ ID NO:78),

$_{179}$ALGAKVLSSVKDRFINFF$_{196}$ (SEQ ID NO:79), and

$_{216}$VRRLKETKDGFM$_{227}$ (SEQ ID NO:80).

More particularly, Mab C8-H241 binds c-Met by interacting with one or more amino acid residues within:

$_{123}$DTYYDD$_{128}$ (SEQ ID NO:81) inclusive,

$_{144}$HVFPHNHTADIQS$_{156}$ (SEQ ID NO: 82) inclusive,

$_{192}$FINF$_{195}$ (SEQ ID NO:83) inclusive, and

$_{220}$KETKDGFM$_{227}$ (SEQ ID NO:84) inclusive.

Binding of Mab C8-H241 to the region $_{144}$HVFPHNHTADIQS$_{156}$ (SEQ ID NO: 82) renders it capable of binding both human (SEQ ID NO:75) and cynomolgus monkey (amino acids 25 to 932 of SEQ ID NO:73) c-Met extracellular domain with comparable affinity, but not rat or mouse c-Met, up to 100 nM of antibody in binding assays.

Mab D11-8B8 binding to the α-chain of the c-Met Sema domain appears to be localized to one region, i.e., amino acid residues within a linear epitope within the amino acid sequence $_{84}$YKTGPVLEHPDCFPCQDCSSKANL$_{107}$ (SEQ ID NO:85) inclusive, more particularly the region $_{95}$CFPCQDCSSKA$_{105}$ (SEQ ID NO:86) inclusive. The epitope for Mab D11-8B8 is further confirmed by epitope extraction experiments (Dhungana et al. (2009) *Methods Mol. Biol.* 524:87-101). c-Met Sema domain is digested with porcine trypsin (Promega) and the digest is then mixed with biotinylated Mab D11-8B8, using the EZ-Link™ Sulfo-NHS-LC-Biotin kit (Pierce, Prod. #1754210), to bind the c-Met peptides. Biotinylated D11-8B8 with or without bound c-Met peptides is captured by high capacity Streptavidin agarose resin (Thermo Scientific, Prod. # 20359). The bound peptides are released by 0.15% formic acid (v/v) in H_2O, and then identified by LC/MS.

Example 4

Preferential Binding of Antibodies to c-Met vs. RON and PlexinA2 ECDs

The human proteins with the greatest sequence identity to c-Met are RON and Plexin A2. This experiment compares the specificity of c-Met antibody binding to c-Met, RON, and PlexinA2 ECDs.

Wells of 96-well EIA/RIA high binding plates (Costar, #2592) are coated with 100 µL of 1 µg/mL human c-Met extracellular domain (ECD)-Fc-Flis fusion (SEQ ID NO:72), RON-ECD-Fc (R&D Systems, #1947-MS), or PlexinA2-ECD-Fc (Abnova, Taipei, Taiwan #H00005362-P01) in coating buffer (BioFX Labs, # COAT-1000-01) overnight at 4°C. The wells are aspirated and non-specific binding sites are blocked by adding 200 µL of blocking buffer (Tris-buffered saline, pH 8.0, with 0.05% (v/v) polysorbate 20 (Tween-20) (TBS-T) (BioFX Labs, # WSHW-1000-01) plus 1% (w/v) bovine serum albumin (BSA) (Jackson Immuno, #001-000-162) and incubating for 1 hour at room temperature. After the plates are washed three times with wash buffer (TBS-T), 100 µL/well of 1:6 serial dilutions of c-Met antibodies in blocking buffer (starting from 100 µg/mL) are added and incubated at room temperature for 2 hours. The plates are washed and incubated with 100 µL/well of HRP-conjugated goat anti-human $F(ab)_2$ IgG (Jackson ImmunoResearch Labs #109-036-097) in blocking buffer for 90 min. After the plates are washed, 100 µL/well of substrate solution (BioFx, #TMBW-1000-01) are added and the plates are incubated for 10 min. at room temperature. 100 µL/well of stop solution (BioFx, # LSTP-1000-01) are added to stop the reaction. The colorimetric signals are developed and read at 450 nm using a SpectraMax 190 plate reader (Molecular Devices, Sunnyvale, CA). c-Met antibody binding to c-Met, RON and PlexinA2 ECDs is proportional to color signal production.

As shown in Table 6, both C8 and D11 c-Met antibodies of the present invention preferentially bind to human c-Met ECD compared to RON ECD or PlexinA2 ECDs.

Table 6
Preferential Binding of c-Met Antibodies to Human c-Met ECD vs. RON and PlexinA2 ECDs

	Average A450 nm								
Antibody dose	C8-H241			C8-6			C8-co16		
(ng/mL)	c-Met ECD	RON ECD	Plexin A2	c-Met ECD	RON ECD	Plexin A2	c-Met ECD	RON ECD	Plexin A2
0.000	0.300	0.098	0.092	0.322	0.110	0.109	0.335	0.106	0.104
0.002	0.317	0.088	0.082	0.326	0.106	0.099	0.369	0.100	0.094
0.010	0.317	0.081	0.090	0.335	0.097	0.095	0.374	0.096	0.078
0.060	0.484	0.104	0.099	0.551	0.087	0.096	0.446	0.089	0.082
0.357	0.902	0.091	0.097	0.813	0.080	0.107	0.907	0.083	0.095
2.143	2.861	0.101	0.103	2.415	0.098	0.106	2.888	0.114	0.092
12.860	4.000	0.079	0.083	4.000	0.087	0.085	4.000	0.102	0.069
77.160	4.000	0.085	0.079	4.000	0.090	0.078	4.000	0.084	0.096
462.963	4.000	0.090	0.104	4.000	0.088	0.086	4.000	0.103	0.092
2777.778	4.000	0.085	0.092	4.000	0.088	0.094	4.000	0.095	0.114
16666.667	4.000	0.117	0.125	4.000	0.124	0.118	4.000	0.198	0.284
100000.000	4.000	0.267	0.310	4.000	0.170	0.171	4.000	0.421	0.983
Standard Error									
0.000	0.006	0.015	0.007	0.011	0.000	0.004	0.003	0.005	0.011
0.002	0.003	0.006	0.002	0.012	0.005	0.006	0.023	0.000	0.006
0.010	0.002	0.000	0.005	0.001	0.004	0.009	0.015	0.012	0.002
0.060	0.055	0.001	0.002	0.144	0.004	0.005	0.029	0.003	0.002
0.357	0.004	0.004	0.002	0.022	0.005	0.003	0.005	0.007	0.000
2.143	0.032	0.004	0.005	0.041	0.004	0.003	0.128	0.002	0.007
12.860	0.000	0.001	0.012	0.000	0.000	0.007	0.000	0.004	0.004
77.160	0.000	0.002	0.014	0.000	0.008	0.007	0.000	0.003	0.001
462.963	0.000	0.004	0.003	0.000	0.006	0.001	0.000	0.013	0.001
2777.778	0.000	0.004	0.002	0.000	0.004	0.001	0.000	0.007	0.000
16666.667	0.000	0.005	0.004	0.000	0.004	0.003	0.000	0.003	0.002
100000.000	0.000	0.008	0.012	0.000	0.002	0.003	0.000	0.011	0.093

D11-8B8			D11-C27G3			D11-S17Y		
c-Met ECD	RON ECD	Plexin A2	c-Met ECD	RON ECD	Plexin A2	c-Met ECD	RON ECD	Plexin A2
0.297	0.097	0.081	0.2844	0.0791	0.0771	0.31475	0.1129	0.10795
0.299	0.082	0.078	0.2708	0.07065	0.07965	0.3091	0.09705	0.09345
0.304	0.074	0.078	0.2904	0.0715	0.0763	0.31405	0.0868	0.0986
0.445	0.094	0.073	0.45965	0.0695	0.06605	0.37355	0.0857	0.09455
1.314	0.073	0.074	1.43965	0.07085	0.0792	0.78915	0.08475	0.0924
2.416	0.069	0.073	2.5266	0.0655	0.0742	2.1432	0.1085	0.08235
3.931	0.079	0.075	4	0.07835	0.0641	4	0.0825	0.0768
4.000	0.079	0.066	4	0.06725	0.076	4	0.08195	0.07575
4.000	0.071	0.077	4	0.0981	0.1045	4	0.08185	0.0783
4.000	0.088	0.093	4	0.20445	0.30325	4	0.09255	0.0955
4.000	0.165	0.181	4	0.67565	1.33475	4	0.1274	0.1491
4.000	0.544	0.498	3.9211	2.30295	3.37085	4	0.23775	0.3146
Standard Error								
0.006	0.003	0.003	0.0087	0.0017	0.0106	0.00515	0.0012	0.00035
0.011	0.002	0.002	0.0194	0.00595	0.00185	0.0228	0.00065	0.01725
0.012	0.002	0.001	0.0051	0.0018	0.0017	0.01195	0.0058	0.0079
0.007	0.017	0.003	0.01495	0.0006	0.00335	0.00375	0.0013	0.00035
0.092	0.007	0.004	0.02935	0.00695	0.0017	0.04195	0.00075	0.0039
0.044	0.002	0.001	0.2742	0.0002	0.0015	0.0841	0.0054	0.00015
0.069	0.005	0.002	0	0.01505	0.0043	0	0.0029	0.0061
0.000	0.007	0.000	0	0.00275	0.0066	0	0.00445	0.00455
0.000	0.005	0.004	0	0.0063	0.0032	0	0.00485	0.0089
0.000	0.003	0.005	0	0.00975	0.00535	0	0.00705	0.0055
0.000	0.007	0.004	0	0.03125	0.03495	0	0.0007	0.0035
0.000	0.016	0.006	0.0789	0.07335	0.14345	0	0.00865	0.004

5

Example 5

c-Met Antibodies Block HGF/c-Met Binding

In vitro binding assays are used to determine inhibition of HGF binding to c-Met by the present c-Met antibodies.

Wells of 96-well EIA/RIA high binding plates (Costar, #2592) are coated with 100 µL of human c-Met ECD-Fc-Flis (SEQ ID NO:72) (2 µg/mL) in Dulbecco's phosphate buffered saline (DPBS) overnight at room temperature; washed four times with wash buffer (Tris-buffered saline, pH 8.0, with 0.05% (v/v) polysorbate 20 (TWEEN®-20) (TBS-T) (BioFX Labs, # WSHW-1000-01) in a plate washer; blocked by adding 300 µL of blocking buffer (TBS-T plus 1% (w/v) bovine serum albumin (BSA) (Jackson Immuno, #001-000-162); and incubating for 60 min. at 37°C. Blocking buffer is then removed from the wells and 50 µL of antibodies in blocking buffer at final concentrations as indicated in Table 7 are added into each well, respectively. Blocking buffer is added to the HGF-only control wells. The plates with c-Met antibodies are incubated for 90 min. at 37°C. 50 µL of human hepatocyte growth factor (HGF) (R&D Systems, #294) in blocking buffer at a final concentration of 10 ng/mL are then added to each well except the antibody-only control wells. The plates containing the c-Met antibody/HGF mixture are incubated on a plate shaker for two hours at room temperature. The wells are then washed four times with TBS-T in a plate washer. Next, 100 µL of biotinylated anti-HGF antibody (R&D Systems, # BAF294) in blocking buffer at a final concentration of 100 ng/mL are added into each well. The plates are incubated for 90 min. at room temperature. The wells are again washed four times with TBS-T in a plate washer. 100 µL of a 1:200 dilution of streptavidin-horseradish peroxidase (HRP) (R&D Systems, DY998) in blocking buffer are added to each well. The plates are incubated for 30 min. at room temperature. The wells are washed four times with TBS-T in a plate washer. 100 µL/well of substrate solution (BioFx, #TMBW-1000-01) are then added, and the plates are incubated for 10 min. at room temperature. To stop the reaction, 100 µL/well of stop solution (BioFx, # LSTP-1000-01) are added. The samples are read at a wavelength of 450-570 nm on a microplate reader (Spectra, MAX 190) and no background is subtracted.

As shown in Table 7, both C8 and D11 c-Met antibodies of the present invention block human HGF/c-Met binding.

Table 7

Inhibition of Human HGF Binding to Human c-Met by C8- and D11- Antibodies

	AVG A450 nm							
Antibody dose (ng/mL)	hIgG2	D11-8B8	D11-27G3	D11-S17Y	hIgG4	C8-6	C8-H241	C8-co16
0.00	2.43	2.43	2.43	2.43	2.61	2.61	2.61	2.61
0.48	2.46	2.51	2.37	2.15	2.41	2.56	2.45	2.05
1.91	2.87	2.48	2.31	1.98	2.49	2.59	2.46	2.18
7.63	2.70	2.41	2.27	2.08	2.88	2.64	2.53	2.20
30.52	2.41	2.32	2.38	2.07	2.63	2.42	2.42	2.08
122.07	2.52	1.92	1.60	1.69	2.44	2.00	1.77	1.30
488.28	2.53	1.30	0.94	1.29	2.44	1.33	0.73	0.70
1953.13	2.43	1.43	0.97	1.23	2.27	1.08	0.68	0.60
7812.50	2.50	1.24	0.91	1.12	2.49	0.95	0.70	0.57
31250.00	2.50	1.31	0.94	1.17	2.67	0.98	0.77	0.56
125000.00	2.43	1.38	0.89	1.35	3.00	1.32	0.69	0.38
	STDErr							
0.00	0.06	0.06	0.06	0.06	0.10	0.10	0.10	0.10
0.48	0.02	0.13	0.01	0.09	0.24	0.02	0.14	0.15
1.91	0.27	0.08	0.08	0.03	0.00	0.03	0.16	0.12
7.63	0.14	0.01	0.21	0.08	0.14	0.03	0.06	0.09
30.52	0.09	0.02	0.31	0.09	0.16	0.01	0.03	0.01
122.07	0.09	0.07	0.03	0.02	0.08	0.10	0.02	0.03
488.28	0.00	0.04	0.04	0.02	0.01	0.02	0.00	0.01
1953.13	0.13	0.16	0.02	0.05	0.00	0.01	0.01	0.01
7812.50	0.04	0.01	0.01	0.02	0.25	0.07	0.01	0.02
31250.00	0.06	0.02	0.02	0.04	0.03	0.03	0.03	0.03
125000.00	0.07	0.03	0.01	0.08	0.13	0.47	0.01	0.00

Abbreviations: AVG = average; STDErr = Standard Error

Example 6

c-Met Antibodies Induce Weak c-Met Phosphorylation Compared to Hepatocyte Growth Factor and Agonist Mab 5D5

Upon stimulation with HGF, the A549 lung carcinoma cell line exhibits increased c-Met phosphorylation at tyrosine residue 1349. A phosphorylated c-Met (p-Met) ELISA assay is used to measure the agonistic activity of various c-Met antibodies in the absence of HGF, as well as of Mab 5D5 and HGF itself.

A549 cells (ATCC, #CCL-185) are cultured in Ham's F12K + 2 mM glutamine (Invitrogen, # 21127-022) + 10% FBS medium (Invitrogen, #10082). Cells are plated at

6×10^4 cells/well in the full serum medium as above in 96-well plates and incubated overnight at 37°C under 5% (v/v) CO_2. The medium is removed from the wells and the cells are starved in 100 µL of Ham's F12K + 2 mM glutamine medium + 0.5% FBS for six hours at 37°C under 5% (v/v) CO_2. Antibodies or HGF are diluted in the starvation culture medium, added at final concentrations as indicated in Table 8, and incubated for 15 min. at 37°C. The medium is aspirated and cells are lysed in 50 µL lysis buffer (10 mM Tris (Invitrogen, #15567-027), 150 mM NaCl, 2 mM ethylenediaminetetraacetic acid (EDTA) (Invitrogen, #15575-038), 50 mM NaF (Sigma, #S1504), 1% (v/v) octylphenoxy polyethoxy ethanol (TRITON®-X 100; Sigma, #T8787), 2 mM sodium orthovanadate (EMD Chemicals, Gibbstown, NJ, #567540), protease inhibitor (Sigma, St. Louis, MO; #P8340), phosphatase inhibitor cocktail I (Sigma #P2850), and phosphatase inhibitor cocktail II (Sigma #P5726)) per well and incubated on ice for 15-20 min. Cell lysates are used in the ELISA assay described below to determine the phosphorylation of c-Met at residue Y1349.

c-Met capture antibody is diluted in Bup H coating buffer (Thermo Fisher Scientific, Waltham, MA, #28382) to 2 µg/mL, added to ELISA plates (Greiner Bio-One, Monroe, NC, #655081) and incubated overnight at 4°C. The wells are aspirated, washed three times with TBS-T (BioFX, Owings Mills, MD, WSH-1000-01), and then blocked with 200 µL of blocking buffer (TBS-T plus 2% (w/v) BSA) for 1 hour at room temperature. The plates are washed three times with TBS-T. Next, 25 µL of cell lysates plus 75 µL blocking buffer are added, and the plates are incubated overnight at 4°C. The plates are then washed three times with TBS-T. 100 µl of 0.5 µg/mL of pY1349 c-Met polyclonal antibody (Cell Signaling Technology, Danvers, MA, #3133) in blocking buffer are added to each well, and incubated for 2 hours at room temperature. The plates are then washed three times with TBS-T. 100 µL of 1/10,000 diluted peroxidase-conjugated goat anti-rabbit IgG polyclonal antibody (Jackson ImmunoResearch Laboratories, West Grove, PA, #111-035-144) in blocking buffer are added to each well and incubated 1 hour at room temperature. The plates are then washed three times with TBS-T. 100 µL of 3,3′,5,5′-tetramethylbenzidine solution (BioFX, #TMBW-1000-01) are added to each well, followed by the addition of 100 µl stop solution (BioFX, #LSTP-1000-01). The plates are read at 450 nm on a SpectraMax M5 plate reader (Molecular Devices,

Sunnyvale, CA) and sample values are determined using SOFTmax Pro 4.8 software (Molecular Devices).

The results are shown in Table 8.

Table 8

Effect of c-Met Antibodies, HGF, and Agonist Mab 5D5 on c-Met Phosphorylation in A549 Cells

Ab Conc. (µg/mL)	p-Met signal C8-H241	p-Met signal C8-6	p-Met signal hIgG4
30	0.333	0.272	0.061
10	0.418	0.334	0.077
3.333	0.450	0.328	0.068
1.113	0.465	0.365	0.087
0.370	0.340	0.211	0.082
0.123	0.304	0.127	0.067
0.041	0.130	0.125	0.268
0.014	0.078	0.105	0.074
0.005	0.074	0.070	0.074
0.002	0.070	0.081	0.117

HGF Conc. (ng/mL)	p-Met signal	Ab Conc. (ng/mL)	p-Met signal Mab 5D5
300	10.615	5	3.290
100	6.129		
33.33333	2.267		
11.11111	1.0667		
3.703704	0.461		
1.234568	0.211		
0.411523	0.142		
0.137174	0.128		
0.045725	0.100		
0.015242	0.148		

The data demonstrate that HGF and agonist c-Met Mab 5D5 strongly stimulate c-Met phosphorylation, while present c-Met Mabs C8-H241 and C8-6 weakly stimulate c-Met phosphorylation. Similar results are obtained in HCT116, H460, and MDA-MB-231 cell lines.

Mab D11-8B8 yields similar results.

Example 7

c-Met Antibodies Inhibit HGF-Induced c-Met Phosphorylation

Binding of HGF to c-Met results in tyrosine phosphorylation of c-Met molecules and activation of the c-Met signaling pathway. In this example, human colon cancer cell line HCT116, which is responsive to HGF, is used to assess the inhibition of HGF-induced phosphorylation at tyrosine residues 1230, 1234, and 1235 of c-Met by the present c-Met antibodies.

HCT116 cells (ATCC, Manassas, VA, #CCL-247) are resuspended to 150,000 cells/mL in McCoy's 5A medium (Invitrogen, Carlsbad, CA, #16600-082) plus penicillin/streptomycin (Invitrogen, #15140-122) with 10% (v/v) fetal bovine serum (FBS) (Invitrogen, #10082-147). 0.2 mL of the resuspended HCT116 cells are added to 96-well microtiter plates (Corning, Lowell, MA, #3596) at 30,000 cells/well, and the cells are then incubated for 48 hours at 37°C under 5% (v/v) CO_2. The culture medium is then aspirated and the cells are starved in 100 µL of McCoy's 5A medium plus penicillin/streptomycin with 0.1% (w/v) BSA for 24 hours at 37°C under 5% (v/v) CO_2. 25 µL of sodium azide (final concentration of 0.01% (w/v)) (Sigma, #S2002) are added, followed immediately by adding 25 µL of 8X c-Met antibody dilutions to McCoy's 5A medium plus penicillin/streptomycin with 0.1% (w/v) BSA, and the cells are incubated for 30 min. at 37°C under 5% (v/v) CO_2. 50 µL of HGF at a final concentration of 200 ng/mL are added to each well and the cells are incubated for an additional 15 min. at 37°C under 5% (v/v) CO_2. The medium is then aspirated and 50 µL of cell lysis buffer are added (10 mM Tris (Invitrogen, #15567-027), 150 mM NaCl, 2 mM ethylenediaminetetraacetic acid (EDTA) (Invitrogen, #15575-038), 50 mM NaF (Sigma, #S1504), 1% (v/v) octylphenoxy polyethoxy ethanol (TRITON®-X 100; Sigma, #T8787), 2 mM sodium orthovanadate (EMD Chemicals, Gibbstown, NJ, #567540), and complete protease inhibitor, EDTA free (Roche, Basel, Switzerland, #11836170001). Cells are incubated in the lysis buffer at room temperature for 15-30 min. Cell lysates are assayed for c-Met tyrosine phosphorylation by ELISA as follows.

c-Met capture antibody is diluted in coating buffer (BioFX, Glendora, CA, COAT-1000-01) to 2 µg/mL. 110 µL of the diluted antibody are added per well to ELISA plates (Greiner Bio-One, Monroe, NC, #655081) and the plates are incubated

overnight at 4°C. The wells are aspirated, washed twice with TBS-T, and then blocked with 200 µL of blocking buffer (TBS-T plus 2% (w/v) BSA) for 1 hour. The plates are washed twice with TBS-T. Next, 25 µL of cell lysates plus 75 µL blocking buffer or 100 µL of MKN45 cell lysate dilutions (diluted with blocking buffer, as a standard) are added, and the plates are incubated for 2 hours at room temperature. The plates are then washed four times with TBS-T. 100 µL of 0.5 µg/mL of pYpYpY1230/1234/1235 c-Met polyclonal antibody (Invitrogen, #44-888G) in blocking buffer are then added to each well, and the plates are incubated for 2 hours at room temperature. Next, the plates are washed four times with TBS-T. 100 µL of 1/12,000 diluted peroxidase-conjugated goat anti-rabbit IgG polyclonal antibody (Jackson ImmunoResearch Laboratories, West Grove, PA, #111-035-144) in blocking buffer are then added, and the mixture is incubated for 1 hour at room temperature. The plates are then washed six times with TBS-T. 100 µL of 3,3′,5,5′-tetramethylbenzidine solution (BioFX, #TMBW-1000-01) are added to each well, followed by the addition of 100 µL stop solution (BioFX, #LSTP-1000-01). The plates are read at 450 nm with a 570 nm correction using a SpectraMax 190 plate reader (Molecular Devices, Sunnyvale, CA). The standard curve is established using 4-parameter analysis, and sample values are determined using SOFTmax Pro 3.1.2 software (Molecular Devices).

The percentage of inhibition of c-Met tyrosine phosphorylation is set at the average of HGF treatment without c-Met antibody addition (0% inhibition), and 100% inhibition is set at the average of sodium azide treatment (no HGF or c-Met antibody treatment).

Table 9 shows the average of triplicate treatments per experiment for three experiments with standard errors. The data demonstrate that five of six c-Met antibodies of the present invention significantly inhibit HGF-induced tyrosine phosphorylation of c-Met compared to that of the human IgG2 and IgG4 isotype controls.

Table 9

Percent Inhibition of HGF-Induced c-Met Tyrosine Phosphorylation By C8- and D11- Antibodies

		Antibody doses:											
		10 µg/mL		2 µg/mL		0.4 µg/mL		0.08 µg/mL		0.016 µg/mL		0.0032 µg/mL	
Ab treatment	isotype	average	sterr	average	sterr	average	sterr	average	sterr	average	sterr	average	sterr
D11-8B8	IgG2	19.56	4.68	40.74	8.24	35.04	6.43	10.52	7.41	-6.68	9.48	-10.37	6.79
D11-C27G3	IgG2	1.25	7.55	31.78	7.71	38.10	4.97	20.79	5.72	5.73	8.95	9.72	7.09
D11-S17Y	IgG2	16.73	8.72	31.39	6.43	15.91	6.05	-3.24	10.54	2.53	8.52	9.48	8.51
C8-H241	IgG4	60.08	5.27	62.20	2.10	59.09	3.05	22.09	4.92	14.68	12.40	-12.33	10.18
C8-co-16	IgG4	65.40	3.94	58.96	3.37	45.08	3.44	9.77	9.14	-2.89	10.95	-10.72	5.41
C8-6	IgG4	14.88	10.78	27.94	3.80	16.83	4.36	7.79	7.68	-6.06	8.84	-11.34	8.65
5D5	mIgG1	64.12	3.31	63.75	4.44	42.55	4.27	11.87	7.73	-3.50	9.70	-6.07	5.68
hIgG2	isotype control	6.24	5.31	17.53	5.74	9.31	6.56	-1.58	8.16	9.49	6.77	-10.21	6.78
hIgG4	isotype control	-1.47	5.76	-6.62	9.07	-16.07	7.01	-21.70	9.09	-7.96	10.97	-14.40	7.37
mIgG1	isotype control	5.46	6.31	9.76	9.27	13.43	8.18	-10.59	10.31	-5.42	10.53	-9.76	8.42

		ave	sterr
untreated		100.00	0.98
HGF	200 ng/mL	0.00	2.76

% inhibition of HGF stimulated phospho-Met as measured by ELISA

Example 8

Induction of c-Met Internalization by c-Met Antibodies

The experiments described in this example employ fluorescence-activated cell sorting (FACS) analysis to demonstrate that the present c-Met antibodies bind to cell surface c-Met molecules and induce c-Met internalization. The MKN45 cells employed constitutively express high levels of total c-Met, and exhibit HGF-independent phosphorylation of c-Met as a result of *MET* gene amplification. c-Met internalization appears to induce c-Met degradation (see Examples 10 and 19), resulting in inhibition of the c-Met signaling pathway.

Wells of 6-well tissue culture plates (Costar, #3598) are seeded with 1.5 x 10^5 human gastric tumor MKN45 cells (Japan Health Sciences Foundation, Health Science Research Resource Bank, #JCRB0254) in 2 mL of culture medium (RPMI-1640 (Invitrogen, #11835); 10% (v/v) FBS (Invitrogen, #10082); 2 mM L-glutamine (Invitrogen, #25030); 100 U/500mL penicillin G, and 100 µg/500 mL streptomycin (Invitrogen, #15140)). The plates are incubated for 24 hours at 37°C under 95% relative humidity and 5% (v/v) CO_2. Antibodies are then added to the wells at a final concentration of 5 µg/mL. After overnight treatment, the culture medium is removed from the wells and replaced with 1 mL of enzyme-free cell dissociation solution (Chemicon, #S-014-B). The cells are collected into centrifuge tubes after being incubated for 5 min. at room temperature, and washed once in culture medium followed by one more wash in binding buffer (DPBS with 1% (w/v) BSA and 0.01% (w/v) sodium azide). Before staining cells, a c-Met antibody that recognizes a different epitope from the present c-Met antibodies is labeled by using an Alexa Fluor 488 Monoclonal Antibody Labeling Kit (Molecular Probes, Eugene, OR, #A-20181) according to the supplier's instructions. 100 µL of binding buffer containing 2 µg/mL of the Alexa Fluor 488-labeled antibody are added to the cells, which are then incubated for 60 min. on ice. The cells are then washed once with binding buffer and resuspended in DPBS containing 2 µg/mL propidium iodide (to stain the dead cells). The amount of c-Met molecules remaining on the cell surface is analyzed by FACS analysis, and 10,000 events are acquired for each sample.

The mean fluorescence intensity on the cell surface reflects the quantity of c-Met molecules that remain on the cell surface after treatment with c-Met antibodies.

The data from one representative experiment are shown in Table 10.

Table 10

Effect of C8- and D11- Antibodies on c-Met Internalization as Determined by FACS Analysis

	Mean Fluorescence									
Antibody (ug/mL)	hIgG2	D11-8B8	D11-C27G3	D11-S17Y	hIgG4	C8-6	C8-H241	C8-co16	mIgG	5D5
5.00	130.25	44.66	51.09	56.35	135.03	67.88	57.63	56.16	136.47	92.87

The data demonstrate that the mean fluorescence intensity on the surface of cells treated with the present c-Met antibodies is lower than that in cells treated with a corresponding IgG isotype control antibody or mIgG antibody, indicating that the present c-Met antibodies induce c-Met internalization. Moreover, the mean fluorescence intensity on the surface of the cells treated with the present c-Met antibodies is lower than that in cells treated with control agonist c-Met antibody 5D5. % internalization may be calculated as follows

% internalization = [1- (mean fluorescence of test antibody divided by mean fluorescence of isotype antibody control)] multiplied by 100].

The internalization data suggest that the present antibodies induce dimerization of human c-Met. The data in Examples 9 and 19 demonstrate that C8- and D11- antibodies also induce degradation, and reduce phosphorylation, of c-Met.

Complementary internalization results are obtained by confocal microscopy using fluorescently labeled murine C8 antibody in MKN45 and Caki-1 cells.

Using C8-H241, C8-6, C8-co-16, and murine D11 antibodies, similar internalization results are obtained in cynomolgous monkey NIH3T3 cells transfected with nucleic acid encoding cynomolgus monkey c-Met.

C8-H241, C8-6, C8-co-16, and optD11 antibodies also induce internalization of c-Met in NIH3T3 cells transfected with nucleic acid encoding human c-Met kinase domain mutation M1149T. Antibodies C8-H241, C8-6, C8-co-16, and murine D11 also induce internalization of c-Met in non-small cell lung cancer H1437 cells containing c-Met juxtamembrane domain mutation R988C. Both mutations cause gain-of-function constitutive activation of the c-Met.

Example 9

Antibody Inhibition of Ligand-Independent Tumor Cell Proliferation *In Vitro*

The inhibition of tumor cell growth *in vitro* by c-Met antibodies in HGF-independent MKN45 cells in the absence of HGF ligand is examined in this assay.

c-Met and isotype control antibodies are diluted with culture medium (RPMI-1640 (Invitrogen, #11835), 10% (v/v) FBS, 2 mM L-glutamine (Invitrogen, #25030), 100 U/500mL penicillin G, and 100 µg/500 mL streptomycin (Invitrogen, #15140)) to achieve 2X the final concentrations indicated in Table 11, and 50 µL of the 2X antibody solutions are added to each well of 96-well tissue culture plates (PerkinElmer#1450-517). MKN45 cells (Japan Health Sciences Foundation, Health Science Research Resource Bank, #JCRB0254) are maintained in the medium indicated above, and are resuspended to $1x10^5$ cells/mL in the same medium. 50 µL of this MKN45 resuspension are added to each well to achieve $5x10^3$ cells/well. The plates are then incubated for 48 hours at 37°C under 95% relative humidity and 5% (v/v) CO_2. For the last six hours of the culture, the cells are pulsed with ^{3}H-thymidine (MP Biomedicals, Solon, OH #24066) at 1 µci/well at 37°C, 95% relative humidity, 5% (v/v) CO_2. The medium is then removed and the cells are washed once with DPBS. After this, 200 µL of Optiphase Supermix (PerkinElmer, #1200-439) are added to each well. The plates are sealed and incubated for at least one hour at room temperature. ^{3}H-thymidine incorporated in the cells is counted for one min. using a scintillation counter.

The data from one representative experiment are shown in Table 11.

Table 11
Inhibition of ^{3}H-Thymidine Incorporation in MKN45 Cells by Various C8- and D11- Antibodies

	AVG CPM									
Antibody dose (ng/mL)	hIgG2	D11-8B8	D11-27G3	D11-S17Y	hIgG4	C8-co16	C8-H241	C8-6	mIgG	5D5
20000.0	45054	11239	18407	19752	50628	11738	11425	16387	52448	35086
3333.3	49384	11441	16920	21239	54026	12956	12063	18216	53670	38195
555.6	51720	11925	15987	20936	54204	13476	13217	18334	54655	39496
92.6	47562	11094	14550	21911	54264	12419	11962	23041	52241	39607
15.4	50488	24402	28685	39695	53806	22528	23150	44771	51329	49766
2.6	48491	44741	47034	49868	54765	53465	48761	54598	55463	54967
0.4	46468	43334	43998	45304	55330	47485	46645	53389	51549	49563
0.1	44822	41578	41515	44566	53856	45725	44418	51668	47709	51883
0.0	50427	50427	44213	44213	48708	51478	51478	48708	50300	50300
	STDErr									
20000.0	2927	114	1265	1206	2491	262	654	282	1845	764
3333.3	2462	732	641	689	1421	388	386	314	1310	1491
555.6	2166	605	578	267	4364	281	341	1116	1192	534
92.6	1835	22	150	564	1733	352	636	475	1036	370
15.4	2144	2024	941	463	1376	207	1771	422	1281	968
2.6	2587	1914	1133	1910	1978	2164	1945	444	919	2577
0.4	2041	650	1177	2551	1501	378	2392	162	438	1943
0.1	1628	1734	1817	2402	678	1340	2442	1589	2092	3143
0.0	1203	1203	841	841	1377	886	886	1377	777	777

Abbreviations: AVG = average; CPM = count per minute; STDErr = Standard Error

These data demonstrate that various C8- and D11- c-Met antibodies of the present invention inhibit HGF-independent MKN45 cell proliferation as evidenced by a reduction in ^{3}H-thymidine incorporation. Similar results are obtained in SNU5 and NUGC-4 tumor cells, which each exhibit constitutive overexpression and phosphorylation of c-Met.

Example 10

Reduction of Phosphorylated and Total c-Met, and Lack of Shedding of c-Met Extracellular Domain, in Response to c-Met Antibodies

This example investigates whether treatment of MKN45 cells with c-Met antibodies of the present invention results in reduction of phosphorylated c-Met (p-Met) and total c-Met. In addition, this assay is used to determine if c-Met antibody treatment induces the shedding of c-Met ECD into MKN45-conditioned medium.

c-Met and isotype control antibodies are diluted with culture medium (RPMI-1640 (Invitrogen, #11835), 10% (v/v) FBS (Invitrogen, #10082), 2 mM L-glutamine (Invitrogen, #25030), 100 U/500mL penicillin G, and 100 µg/500 mL streptomycin (Invitrogen, #15140)) to achieve 2X the final concentrations indicated in Table 12, and 50 µL of the 2X antibody solutions are added to each well of 96-well tissue culture plates (Costar, #3596). MKN45 cells (Japan Health Sciences Foundation, Health Science Research Resource Bank, #JCRB0254) are maintained in the medium indicated above and are resuspended to $1x10^5$ cells/mL in the same medium. 50 µL of this MKN45 resuspension are added to each well to achieve $5x10^3$ cells/well. The plates are then incubated for 24 hours at 37°C under 95% relative humidity and 5% (v/v) CO_2, and cell lysates are prepared as described in Example 7. In addition, the conditioned medium from each treatment is collected for c-Met-ECD quantitation. p-Met and total c-Met levels in the cell lysates are determined by ELISA, and normalized to lysate protein concentration (as determined by BCA, Pierce #23225).

Phosphorylated c-Met

Phosphorylation of c-Met at tyrosine residues 1230, 1234, and 1235 is determined as described in Example 7.

Total c-Met and c-Met ECD Shedding

For the total c-Met and c-Met ECD ELISAs, a c-Met capture antibody is diluted in coating buffer (BioFX, Glendora, CA, COAT-1000-01) to 2 µg/mL. 110 µL of the diluted antibody are added per well to ELISA plates (Greiner Bio-One, Monroe, NC, #655081) and the plates are incubated overnight at 4°C. The wells are aspirated, washed twice with TBS-T, and then blocked with 200 µL of blocking buffer (TBS-T plus 2% (w/v) BSA) for 1 hour at room temperature. The plates are then washed twice with TBS-T. Next, MKN45 cell lysates, MKN45 conditioned medium, or c-Met extracellular domain (ECD) (SEQ ID NO:75) (as a standard), are added, and the plates are incubated for 2 hours at room temperature. The plates are then washed four times with TBS-T. 100 µl of 0.5 µg/mL of a biotinylated second c-Met antibody (Mab 5D5) that binds a different c-Met epitope from the capture antibody diluted in blocking buffer are then added to each well, and the plates are incubated for 2 hours at room temperature. Next, the plates are

washed four times with TBS-T. 100 µL of 1/12,000 diluted peroxidase-conjugated streptavidin (Jackson ImmunoResearch Laboratories, West Grove, PA, #016-030-084) in blocking buffer are then added, and the mixture is incubated for 1 hour at room temperature. The plates are then washed six times with TBS-T. 100 µL of 3,3′,5,5′-tetramethylbenzidine solution (BioFX, #TMBW-1000-01) are added to each well, followed by the addition of 100 µL stop solution (BioFX, #LSTP-1000-01). The plates are read at 450 nm with a 570 nm correction using a SpectraMax 190 plate reader (Molecular Devices, Sunnyvale, CA). The standard curve is established using 4-parameter analysis, and sample values are determined using SOFTmax Pro 3.1.2 software (Molecular Devices).

As shown in Table 12, the p-Met and total c-Met ELISA data reveal that Mab C8-H241 treatment maximally reduces p-Met by 77% and total c-Met by approximately 67%. D11-8B8 treatment maximally reduces p-Met by approximately 75% and total c-Met by 63%.

As noted in Example 8, these data demonstrate that C8- and D11- antibodies induce c-Met degradation and reduce phosphorylation of c-Met.

The data in Table 12 also indicate that treatment with C8-H241 and D11-8B8 c-Met antibodies does not induce cleavage and shedding of the c-Met ECD.

Table 12

Effect of C8- and D11- Antibodies on Phosphorylated c-Met, Total c-Met, and Shedding of c-Met Extracellular Domain in MKN45 Cell Lysates and Conditioned Medium

Percent Inhibition of MKN45 Phosphorylated c-Met

	C8-H241		hIgG4		D11-8B8		hIgG2	
Mab Dose (ng/mL)	ave	stdev	ave	stdev	ave	stdev	ave	stdev
10000	77.0	2.4	-2.1	10.9	74.8	3.4	14.0	7.1
1000	74.1	1.3	11.1	9.7	72.8	2.0	-4.6	10.9
100	71.7	2.5	13.4	13.3	69.1	3.7	-7.6	24.4
10	42.3	3.0	5.8	11.3	37.6	5.5	6.1	11.0
	ave	stdev						
untreated	0.0	8.0						

Percent Inhibition of MKN45 Total c-Met

	C8-H241		hIgG4		D11-8B8		hIgG2	
Mab Dose (ng/mL)	ave	stdev	ave	stdev	ave	stdev	ave	stdev
10000	63.1	1.6	-23.7	4.1	63.0	7.0	14.1	9.1
1000	66.7	4.3	10.4	16.0	62.7	0.6	-2.4	22.4
100	61.5	3.4	-3.7	14.4	62.9	2.5	7.3	9.1
10	32.3	4.9	-3.4	13.5	34.5	8.4	15.1	7.0
	ave	stdev						
untreated	0.0	25.9						

Level of c-Met ECD in MKN45-Conditioned Medium (ng/mL)

	C8-H241		hIgG4		D11-8B8		hIgG2	
Mab Dose (ng/mL)	ave	stdev	ave	stdev	ave	stdev	ave	stdev
10000	5.1	0.5	7.4	0.7	6.2	0.2	7.0	0.1
1000	6.1	0.8	7.6	0.4	7.2	0.5	7.2	0.5
100	5.4	0.4	7.6	0.8	6.9	1.6	7.2	0.6
10	6.6	0.2	8.4	1.1	6.2	0.4	7.2	0.1
	ave	stdev						
untreated	8.3	1.0						

Example 11

Agonist Activity of Antibodies in Caki-1 Tumor Cells in the Absence of HGF

Caki-1 renal carcinoma cells proliferate in response to HGF. The activation of c-Met in Caki-1 cells by c-Met antibodies in the absence of HGF is examined in this example to assess the agonist activity of c-Met antibodies of the present invention.

Wells of 96-well tissue culture plates are seeded with 5,000 human kidney clear cell carcinoma Caki-1 cells (ATCC, #HTB-46) in McCoy's 5A culture medium (Invitrogen, #16600) supplemented with 10% (v/v) FBS, 2 mM L-glutamine (Invitrogen, #25030), 100 U/500mL penicillin G and 100 µg/500 mL streptomycin (Invitrogen, #15140). After culture for 24 hours, cells are starved in low serum medium (0.5% (v/v) FBS) for another 24 hours. The cells are then cultured in the presence of anti-c-Met and control antibodies in low serum medium at final concentrations as indicated in Table 13 for 24 hours at 37°C, 95% relative humidity, 5% (v/v) CO_2. For the last six hours of the culture, the cells are pulsed with ^{3}H-thymidine (MP Biomedicals, Solon, OH #24066) at 1 µci/well at 37°C, 95% relative humidity, 5% (v/v) CO_2. Next, the medium is removed

and the cells are washed once with DPBS. After this, 200 µL of Optiphase Supermix (PerkinElmer, #1200-439) are added to each well. The plates are then sealed and incubated for at least one hour at room temperature. ^{3}H-thymidine incorporated in the cells is counted for one min. using a scintillation counter.

The data from one representative experiment are shown in Table 13.

Table 13

Effect of C8- and D11- Antibodies on ^{3}H-Thymidine Incorporation in Caki-1 Cells in the Absence of HGF

	AVG CPM									
Antibody dose (ng/mL)	hIgG2	D11-8B8	D11-27G3	D11-S17Y	hIgG4	C8-co16	C8-H241	C8-6	mIgG	5D5
20000.0	14658	15866	16054	18616	13704	13112	12797	13194	14224	19893
3333.3	14034	15730	15765	17897	13023	13829	12702	13193	13469	19018
555.6	14048	13997	14536	16620	12393	11359	12116	13494	13200	20043
92.6	14113	13705	14718	15342	12934	11563	12142	12793	13761	15588
15.4	14473	13488	13836	14579	13271	12111	13020	13670	13638	11748
2.6	15517	14097	13325	14867	13858	13713	14407	14126	13766	12520
0.4	14341	14411	13596	14618	13412	14080	14142	14601	14357	12896
0.1	16947	15319	17690	15899	13547	15567	15530	16121	13797	14383
0.0	14992	14992	15237	15237	14622	13889	14622	13889	14531	14531
	STDErr									
20000.0	398	737	549	345	219	642	268	96	465	807
3333.3	358	959	538	466	84	1086	380	382	927	954
555.6	343	809	705	284	478	437	216	4	397	505
92.6	428	502	728	237	447	292	445	212	706	394
15.4	232	737	729	160	487	267	305	107	514	318
2.6	386	173	295	404	339	299	291	711	91	221
0.4	357	568	508	392	317	556	656	281	331	323
0.1	262	550	1108	326	381	601	583	536	229	145
0.0	310	310	394	394	364	554	364	554	238	238

Abbreviations: AVG = average; CPM = count per minute; STDErr = Standard Error

These data demonstrate that c-Met antibodies C8-H241, C8-6, C8-co-16, and D11-8B8 do not significantly increase the uptake of thymidine-[methyl-^{3}H] in Caki-1 cells compared to that of the IgG isotype controls. D11-C27G3 and D11-S17Y exhibit a low, variable, but statistically significant stimulation of thymidine-[methyl-^{3}H] uptake in Caki-1 cells compared to that of the IgG isotype control. The control c-Met agonist antibody 5D5 induces stronger thymidine-[methyl-^{3}H] uptake than that of the present

c-Met antibodies in Caki-1 cells under the same experimental conditions.

Example 12

Agonist Activity of Antibodies in Primary Human Hepatocytes in the Absence of HGF

The agonist activity of the present c-Met antibodies is further assessed in primary human hepatocytes (PHH), which are HGF-responsive, in the absence of HGF.

Cryopreserved, plateable PHH cells (KQG Celsis, Chicago, IL, RD#00002) are thawed at 37°C and resuspended to 175,000 cells/mL in InVitroGRO CP medium (Celsis, #Z99029) with torpedo antibiotic mix (Celsis, #Z99000). 0.2 mL of resuspended PHH cells are added per well to collagen I coated 96-well microtiter plates (BD, Franklin Lakes, NJ, #354407) at 35,000 cells/well, and the cells are incubated for 24 hours at 37°C, 5% (v/v) CO_2. The culture medium is then aspirated and 150 µL of InVitroGRO HI medium (Celsis, #Z99009) with torpedo antibiotic mix plus 0.1% (w/v) BSA are added per well, plus 50 µL of c-Met and control antibodies in a final concentration range of 10 µg/mL to 0.0032 µg/mL, or HGF at a final concentration of 200 ng/mL, in InVitroGRO HI medium with torpedo antibiotic mix plus 0.1% (w/v) BSA. Cells are incubated for 48 hours at 37°C, 5% (v/v) CO_2, and 10 µL of 0.1 mCi thymidine-[methyl-^{3}H]/mL (MP Biomedicals, Solon, OH, #24066) are added per well for the last 6 hours of incubation. Assay plates are frozen at -70°C, thawed at 37°C, and harvested onto UniFilter-96, GF/C plates (Perkin Elmer, Waltham, MA, #6005174) using a Filtermate Harvester (Perkin Elmer). The UniFilter plates are dried, 20 µL of Microscint 0 scintillant (Perkin Elmer, #6013611) are added per well, and plates are counted on a 1450 Microbeta liquid scintillation counter (Perkin Elmer).

Table 14 shows the average of triplicate treatments with standard deviations, and is representative of three repeat experiments.

Table 14

Effect of Various C8- and D11- Antibodies on ^{3}H-Thymidine Incorporation in Primary Human Hepatocytes in the Absence of HGF

		Doses: 10 μg/mL		2 μg/mL		0.4 μg/mL		0.08 μg/mL		0.016 μg/mL		0.0032 μg/mL	
Ab treatment	isotype	average	stdev	average	stdev	average	stdev	average	stdev	average	stdev	average	stdev
D11-8B8	IgG2	1703.7	140.1	1427.0	120.3	1231.0	232.4	1122.7	188.3	715.3	25.8	611.0	61.5
D11-C27G3	IgG2	2145.7	171.0	1874.0	443.4	1753.0	199.8	1283.3	131.6	892.0	109.3	692.7	23.0
D11-S17Y	IgG2	3155.0	594.2	2566.0	173.1	1911.0	348.3	1458.7	132.7	919.7	47.5	726.3	131.7
C8-H241	IgG4	671.0	61.0	710.3	81.8	681.3	13.7	669.3	77.0	630.0	37.2	625.0	65.5
C8-co-16	IgG4	952.0	36.0	822.0	88.4	670.0	23.3	767.3	12.6	715.7	12.5	828.7	85.2
C8-6	IgG4	1042.3	91.3	892.3	107.0	801.7	77.4	792.3	48.4	769.7	109.9	736.3	43.1
5D5	mIgG1	4978.0	59.9	4912.3	287.7	3763.7	292.1	3320.7	40.1	1716.3	324.1	821.7	175.3
hIgG2	isotype control	650.0	39.4	643.7	11.7	711.7	16.6	836.0	61.0	748.3	57.7	799.7	80.3
hIgG4	isotype control	647.3	77.1	735.0	33.8	717.3	19.4	819.0	44.2	848.3	75.1	806.7	79.7
mIgG1	isotype control	616.7	24.8	581.0	81.8	601.0	82.0	596.0	78.6	588.7	43.0	675.0	73.0

		ave	stdev
HGF	200 ng/mL	6292.7	733.0
untreated		615.1	83.9

The data demonstrate that compared with the IgG isotype controls, present c-Met antibodies C8-H241 does not significantly increase thymidine-[methyl-^{3}H] uptake in PHH cells; C8-6, C8-co-16, D11-8B8, D11-C27G3, and D11-S17Y exhibit a low, variable, but statistically significant stimulation of thymidine-[methyl-^{3}H] uptake. However, the agonist activity of the present c-Met antibodies is significantly lower than that of the 5D5 control c-Met antibody. Agonist antibody 5D5 stimulates PHH proliferation in a dose-dependent manner, with a 5-fold increase at a concentration of 3 μg/mL. At 200 ng/mL, HGF stimulates a 5-fold increase in ^{3}H-thymidine uptake. Mab C8-H241 does not induce proliferation even when used at 10 μg/mL.

Similar results are obtained in human renal epithelial HK2 cells, which also proliferate in response to HGF stimulation.

Example 13

Effect of Antibodies on Tubular Morphogenesis in HepG2 Cells in the Absence of HGF

HGF induces tubular morphologenic changes in HepG2 cells grown in Matrigel™ (Becton-Dickinson, #354234), an extracellular matrix material containing components of the basement membrane. In this experiment, the HGF-like agonist activity of antibodies of the present invention in inducing tubular morphologenic changes in HepG2 cells is assessed.

HepG2 cells (ATCC, #HB-8065) are cultured in DMEM supplemented with 10% FBS. 100 µL of a Matrigel™ solution (Matrigel™, Becton-Dickinson) diluted in Opi-MEMI (Invitrogen, #31985) supplemented with 10% (v/v) FBS, 2 mM L-glutamine (Invitrogen, #25030), 100 U/500mL penicillin G and 100 µg/500 mL streptomycin (Invitrogen, #15140) are plated in wells of 96-well tissue culture plates (Costar, #3596). After the Matigel™ solution solidifies, 2000 HepG2 cells in 50 µL of culture medium supplemented with 10% serum are added. Next, c-Met and control antibodies at a final concentration of 50 µg/mL, or HGF at a final concentration of 50 ng/mL, are added to the cells. The cells are grown for 4 days at 37°C in a humidified atmosphere containing 5% (v/v) CO_2. After 4 days, the top medium is removed and replaced with 50 µL of 1 mg/mL p-Iodonitro-tetrazolium violet (Sigma, #I8377) in PBS, and the cells are incubated for another 48 hours under the same conditions. Photographs are taken of the stained 32 mm area, and analyzed using Image-Pro Plus 6 (Media Cybernetics, Inc., MD).

The data from one representative experiment, for antibodies at 50 µg/mL, are shown in Table 15.

Table 15
Effect of C8- Antibodies on Tubular Morphogenesis in HepG2 Cells

Antibody (50ug/ml)	hIgG4	C8-H241	C8-co16	C8-6	mIgG	5D5	HGF (50ng/ml)
Tubular Morphogenesis	no stimulation	no stimulation	no stimulation	1.2	1.2	4.9	5.4
Agonist activity							
(fold stimulation)							

These data demonstrate that HGF and control agonist antibody 5D5 induce approximately 5-fold tubular morphologenic changes in HepG2 cells as compared to the isotype control. In contrast, present c-Met antibodies C8-H241 and C8-co16 do not induce significant tubular morphologenic changes in HepG2 cells under the same conditions, while c-Met antibody C8-6 induces only a low level of stimulation.

Similar results are obtained with Mab D11-8-B8.

Example 14

Effect of Antibodies on Cell Motility:

DU145 Scatter Assay and H441 Cell Scratch Assay

Upon stimulation with HGF, DU145 prostate cancer cells dissociate from each other and H441 cells fill in a scratch made in a confluent cell layer. H441 cells exhibit a high level of c-Met expression and constitutive phosphorylation of this receptor, but are still HGF-responsive. The following experiments assess the agonist effect of antibodies of the present invention on cell motility in a scatter assay and a scratch assay.

DU145 Cell Scatter Assay

DU145 cells (ATCC, # HTB-81) grown in MEM medium (Invitrogen, #11095) + 10% FBS (Invitrogen, #10082) at 37°C under 5% (v/v) CO_2 are plated at 2 x 10^3 cells/well in 70 µL volume in black ViewPlate 96-well plates (Perkin Elmer, Waltham, MA, #6005182) and incubated overnight at 37°C under 5% (v/v) CO_2. c-Met and control antibodies are diluted in the cell culture medium and added at a final concentration of 20 µg/mL, and HGF is added at a final concentration of 20 ng/mL, each in 30 µL volume with twelve replicates, and incubated for 48 hours at 37°C under 5% (v/v) CO_2. The medium is then aspirated and cells are fixed in 2% formaldehyde for 15 min. at room temperature. Wells are washed three times with PBS, and 50 µL of 5 U/mL Alexa Fluor 488 phalloidin (Invitrogen, #A12379) are added for 30 min. at room temperature. Wells are washed three times with PBS, and 50 µL of 15 µM propidium iodide (Invitrogen, #P3566) are added. The plate is subsequently read on an Acumen Explorer™ laser-scanning fluorescence microplate cytometer (TTP Labtech Ltd, Cambridge, MA) using Jockyss software in order to determine the percent of DU145 cells in colonies.

The results are shown in Table 16.

Table 16

Effect of C8- and D11- Antibodies on DU145 Cell Scattering

	Percent of DU145 Cells in Colonies								
	C8-H241	**C8-6**	**C8-co-16**	**hIgG4**	**D11-8B8**	**hIgG2**	**5D5**	**HGF**	**Untreated**
Average	24.13	23.29	23.69	24.41	25.57	25.67	14.45	8.15	26.53
Std. Dev.	3.35	1.37	2.30	2.02	1.98	3.13	0.34	1.23	2.44

The data demonstrate that agonist c-Met Mab 5D5 and HGF, but not c-Met Mabs C8-H241, C8-co-16, C8-6, or D11-8B8, significantly stimulate DU145 cell scattering/motility.

H441 Cell Scratch Assay

For the H441 Scratch assay, H441 cells (ATCC, # HTB-174) are grown in RPMI-1640 (Invitrogen, #11835); 10% (v/v) FBS (Invitrogen, #10082); 2 mM L-glutamine (Invitrogen, #25030); 100 U/500mL penicillin G, and 100 µg/500 mL streptomycin (Invitrogen, #15140)), and seeded at $1x10^6$ cells/2mL/well in wells of 6-well tissue culture plates (Costar, #3598) in the culture medium. The plates are incubated for 3 days under 95% relative humidity and 5% (v/v) CO_2. The medium is then aspirated, and the cells are starved in low serum medium (0.5% (v/v) FBS in RPMI medium) for 16 hours. The confluent cell layers on the bottom of the wells are scratched with 5 mL pipette tips in the middle of each well, and floating cells are aspirated. The remaining cells are washed 1X with low serum medium. Low serum medium is added, and the scratched areas are imaged using a bright field microscope with a 4X objective. These gaps are defined as Gaps at 0 hours.

The testing antibodies are added to the cells at a final concentration of 10 µg/mL, followed by incubation at 37°C under 5% CO_2 (v/v) for 16 hours. HGF is tested at a final concentration of 200 ng/mL. Each treatment group is tested at least in duplicate wells. The scratched areas are imaged again using a bright field microscope at 16 hours. These gaps are defined as Gaps at 16 hours.

The effect of c-Met antibodies or HGF on the movement of H441 cells to fill the gaps are calculated as follows:

$$\textbf{Average percentage change} = \frac{\text{Treatment group (Gap at 0 hour – Gap at 16 hours)}}{\text{Average medium group (Gap at 0 hour – Gap at 16 hours)}} \times 100$$

The results are shown in Table 17.

Table 17
Effect of C8- antibodies in H441 Scratch Assay

Antibody (10 µg/mL)	Avg. %	St. Dev.
Medium	100	7
hIgG4	98	9
C8-H241	98	9
C8-6	102	4
mIgG1	98	18
Mab 5D5	244	4
HGF (200 ng/mL)	364	9

The data demonstrate that agonist c-Met Mab 5D5 and HGF stimulate movement of H441 cells/filling in of the scratched areas. Under the same conditions, c-Met Mabs C8-H241 and C8-6 do not stimulate H441 cell motility.

Example 15

Effect of c-Met Antibodies on HepG2 Cell Invasiveness

HGF and agonist c-Met antibodies stimulate invasion of c-Met bearing cells. This example examines the agonist activity of the present c-Met antibodies in a cell invasion assay employing HepG2 cells, which are HGF-responsive in an invasion assay.

HepG2 cells (ATCC, #HB-8065) are starved overnight in serum-free MEM medium (Invitrogen, #11095) and then 5 x 10^4 cells in a total volume of 500 µL are added to each well of the top chamber of a matrigel invasion chamber (BD, Franklin Lakes, NJ, #354483), with the bottom chamber containing antibodies in a total volume of 750 µL of serum-free medium at a concentration of 10 µg/mL, or HGF at 50 ng/mL in serum-free medium, followed by incubation for forty-eight hours at 37°C under 5% (v/v) CO_2. Non-invading cells are removed from the top chamber with a swab, followed by membrane fixation with 95% ethanol and staining with 0.2% (w/v) crystal violet. After washing and drying, the number of invading cells is counted using Image-Pro Plus 6 Manual Tag (Media Cybernetics, Inc., MD) software analysis of photographs taken of stained cells with a 2.5X objective.

The results are summarized in Table 18.

Table 18

Effect of C8-, C8, and D11 Antibodies on HepG2 Cell Invasiveness

Antibody Concentration (µg/mL)	Average Cell Number Per 2.5x Field							
	hIgG4	C8-H241	C8	mIgG1	optD11	5D5	HGF (50 ng/mL)	Medium
10.00	5.5	3.5	4.3	5.5	19.8	85.0	509.5	3.5
	Std. Err.							
	0.5	0.9	1.3	0.5	4.1	20.4	4.5	0.5

The data demonstrate that agonist c-Met Mab 5D5 and HGF, but not c-Met Mabs C8-H241 and (murine) C8, stimulate HepG2 invasion. Murine c-Met Mab optD11 weakly induces HepG2 cell invasion.

Example 16

C8- and D11- Antibodies Do Not Protect Caki-1 Cells from Staurosporine-Induced Apoptosis

HGF and agonist c-Met antibodies protect cells from staurosporine-induced cell death. This example examines the agonist activity of c-Met antibodies of the present invention in a staurosporine-induced apoptosis assay employing HGF-responsive Caki-1 cells.

Caki-1 cells (ATCC, #HTB-46) are grown as described in Example 11, seeded at 1 x 10^4 cells/well in 96 well plates (Costar, #3596) in culture medium, and pre-treated with antibodies (diluted from 30000 ng/mL to 3 ng/mL in the cell culture medium), or HGF (diluted from 225 ng/mL to 0.02 ng/mL), for one hour followed by treatment with 0.1 μM staurosporine (final concentration) for forty-eight hours at 37° C. The medium is aspirated and the cells are lysed for 30 min. with 0.2 mL of the lysis buffer component of the Cell Death Detection ELISA kit (Roche Applied Science, Indianapolis, IN, #11774425001). This kit utilizes 20 μL of each lysate to measure cell death by detection of cytoplasmic histone-associated DNA fragments as determined by the absorption at 450 nm. A higher optical density at 450 nm indicates greater apoptosis.

The results shown in Table 19 demonstrate that HGF, but not c-Met Mabs C8-H241, C8-6, and D11-8B8, protects Caki-1 cells from staurosporine-induced apoptosis.

Table 19

Effect of c-Met Antibodies on Staurosporine-Induced Apoptosis in Caki-1 Cells

Antibody dose (ng/mL)	Average A450 nm							
	hIgG4	C8-H241	C8-6	HGF	hIgG2	D11-8B8	Medium	STS*
3	0.95	0.83	1.09	0.97 (0.02 ng/mL)	0.95	1.02	0.24	0.87
30	0.98	0.84	1.02	0.93 (0.23 ng/mL)	0.91	0.91		
300	0.87	0.86	0.97	0.73 (2.25 ng/mL)	0.95	0.81		
3000	0.98	0.85	0.92	0.58 (22.5 ng/mL)	0.95	0.91		
30000	0.91	0.90	0.96	0.45 (225 ng/mL)	0.94	0.87		
	STDErr							
3	0.03	0.02	0.19	0.02	0.07	0.01	0.01	0.03
30	0.03	0.01	0.14	0.06	0.00	0.11		
300	0.10	0.03	0.06	0.03	0.02	0.02		
3000	0.04	0.01	0.12	0.01	0.06	0.02		
30000	0.00	0.01	0.13	0.06	0.04	0.03		

*STS: Staurosporine

Example 17

Effect of C8- Antibodies on Angiogenesis

HGF and agonist c-Met antibodies stimulate angiogenesis. c-Met antibodies of the present invention are evaluated for this functional agonist property in the ADSC/ECFC co-culture tube formation assay. Adipose-derived stem cells (ADSC) express HGF; endothelial colony forming cells (ECFC) form tubes in response to stimulation by HGF.

ADCS (Lonza, Allendale, NJ, # PT-5006) are dissociated, resuspended in basal medium (MCDB-131 medium (Sigma, St. Louis, MO #M8537)) + 30 µg/mL L-ascorbic acid 2-phosphate (Sigma #A8960), 1 µM dexamethasone (Sigma #D4902), 50 µg/mL tobramycin (Sigma #T4014), 10 µg/mL Cell Prime r-transferrin AF (Millipore #9701) + 10 µg/mL Nucellin (Lilly human recombinant insulin)), plated at 4 x 10^4 cells/well in 96-well plates, and incubated overnight at 37°C under 5% (v/v) CO_2. The medium is aspirated and 500 µg/mL sodium heparin (Sigma, #H3393) are added in basal medium at 100 µL/well; the cells are then incubated for 1 hour at 37°C. The wells are aspirated, washed once with 100 µL basal medium, and ECFC are added as follows: ECFC

(EndGenitor Technologies, Inc., Indianapolis, IN #EGT-ECFC100506) are dissociated, washed in basal medium, resuspended in basal medium, and added at 4 x 10^3 cells/well on top of ADSC in 96-well plates. After 4 hours incubation at 37ºC, HGF and antibodies are diluted in the cell culture medium and added to separate wells at the following final concentrations: HGF:100 ng/mL; antibodies: 10 µg/mL. The HGF antibody (R&D Systems #AB-294-NA) is also added at 10 µg/mL final concentration. The cells are incubated for an additional 4 days at 37ºC. The wells are aspirated, and 100 µL/well of 1% paraformaldehyde are added, followed by incubation for 20-30 min. Cells are washed three times with PBS-BSA (0.1% BSA, Invitrogen #15260-037) and treated with 50 µL of 1 µg/mL anti-human CD31 antibody (R&D Systems, #AF806) for 1 hour at 37˚C or overnight at 4˚C. Cells are washed twice with PBS-BSA and treated with 50 µL of 4 µg/mL anti-sheep IgG AlexaFluor488 conjugate (Invitrogen, #A11015) for 1 hour at room temperature. Cells are washed twice with PBS-BSA and stained with 100 µL of Hoechst3342 dye and read on a Cellomics ArrayScan (Thermo Fisher Scientific, Waltham, MA). vHCS View Version 1.4.6 software is used to determine total tube areas, which are used to evaluate the effect of the various antibodies and HGF on stimulation of angiogenesis.

The results are shown in Table 20.

Table 20

Effect of C8- Antibodies on Tube Formation in ECFC Cells

Total Tube Area								
	Basal Medium	**hIgG4**	**C8-H241**	**C8-6**	**mIgG1**	**5D5**	**HGF Ab**	**HGF**
Avg.	**67753.3**	**90134.3**	**22979.7**	**65224.0**	**125538.3**	**147237.3**	**22824.3**	**212104.7**
Std. Dev.	**24221.6**	**17741.1**	**604.9**	**18275.9**	**34702.4**	**18748.1**	**6586.0**	**16588.5**

The results demonstrate that C8-H241 and C8-6 do not stimulate tube formation compared to the medium or their corresponding isotype control antibodies, whereas HGF and agonist Mab 5D5 significantly stimulate tube formation.

Example 18

Inhibition of HGF-Independent and HGF-Dependent Tumor Cell Growth in Xenograft Models

The inhibition of HGF-independent and HGF-dependent tumor cell growth by c-Met antibodies of the present invention is examined in *in vivo* assays employing MKN45 cell and U87MG (human glioblastoma) cell mouse xenograft models, respectively. MKN45 cells constitutively express high levels of c-Met and c-Met phosphorylation in the absence of HGF. U87MG cells secrete HGF in an autocrine manner, and are HGF-responsive.

MKN45 cells (Japan Health Sciences Foundation, Health Science Research Resource, #JCRB0254) are expanded in culture as described in Example 8, trypsinized to single cells, harvested, and resuspended in PBS. Two million MKN45 cells in PBS are injected subcutaneously into the rear flank of athymic nude mice (Harlan, Indianapolis, IN). c-Met antibodies and corresponding IgG2 and IgG4 antibodies are diluted in PBS, pH 7.2, and administered on a weekly basis by intravenous injection starting from 3 or 7 days after tumor cell implantation at 1, 5, or 20 mg/mL. Inhibition of tumor cell growth is determined by three dimensional caliper measurement of tumor volumes twice weekly during the course of treatment. Body weight is measured as a general measurement of toxicity.

U87MG cells (ATCC, #HTB-14) are grown in MEM (Invitrogen, #11095) at 37°C, expanded in culture, trypsinized to single cells, harvested, and resuspended in PBS (Invitrogen, # 14190). Five million cells are injected subcutaneously into the rear flank of athymic nude mice (Harlan, Indianapolis, IN). c-Met antibodies are diluted in PBS, and administered on a weekly basis by intravenous injection at the doses indicated in Table 22 starting 7 days after tumor cell implantation. Control IgG4 antibody is administered at 10 mg/kg. Inhibition of tumor cell growth is determined by three dimensional caliper measurement of tumor volumes twice weekly during the course of treatment. Body weight is measured as a general measurement of toxicity.

The anti-tumor efficacy of four antibodies, D11-8B8, C8-H241, C8-6, and C8-Co-16 in the MKN45 cell xenograft model is summarized in Table 21. "Maximum %

Inhibition" represents the percent inhibition of tumor growth compared to treatment with corresponding control antibody (0% inhibition).

When dosed at 5 mg/kg or 20 mg/kg, all four c-Met antibodies produce significant inhibition of MKN45 tumor cell growth as compared to their corresponding IgG isotype controls.

Table 21

Effect of C8- and D11- Antibodies

On HGF-Independent MKN45 Tumor Growth *In Vivo*

Antibody	Dose level (mg/kg)	Maximum % Inhibition	p value
D11-8B8	5	53	p<0.05
	20	56	p<0.01
C8-H241	1	39	NS
	5	63	p<0.01
	20	51	p<0.05
C8-6	1	60	p<0.001
	5	59	p<0.01
	20	73	p<0.001
C8-Co-16	1	36	NS
	5	60	p<0.001
	20	85	p<0.001

A dose-dependent inhibition of tumor cell growth by C8-H241 is also observed in the HGF-dependent U87MG cell xenograft model, as summarized in Table 22. "Maximum % Inhibition" represents the percent inhibition of tumor growth compared to treatment with corresponding IgG4 control antibody (0% inhibition).

Table 22

Effect of C8-H241 Antibody

On HGF-Depdendent U87MG Tumor Growth *In Vivo*

Antibody	Dose level (mg/kg)	Maximum % Inhibition	p value
C8-H241	0.1	45.2	NS
C8-H241	0.3	86.8	p<0.001
C8-H241	1	91.9	p<0.001
C8-H241	3	91	p<0.001
C8-H241	10	94.8	p<0.001

At 5 and 20 mg/kg, C8-H241 antibody also inhibits H441 non-small cell lung cancer xenograft tumor growth 58% and 60%, respectively. H441 cells exhibit a high level of c-Met expression and constitutive phosphorylation of c-Met, but are still responsive to HGF.

Example 19

Antibody Reduction of Total and Phosphorylated c-Met in MKN45 Xenograft Tumors

The *in vivo* activity of c-Met antibody C8-H241 on total c-Met and phosphorylated c-Met in mice bearing MKN45 (HGF-independent) xenograft tumors is investigated in this example. A dose-dependent reduction of both total c-Met and phosphorylated c-Met (at tyrosine 1349) is observed 24 hours after antibody administration.

MKN45 cells are expanded in culture as described in Example 8, trypsinized, and harvested. Two million MKN45 cells in PBS are injected subcutaneously into the rear flank of athymic nude mice (Harlan, Indianapolis, IN). c-Met antibody C8-H241 is diluted in PBS, pH 7.2, and administered by intravenous injection eight days after tumor cell implantation at 2.5, 5, 10, 20, and 40 mg/kg. Control antibody hIgG4 is administered at 40 mg/kg. After 24 hours of treatment, tumors are removed, flash frozen, stored temporarily at -80°C, and lysed in lysis buffer (5 mM ethylenediaminetetraacetic acid (EDTA), 5 mM ethyleneglycol-bis(b-aminoethyl)-N,N,N',N'-tetracetic acid (EGTA), 50 mM HEPES, 20 mM sodium pyrophosphate (ThermoFisherScientific, #S390-500), 150 mM NaCl, 20 mM NaF, 1% (v/v) octylphenoxy polyethoxy ethanol (TRITON®-X 100), complete protease inhibitor, EDTA free (Roche, Basel, Switzerland, # 1836153) phosphatase inhibitor cocktail I (Sigma #P2850), and phosphatase inhibitor cocktail II (Sigma #P5726)).

Total c-Met ELISA

For the total c-Met ELISA, a c-Met capture antibody is diluted in Bup H coating buffer (Thermo Fisher Scientific, Waltham, MA, #28382) to 2 μg/mL. 100 μL of the diluted antibody is added per well to ELISA plates (ThermoFisherScientific, Waltham, MA #439454), and the plates are incubated overnight at 4°C. The wells are aspirated,

washed twice with TBS-T, and then blocked with 200 µL of blocking buffer (TBS-T plus 2% (w/v) BSA) for 1 hour at room temperature. The plates are washed twice with TBS-T. Next, dilutions of tumor lysates or c-Met extracellular domain (amino acids 25-932 of SEQ ID NO:75) are added, and the plates are incubated overnight at 4°C. The plates are then washed three times with TBS-T. 100 µL of 0.5 µg/mL of biotinylated Mab 5D5 (as second c-Met antibody that binds a different c-Met epitope from the capture antibody) diluted in blocking buffer are then added to each well, and the plates are incubated for 2 hours at room temperature. Next, the plates are washed three times with TBS-T. 100 µL of 1/10,000 diluted peroxidase-conjugated streptavidin (Jackson ImmunoResearch Laboratories, West Grove, PA, #016-030-084) in blocking buffer are then added, and the mixture is incubated for 1 hour at room temperature. The plates are then washed three times with TBS-T. 100 µL of 3,3′,5,5′-tetramethylbenzidine solution (BioFX, #TMBW-1000-01) are added to each well, followed by the addition of 100 µL stop solution (BioFX, #LSTP-1000-01). The plates are read at 450 nm using a SpectraMax 250 plate reader (Molecular Devices, Sunnyvale, CA) with SOFTmax Pro 3.1.2 software (Molecular Devices).

Phosphorylated c-Met ELISA

For the phospho-c-Met ELISA, a c-Met capture antibody is diluted in Bup H coating buffer (Thermo Fisher Scientific, Waltham, MA, #28382) to 2 µg/mL. 100 µL of the diluted antibody are added per well to ELISA plates (ThermoFisherScientific, Waltham, MA #439454), and the plates are incubated overnight at 4°C. The wells are aspirated, washed twice with TBS-T, and then blocked with 200 µL of blocking buffer (TBS-T plus 2% (w/v) BSA) for 1 hour at room temperature. The plates are washed twice with TBS-T. Next, MKN45 cell lysates are added, and the plates are incubated overnight at room temperature. The plates are then washed three times with TBS-T. 100 µL of 0.5 µg/mL anti-pY1349 c-Met antibody (Cell Signaling Technology, Danvers, Massachusetts, #3121) diluted in blocking buffer are then added to each well, and the plates are incubated for 2 hours at room temperature. Next, the plates are washed three times with TBS-T. 100 µL of 1/10,000 diluted peroxidase conjugated anti-rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, PA, #111-035-144) in blocking

buffer are then added, and the mixture is incubated for 1 hour at room temperature. The plates are then washed three times with TBS-T. 100 µL of 3,3′,5,5′-tetramethylbenzidine solution (BioFX, #TMBW-1000-01) are added to each well, followed by the addition of 100 µL stop solution (BioFX, #LSTP-1000-01). The plates are read at 450 nm using a SpectraMax 250 plate reader (Molecular Devices, Sunnyvale, CA) with SOFTmax Pro 3.1.2 software (Molecular Devices).

The results are shown in Table 23.

The data demonstrate that *in vivo* treatment of MKN45 xenograft tumors with Mab C8-H241 for 24 hours maximally reduces total c-Met by approximately 43%, and phosphorylated c-Met by approximately 73%, under these conditions.

Table 23

Reduction of Total and Phosphorylated c-Met in MKN45 Xenograft Tumors After 24 Hour Treatment with c-Met antibody C8-H241 *In Vivo*

Reduction of Total c-Met

% Inhibition		C8-H241					hIgG4
	PBS	2.5 mpk	5 mpk	10 mpk	20 mpk	40 mpk	40 mpk
average % inhibition	-88.51	-8.43	4.26	25.07	42.85	30.62	0.00
st. dev. % inhibition	62.63	-8.43	36.84	26.88	17.67	35.95	41.23

Reduction of Phosphorylated c-Met

% Inhibition		C8-H241					hIgG4
	PBS	2.5 mpk	5 mpk	10 mpk	20 mpk	40 mpk	40 mpk
average % inhibition	12.16	22.89	17.56	53.21	67.04	73.42	0.00
st. dev. % inhibition	23.89	22.89	37.05	9.90	6.58	7.04	48.81

mpk: mg/kg

Amino Acid and Nucleotide Sequences

Light Chain Variable Region Amino Acid Sequences

D11-S17Y (SEQ ID NO:1)

EIVLTQSPGTLSLSPGERATLSCSVSSSISSTNLHWYQQKPGQAPRLLIYGTSYLAS
GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQWSSYPYSFGQGTKLEIK

D11-8B8 (SEQ ID NO:2)

EIVLTQSPGTLSLSPGERATLSCSVSSSISSTNLHWYQQKPGQAPRLLIYGTSYLAS
GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQWSSYPYSFGQGTKLEIK

D11-C27G3 (SEQ ID NO:3)

EIVLTQSPGTLSLSPGERATLSCSVSSSISSTNLHWYQQKPGQAPRLLIYGTSRLRS
GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQWSSYPYSFGQGTKLEIK

C8-6 (SEQ ID NO:4)

DIQMTQSPSSLSASVGDRVTITCSVSSSVSSIYLHWYQQKPGKAPKLLIYSTSNLAS
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCIQYSGYPLTFGGGTKVEIK

C8-H241 (SEQ ID NO:5)

DIQMTQSPSSLSASVGDRVTITCSVSSSVSSIYLHWYQQKPGKAPKLLIYSTSNLAS
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQVYSGYPLTFGGGTKVEIK

C8-co-16 (SEQ ID NO:6)

DIQMTQSPSSLSASVGDRVTITCSVSSSVRSIYLHWYQQKPGKAPKLLIYSTSNLA
SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQVYRGYPLTFGGGTKVEIK

Light Chain Variable Region Nucleic Acid Sequences

D11-S17Y (SEQ ID NO:7)

GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACC
CTCTCCTGCAGTGTCAGCTCAAGTATAAGTTCCACCAACTTACACTGGTACCAGCAGAAA
CCTGGCCAGGCTCCCAGGCTCCTCATCTATGGCACATCCTATCTGGCTTCTGGCATCCCA
GACAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAG
CCTGAAGATTTTGCAGTGTATTACTGTCAACAGTGGAGTAGTTACCCGTACAGTTTCGGC
CAAGGGACCAAGTTGGAGATCAAA

D11-8B8 (SEQ ID NO:8)

GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACC
CTCTCCTGCAGTGTCAGCTCAAGTATAAGTTCCACCAACTTACACTGGTACCAGCAGAAA
CCTGGCCAGGCTCCCAGGCTCCTCATCTATGGCACATCCTACCTGGCTTCTGGCATCCCA
GACAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAG
CCTGAAGATTTTGCAGTGTATTACTGTCAACAGTGGAGTAGTTACCCGTACAGTTTCGGC
CAAGGGACCAAGTTGGAGATCAAA

D11-C27G3 (SEQ ID NO:9)

GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACC
CTCTCCTGCAGTGTCAGCTCAAGTATAAGTTCCACCAACTTACACTGGTACCAGCAGAAA
CCTGGCCAGGCTCCCAGGCTCCTCATCTATGGCACATCCAGACTGAGATCTGGCATCCCA
GACAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAG
CCTGAAGATTTTGCAGTGTATTACTGTCAACAGTGGAGTAGTTACCCGTACAGTTTCGGC
CAAGGGACCAAGTTGGAGATCAAA

C8-6 (SEQ ID NO:10)

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCACTTGCAGTGTCAGCTCAAGTGTAAGTTCCATTTACTTGCACTGGTATCAGCAGAAA
CCAGGGAAAGCCCCTAAGCTCCTGATCTATAGCACATCCAACTTGGCTTCTGGAGTCCCA
TCAAGGTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAA

CCTGAAGATTTTGCAACTTACTACTGTATTCAGTACAGTGGTTACCCGCTCACGTTCGGC
GGAGGGACCAAGGTGGAGATCAAA

C8-H241 (SEQ ID NO:11)

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCACTTGCAGTGTCAGCTCAAGTGTAAGTTCCATTTACTTGCACTGGTATCAGCAGAAA
CCAGGGAAAGCCCCTAAGCTCCTGATCTATAGCACATCCAACTTGGCTTCTGGAGTCCCA
TCAAGGTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAA
CCTGAAGATTTTGCAACTTACTACTGTCAGGTGTACAGTGGTTACCCGCTCACGTTCGGC
GGAGGGACCAAGGTGGAGATCAAA

C8-co-16 (SEQ ID NO:12)

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCACTTGCAGTGTCAGCTCAAGTGTACGTTCCATTTACTTGCACTGGTATCAGCAGAAA
CCAGGGAAAGCCCCTAAGCTCCTGATCTATAGCACATCCAACTTGGCTTCTGGAGTCCCA
TCAAGGTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAA
CCTGAAGATTTTGCAACTTACTACTGTCAGGTGTACAGGGGTTACCCGCTCACGTTCGGC
GGAGGGACCAAGGTGGAGATCAAA

Heavy Chain Variable Region Amino Acid Sequences

D11-S17Y (SEQ ID NO:13)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSRYIHWVRQAPGQGLEWMGWIYP
VTGDTYYNEKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARGYGAFYYW
GQGTLVTVS

D11-8B8 (SEQ ID NO:14)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSRYIHWVRQAPGQGLEWMGWIYP
VTGDTYYIEKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARGYGAFFYWG
QGTLVTVS

D11-C27G3 (SEQ ID NO:15)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSRYIHWVRQAPGQGLEWMGWIYP
VTGDTYYREPFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARGYGAFYYWG
QGTLVTVS

C8-6 (SEQ ID NO:16)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYMHWVRQAPGQGLEWMGRV
NPNRGGTTYNQKFEGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARTNWLDY
WGQGTTVTVS

C8-H241 (SEQ ID NO:17)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYMHWVRQAPGQGLEWMGRV
NPNRRGTTYNQKFEGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARANWLDY
WGQGTTVTVS

C8-co-16 (SEQ ID NO:18)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYMHWVRQAPGQGLEWMGRV
NPYRGSTTYNQKFEGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARANILDYW
GQGTTVTVS

Heavy Chain Variable Region Nucleic Acid Sequences

D11-S17Y (SEQ ID NO:19)

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAAGGTC
TCCTGCAAGGCTTCTGGCTACACCTTCACAAGTAGGTATATACACTGGGTGCGACAGGCC
CCTGGACAAGGGCTTGAGTGGATGGGATGGATTTATCCTGTAACTGGTGATACTTACTAC
AACGAGAAGTTCAAGGGCAGAGTCACGATTACCGCGGACAAATCCACGAGCACAGCCTAC
ATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGAGAGGCTAC
GGAGCTTTTTACTACTGGGGCCAGGGCACCCTGGTCACCGTCTCC

D11-8B8 (SEQ ID NO:20)

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAAGGTC
TCCTGCAAGGCTTCTGGCTACACCTTCACAAGTAGGTATATACACTGGGTGCGACAGGCC
CCTGGACAAGGGCTTGAGTGGATGGGATGGATTTATCCTGTAACTGGTGATACTTACTAC
ATCGAGAAGTTCAAGGGCAGAGTCACGATTACCGCGGACAAATCCACGAGCACAGCCTAC
ATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGAGAGGCTAT
GGTGCTTTTTTCTACTGGGGCCAGGGCACCCTGGTCACCGTCTCC

D11-C27G3 (SEQ ID NO:21)

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAAGGTC
TCCTGCAAGGCTTCTGGCTACACCTTCACAAGTAGGTATATACACTGGGTGCGACAGGCC
CCTGGACAAGGGCTTGAGTGGATGGGATGGATTTATCCTGTAACTGGTGATACTTACTAC
AGAGAGCCTTTCAAGGGCAGAGTCACGATTACCGCGGACAAATCCACGAGCACAGCCTAC
ATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGAGAGGCTAT
GGGGCTTTTTACTACTGGGGCCAGGGCACCCTGGTCACCGTCTCC

C8-6 (SEQ ID NO:22)

CAGGTTCAGCTGGTGCAGTCTGGTGCTGAGGTGAAGAAGCCTGGTGCCTCAGTGAAGGTC
TCCTGCAAGGCTTCTGGTTACACCTTTACCGACTACTACATGCACTGGGTGCGTCAGGCC
CCTGGTCAAGGTCTTGAGTGGATGGGTCGTGTTAATCCTAACCGGGGTGGTACTACCTAC
AACCAGAAATTCGAGGGCCGTGTCACCATGACCACAGACACATCCACGAGCACAGCCTAC
ATGGAGCTGCGTAGCCTGCGTTCTGACGACACGGCCGTGTATTACTGTGCGCGTACGAAC
TGGCTTGACTACTGGGGCCAGGGCACCACCGTCACCGTCTCC

C8-H241 (SEQ ID NO:23)

CAGGTTCAGCTGGTGCAGTCTGGTGCTGAGGTGAAGAAGCCTGGTGCCTCAGTGAAGGTC
TCCTGCAAGGCTTCTGGTTACACCTTTACCGACTACTACATGCACTGGGTGCGTCAGGCC
CCTGGTCAAGGTCTTGAGTGGATGGGTCGTGTTAATCCTAACCGGAGGGGTACTACCTAC
AACCAGAAATTCGAGGGCCGTGTCACCATGACCACAGACACATCCACGAGCACAGCCTAC
ATGGAGCTGCGTAGCCTGCGTTCTGACGACACGGCCGTGTATTACTGTGCGCGTGCGAAC
TGGCTTGACTACTGGGGCCAGGGCACCACCGTCACCGTCTCC

C8-co-16 (SEQ ID NO:24)

CAGGTTCAGCTGGTGCAGTCTGGTGCTGAGGTGAAGAAGCCTGGTGCCTCAGTGAAGGTC
TCCTGCAAGGCTTCTGGTTACACATTCACTGACTACTACATGCACTGGGTGCGTCAGGCC
CCTGGTCAAGGTCTTGAGTGGATGGGTCGTGTTAATCCTTATCGGGGTAGTACTACCTAC
AACCAGAAATTCGAGGGCCGTGTCACCATGACCACAGACACATCCACGAGCACAGCCTAC
ATGGAGCTGCGTAGCCTGCGTTCTGACGACACGGCCGTGTATTACTGTGCGCGTGCGAAC
ATTCTTGACTACTGGGGCCAGGGCACCACCGTCACCGTCTCC

Complete Light Chain Amino Acid Sequences

D11-S17Y kappa (SEQ ID NO:25)

EIVLTQSPGTLSLSPGERATLSCSVSSSISSTNLHWYQQKPGQAPRLLIYGTSYLAS
GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQWSSYPYSFGQGTKLEIKRTVAA
PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

D11-8B8 kappa (SEQ ID NO:26)

EIVLTQSPGTLSLSPGERATLSCSVSSSISSTNLHWYQQKPGQAPRLLIYGTSYLAS
GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQWSSYPYSFGQGTKLEIKRTVAA
PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

D11-C27G3 kappa (SEQ ID NO:27)

EIVLTQSPGTLSLSPGERATLSCSVSSSISSTNLHWYQQKPGQAPRLLIYGTSRLRS
GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQWSSYPYSFGQGTKLEIKRTVAA
PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

C8-6 kappa (SEQ ID NO:28)

DIQMTQSPSSLSASVGDRVTITCSVSSSVSSIYLHWYQQKPGKAPKLLIYSTSNLAS
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCIQYSGYPLTFGGGTKVEIKRTVAAP

SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

C8-H241 kappa (SEQ ID NO:29)

DIQMTQSPSSLSASVGDRVTITCSVSSSVSSIYLHWYQQKPGKAPKLLIYSTSNLAS
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQVYSGYPLTFGGGTKVEIKRTVAA
PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

C8-co-16 kappa (SEQ ID NO:30)

DIQMTQSPSSLSASVGDRVTITCSVSSSVRSIYLHWYQQKPGKAPKLLIYSTSNLA
SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQVYRGYPLTFGGGTKVEIKRTVA
APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Complete Light Chain Nucleic Acid Sequences

D11-S17Y IgG2 LC (SEQ ID NO:31)

GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACC
CTCTCCTGCAGTGTCAGCTCAAGTATAAGTTCCACCAACTTACACTGGTACCAGCAGAAA
CCTGGCCAGGCTCCCAGGCTCCTCATCTATGGCACATCCTACCTGGCTTCTGGCATCCCA
GACAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAG
CCTGAAGATTTTGCAGTGTATTACTGTCAACAGTGGAGTAGTTACCCGTACAGTTTCGGC
CAAGGGACCAAGTTGGAGATCAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCG
CCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTC
TATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCC
CAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTG
ACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAG
GGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGC

D11-8B8 IgG2 LC (SEQ ID NO:32)

GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACC
CTCTCCTGCAGTGTCAGCTCAAGTATAAGTTCCACCAACTTACACTGGTACCAGCAGAAA
CCTGGCCAGGCTCCCAGGCTCCTCATCTATGGCACATCCTACCTGGCTTCTGGCATCCCA
GACAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAG
CCTGAAGATTTTGCAGTGTATTACTGTCAACAGTGGAGTAGTTACCCGTACAGTTTCGGC
CAAGGGACCAAGTTGGAGATCAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCG
CCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTC

TATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCC
CAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTG
ACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAG
GGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGC

D11-C27G3 IgG2 LC (SEQ ID NO:33)

GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACC
CTCTCCTGCAGTGTCAGCTCAAGTATAAGTTCCACCAACTTACACTGGTACCAGCAGAAA
CCTGGCCAGGCTCCCAGGCTCCTCATCTATGGCACATCCAGACTGAGATCTGGCATCCCA
GACAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAG
CCTGAAGATTTTGCAGTGTATTACTGTCAACAGTGGAGTAGTTACCCGTACAGTTTCGGC
CAAGGGACCAAGTTGGAGATCAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCG
CCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTC
TATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCC
CAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTG
ACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAG
GGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGC

C8-6 IgG4 LC (SEQ ID NO:34)

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCACTTGCAGTGTCAGCTCAAGTGTAAGTTCCATTTACTTGCACTGGTATCAGCAGAAA
CCAGGGAAAGCCCCTAAGCTCCTGATCTATAGCACATCCAACTTGGCTTCTGGAGTCCCA
TCAAGGTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAA
CCTGAAGATTTTGCAACTTACTACTGTATTCAGTACAGTGGTTACCCGCTCACGTTCGGC
GGAGGGACCAAGGTGGAGATCAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCG
CCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTC
TATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCC
CAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTG
ACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAG
GGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGC

C8-H241 IgG4 LC (SEQ ID NO:35)

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCACTTGCAGTGTCAGCTCAAGTGTATCCTCCATTTACTTGCACTGGTATCAGCAGAAA
CCAGGGAAAGCCCCTAAGCTCCTGATCTATAGCACATCCAACTTGGCTTCTGGAGTCCCA
TCAAGGTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAA
CCTGAAGATTTTGCAACTTACTACTGTCAAGTCTACAGTGGTTACCCGCTCACGTTCGGC
GGAGGGACCAAGGTGGAGATCAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCG
CCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTC
TATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCC
CAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTG
ACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAG
GGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGC

C8-co16 IgG4 LC (SEQ ID NO:36)

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCACTTGCAGTGTCAGCTCAAGTGTACGTTCCATTTACTTGCACTGGTATCAGCAGAAA
CCAGGGAAAGCCCCTAAGCTCCTGATCTATAGCACATCCAACTTGGCTTCTGGAGTCCCA
TCAAGGTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAA
CCTGAAGATTTTGCAACTTACTACTGTCAGGTGTACAGGGGTTACCCGCTCACGTTCGGC
GGAGGGACCAAGGTGGAGATCAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCG
CCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTC
TATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCC
CAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTG
ACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAG
GGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGC

Complete Heavy Chain Amino Acid Sequences

D11-S17Y IgG2 (SEQ ID NO:37)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSRYIHWVRQAPGQGLEWMGWIYP
VTGDTYYNEKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARGYGAFYYW
GQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGA
LTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVER
KCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFN
WYVDGMEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGL
PAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPG

D11-8B8 IgG2 (SEQ ID NO:38)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSRYIHWVRQAPGQGLEWMGWIYP
VTGDTYYIEKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARGYGAFFYWG
QGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGAL
TSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERK
CCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNW
YVDGMEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLP
APIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG

QPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
KSLSLSPG

D11-C27G3 IgG2 (SEQ ID NO:39)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSRYIHWVRQAPGQGLEWMGWIYP
VTGDTYYREPFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARGYGAFYYWG
QGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGAL
TSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERK
CCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNW
YVDGMEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLP
APIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG
QPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
KSLSLSPG

C8-6 IgG4 (SEQ ID NO:40)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYMHWVRQAPGQGLEWMGRV
NPNRGGTTYNQKFEGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARTNWLDY
WGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSG
ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVE
RKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFN
WYVDGMEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGL
PAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPG

C8-H241 IgG4 (SEQ ID NO:41)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYMHWVRQAPGQGLEWMGRV
NPNRRGTTYNQKFEGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARANWLDY
WGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSG
ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVE
RKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFN
WYVDGMEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGL

PAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPG

C8-co-16 IgG4 (SEQ ID NO:42)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYMHWVRQAPGQGLEWMGRV
NPYRGSTTYNQKFEGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARANILDYW
GQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGA
LTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVER
KCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFN
WYVDGMEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGL
PAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPG

Complete Heavy Chain Nucleic Acid Sequences

D11-S17Y IgG2 HC (SEQ ID NO:43)

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAAGGTC
TCCTGCAAGGCTTCTGGCTACACCTTCACAAGTAGGTATATACACTGGGTGCGACAGGCC
CCTGGACAAGGGCTTGAGTGGATGGGATGGATTTATCCTGTAACTGGTGATACTTACTAC
AACGAGAAGTTCAAGGGCAGAGTCACGATTACCGCGGACAAATCCACGAGCACAGCCTAC
ATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGAGAGGCTAT
GGGGCTTTTTACTACTGGGGCCAGGGCACCCTGGTCACCGTCTCCTCCGCCTCCACCAAG
GGCCCATCGGTCTTCCCGCTAGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCCGCC
CTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGC
GCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCC
CTCAGCAGCGTGGTGACCGTGCCCTCCAGCAACTTCGGCACCCAGACCTACACCTGCAAC
GTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGACAGTTGAGCGCAAATGTTGTGTC
GAGTGCCCACCGTGCCCAGCACCACCTGTGGCAGGACCGTCAGTCTTCCTCTTCCCCCCA
AAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGAC
GTGAGCCACGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGACGGCATGGAGGTGCAT
AATGCCAAGACAAAGCCACGGGAGGAGCAGTTCAACAGCACGTTCCGTGTGGTCAGCGTC
CTCACCGTCGTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAAC
AAAGGCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAACCAAAGGGCAGCCCCGAGAA
CCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTG
ACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGG
CAGCCGGAGAACAACTACAAGACCACACCTCCCATGCTGGACTCCGACGGCTCCTTCTTC
CTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGC

TCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCCG
GGT

D11-8B8 IgG2 HC (SEQ ID NO:44)

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAAGGTC
TCCTGCAAGGCTTCTGGCTACACCTTCACAAGTAGGTATATACACTGGGTGCGACAGGCC
CCTGGACAAGGGCTTGAGTGGATGGGATGGATTTATCCTGTAACTGGTGATACTTACTAC
ATCGAGAAGTTCAAGGGCAGAGTCACGATTACCGCGGACAAATCCACGAGCACAGCCTAC
ATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGAGAGGCTAT
GGTGCTTTTTTCTACTGGGGCCAGGGCACCCTGGTCACCGTCTCCTCCGCCTCCACCAAG
GGCCCATCGGTCTTCCCGCTAGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCCGCC
CTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGC
GCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCC
CTCAGCAGCGTGGTGACCGTGCCCTCCAGCAACTTCGGCACCCAGACCTACACCTGCAAC
GTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGACAGTTGAGCGCAAATGTTGTGTC
GAGTGCCCACCGTGCCCAGCACCACCTGTGGCAGGACCGTCAGTCTTCCTCTTCCCCCCA
AAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGAC
GTGAGCCACGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGACGGCATGGAGGTGCAT
AATGCCAAGACAAAGCCACGGGAGGAGCAGTTCAACAGCACGTTCCGTGTGGTCAGCGTC
CTCACCGTCGTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAAC
AAAGGCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAACCAAAGGGCAGCCCCGAGAA
CCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTG
ACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGG
CAGCCGGAGAACAACTACAAGACCACACCTCCCATGCTGGACTCCGACGGCTCCTTCTTC
CTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGC
TCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCCG
GGT

D11-C27G3 IgG2 HC (SEQ ID NO:45)

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAAGGTC
TCCTGCAAGGCTTCTGGCTACACCTTCACAAGTAGGTATATACACTGGGTGCGACAGGCC
CCTGGACAAGGGCTTGAGTGGATGGGATGGATTTATCCTGTAACTGGTGATACTTACTAC
AGAGAGCCTTTCAAGGGCAGAGTCACGATTACCGCGGACAAATCCACGAGCACAGCCTAC
ATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGAGAGGCTAC
GGAGCTTTTTACTACTGGGGCCAGGGCACCCTGGTCACCGTCTCCTCCGCCTCCACCAAG
GGCCCATCGGTCTTCCCGCTAGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCCGCC
CTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGC
GCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCC
CTCAGCAGCGTGGTGACCGTGCCCTCCAGCAACTTCGGCACCCAGACCTACACCTGCAAC
GTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGACAGTTGAGCGCAAATGTTGTGTC
GAGTGCCCACCGTGCCCAGCACCACCTGTGGCAGGACCGTCAGTCTTCCTCTTCCCCCCA
AAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGAC
GTGAGCCACGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGACGGCATGGAGGTGCAT
AATGCCAAGACAAAGCCACGGGAGGAGCAGTTCAACAGCACGTTCCGTGTGGTCAGCGTC
CTCACCGTCGTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAAC

AAAGGCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAACCAAAGGGCAGCCCCGAGAA
CCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTG
ACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGG
CAGCCGGAGAACAACTACAAGACCACACCTCCCATGCTGGACTCCGACGGCTCCTTCTTC
CTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGC
TCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCCG
GGT

C8-6 IgG4 HC (SEQ ID NO:46)

CAGGTTCAGCTGGTGCAGTCTGGTGCTGAGGTGAAGAAGCCTGGTGCCTCAGTGAAGGTC
TCCTGCAAGGCTTCTGGTTACACCTTTACCGACTACTACATGCACTGGGTGCGTCAGGCC
CCTGGTCAAGGTCTTGAGTGGATGGGTCGTGTTAATCCTAACCGGGGTGGTACTACCTAC
AACCAGAAATTCGAGGGCCGTGTCACCATGACCACAGACACATCCACGAGCACAGCCTAC
ATGGAGCTGCGTAGCCTGCGTTCTGACGACACGGCCGTGTATTACTGTGCGCGTACGAAC
TGGCTTGACTACTGGGGCCAGGGCACCACCGTCACCGTCTCCTCCGCCTCCACCAAGGGC
CCATCGGTCTTCCCGCTAGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCCGCCCTG
GGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCC
CTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTC
AGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACGAAGACCTACACCTGCAACGTA
GATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGTCCAAATATGGTCCCCCA
TGCCCACCCTGCCCAGCACCTGAGGCCGCCGGGGGACCATCAGTCTTCCTGTTCCCCCCA
AAACCCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGAC
GTGAGCCAGGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCAT
AATGCCAAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTACCGTGTGGTCAGCGTC
CTCACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAAC
AAAGGCCTCCCGTCCTCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAG
CCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATGACCAAGAACCAGGTCAGCCTG
ACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAAAGCAATGGG
CAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTC
CTCTACAGCAGGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGGAATGTCTTCTCATGC
TCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCTG
GGT

C8-H241 IgG4 HC (SEQ ID NO:47)

CAGGTTCAGCTGGTGCAGTCTGGTGCTGAGGTGAAGAAGCCTGGTGCCTCAGTGAAGGTC
TCCTGCAAGGCTTCTGGTTACACATTCACTGACTACTACATGCACTGGGTGCGTCAGGCC
CCTGGTCAAGGTCTTGAGTGGATGGGTCGTGTTAATCCTAACCGGAGGGGTACTACCTAC
AACCAGAAATTCGAGGGCCGTGTCACCATGACCACAGACACATCCACGAGCACAGCCTAC
ATGGAGCTGCGTAGCCTGCGTTCTGACGACACGGCCGTGTATTACTGTGCGCGTGCGAAC
TGGCTTGACTACTGGGGCCAGGGCACCACCGTCACCGTCTCCTCCGCCTCCACCAAGGGC
CCATCGGTCTTCCCGCTAGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCCGCCCTG
GGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCC
CTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTC
AGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACGAAGACCTACACCTGCAACGTA
GATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGTCCAAATATGGTCCCCCA
TGCCCACCCTGCCCAGCACCTGAGGCCGCCGGGGGACCATCAGTCTTCCTGTTCCCCCCA

AAACCCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGAC
GTGAGCCAGGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCAT
AATGCCAAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTACCGTGTGGTCAGCGTC
CTCACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAAC
AAAGGCCTCCCGTCCTCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAG
CCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATGACCAAGAACCAGGTCAGCCTG
ACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAAAGCAATGGG
CAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTC
CTCTACAGCAGGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGGAATGTCTTCTCATGC
TCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCTG
GGT

C8-co16 IgG4 HC (SEQ ID NO:48)

CAGGTTCAGCTGGTGCAGTCTGGTGCTGAGGTGAAGAAGCCTGGTGCCTCAGTGAAGGTC
TCCTGCAAGGCTTCTGGTTACACATTCACTGACTACTACATGCACTGGGTGCGTCAGGCC
CCTGGTCAAGGTCTTGAGTGGATGGGTCGTGTTAATCCTTATCGGGGTAGTACTACCTAC
AACCAGAAATTCGAGGGCCGTGTCACCATGACCACAGACACATCCACGAGCACAGCCTAC
ATGGAGCTGCGTAGCCTGCGTTCTGACGACACGGCCGTGTATTACTGTGCGCGTGCGAAC
ATTCTTGACTACTGGGGCCAGGGCACCACCGTCACCGTCTCCTCCGCCTCCACCAAGGGC
CCATCGGTCTTCCCGCTAGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCCGCCCTG
GGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCC
CTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTC
AGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACGAAGACCTACACCTGCAACGTA
GATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGTCCAAATATGGTCCCCCA
TGCCCACCCTGCCCAGCACCTGAGGCCGCCGGGGGACCATCAGTCTTCCTGTTCCCCCCA
AAACCCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGAC
GTGAGCCAGGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCAT
AATGCCAAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTACCGTGTGGTCAGCGTC
CTCACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAAC
AAAGGCCTCCCGTCCTCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAG
CCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATGACCAAGAACCAGGTCAGCCTG
ACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAAAGCAATGGG
CAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTC
CTCTACAGCAGGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGGAATGTCTTCTCATGC
TCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCTG
GGT

Human cMet-ECD-Fc-Flis (SEQ ID NO:72)

MKAPAVLAPGILVLLFTLVQRSNGECKEALAKSEMNVNMKYQLPNFTAETPIQN
VILHEHHIFLGATNYIYVLNEEDLQKVAEYKTGPVLEHPDCFPCQDCSSKANLSG
GVWKDNINMALVVDTYYDDQLISCGSVNRGTCQRHVFPHNHTADIQSEVHCIFS
PQIEEPSQCPDCVVSALGAKVLSSVKDRFINFFVGNTINSSYFPDHPLHSISVRRLK
ETKDGFMFLTDQSYIDVLPEFRDSYPIKYVHAFESNNFIYFLTVQRETLDAQTFHT

RIIRFCSINSGLHSYMEMPLECILTEKRKKRSTKKEVFNILQAAYVSKPGAQLARQI
GASLNDDILFGVFAQSKPDSAEPMDRSAMCAFPIKYVNDFFNKIVNKNNVRCLQ
HFYGPNHEHCFNRTLLRNSSGCEARRDEYRTEFTTALQRVDLFMGQFSEVLLTSI
STFIKGDLTIANLGTSEGRFMQVVVSRSGPSTPHVNFLLDSHPVSPEVIVEHTLNQ
NGYTLVITGKKITKIPLNGLGCRHFQSCSQCLSAPPFVQCGWCHDKCVRSEECLS
GTWTQQICLPAIYKVFPNSAPLEGGTRLTICGWDFGFRRNNKFDLKKTRVLLGNE
SCTLTLSESTMNTLKCTVGPAMNKHFNMSIIISNGHGTTQYSTFSYVDPVITSISPK
YGPMAGGTLLTLTGNYLNSGNSRHISIGGKTCTLKSVSNSILECYTPAQTISTEFA
VKLKIDLANRETSIFSYREDPIVYEIHPTKSFISGGSTITGVGKNLNSVSVPRMVINV
HEAGRNFTVACQHRSNSEIICCTTPSLQQLNLQLPLKTKAFFMLDGILSKYFDLIY
VHNPVFKPFEKPVMISMGNENVLEIKGNDIDPEAVKGEVLKVGNKSCENIHLHSE
AVLCTVPNDLLKLNSELNIEWKQAISSTVLGKVIVQPDQNFTLEVLFQGPDIEPKS
CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQEYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
TQKSLSLSPGKRI**<u>DYKDDDDKHVHHHHHH</u>**

Bold, italicized amino acids represent the signal sequence; bold underlined amino acids represent the Flis tag.

Cynomolgus Monkey cMet-ECD-Fc-Flis (SEQ ID NO:73)

MKAPAVLVPGILVLLFTLVQRSNGECKEALAKSEMNVNMKYQLPNFTAETAIQN
VILHEHHIFLGATNYIYVLNEEDLQKVAEYKTGPVLEHPDCFPCQDCSSKANLSG
GVWKDNINMALVVDTYYDDQLISCGSVNRGTCQRHVFPHNHTADIQSEVHCIFS
PQIEEPNQCPDCVVSALGAKVLSSVKDRFINFFVGNTINSSYFPHHPLHSISVRRLK
ETKDGFMFLTDQSYIDVLPEFRDSYPIKYIHAFESNNFIYFLTVQRETLNAQTFHTR
IIRFCSLNSGLHSYMEMPLECILTEKRKKRSTKKEVFNILQAAYVSKPGAQLARQI
GASLNDDILFGVFAQSKPDSAEPMDRSAMCAFPIKYVNDFFNKIVNKNNVRCLQ
HFYGPNHEHCFNRTLLRNSSGCEARRDEYRAEFTTALQRVDLFMGQFSEVLLTSI
STFVKGDLTIANLGTSEGRFMQVVVSRSGPSTPHVNFLLDSHPVSPEVIVEHPLNQ
NGYTLVVTGKKITKIPLNGLGCRHFQSCSQCLSAPPFVQCGWCHDKCVRSEECPS

GTWTQQICLPAIYKVFPTSAPLEGGTRLTICGWDFGFRRNNKFDLKKTRVLLGNE
SCTLTLSESTMNTLKCTVGPAMNKHFNMSIIISNGHGTTQYSTFSYVDPIITSISPK
YGPMAGGTLLTLTGNYLNSGNSRHISIGGKTCTLKSVSNSILECYTPAQTISTEFA
VKLKIDLANRETSIFSYREDPIVYEIHPTKSFISGGSTITGVGKNLHSVSVPRMVINV
HEAGRNFTVACQHRSNSEIICCTTPSLQQLNLQLPLKTKAFFMLDGILSKYFDLIY
VHNPVFKPFEKPVMISMGNENVLEIKGNDIDPEAVKGEVLKVGNKSCENIHLHSE
AVLCTVPNDLLKLNSELNIEWKQAISSTVLGKVIVQPDQNFTLEVLFQGPDIEPKS
CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQEYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
TQKSLSLSPGKRI**DYKDDDDKHVHHHHHH**

Bold, italicized amino acids represent the signal sequence; bold underlined amino acids represent the Flis tag.

Rat cMet-ECD-Fc-Flis (SEQ ID NO:74)

MKAPTALAPGILLLLLTLAQRSHGECKEALVKSEMNVNMKYQLPNFTAETPIHN
VVLHGHHIYLGATNYIYVLNDKDLQKVSEFKTGPVVEHPDCFPCQDCSSKANVS
GGVWKDNVNMALLVDTYYDDQLISCGSVNRGTCQRHVLPPDNAADIQSEVHC
MFSPLAEEESGQCPDCVVSALGAKVLLSEKDRFINFFVGNTINSSYPPDYSLHSISV
RRLKETQDGFKFLTDQSYIDVLPEFRDSYPIKYIHAFESNHFIYFLTVQKETLDAQT
FHTRIIRFCSVDSGLHSYMEMPLECILTEKRRKRSTREEVFNILQAAYVSKPGANL
AKQIGASPYDDILYGVFAQSKPDSAEPMNRSAVCAFPIKYVNDFFNKIVNKNNVR
CLQHFYGPNHEHCFNRTLLRNSSGCEVRSDEYRTEFTTALQRVDLFMGRLNHVL
LTSISTFIKGDLTIANLGTSEGRFMQVVLSRTAHFTPHVNFLLDSYPVSPEVIVEHP
SNQNGYTLVVTGKKITKIPLNGLGCGHFQSCSQCLSAPYFIQCGWCHNRCVHSNE
CPSGTWTQEICLPAVYKVFPTSAPLEGGTMLTICGWDFGFKKNNKFDLRKTKVLL
GNESCTLTLSESTTNTLKCTVGPAMSEHFNVSVIVSNSRETTQYSAFSYVDPVITSI
SPRYGPHAGGTLLTLTGKYLNSGNSRHISIGGKTCTLKSVSDSILECYTPGHTVSA
EFPVKLKIDLADRVTSSFSYREDPVVSEIHPTKSFISGGSTITGIGKNLNSVSTPKLV
IEVHDVGVNYTVACQHRSSSEIICCTTPSLQQLDLQLPLKTKAFFLLDGILSKHFDL
TYVHDPMFKPFEKPVMISMGNENVVEIKGDDIDPEAVKGEVLKVGNKSCENLH

WHSEALLCTVPSDLLKLNGGELNIEWKQAVSSTVLGKVIVQPDQNFALEVLFQG
PDIEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNKALPAPIEKTISKAKGQPREPQEYTLPPSREEMTKNQVSLTCLVKGFYPSDI
AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGKRI**DYKDDDDKHVHHHHHH**

Bold, italicized amino acids represent the signal sequence; bold underlined amino acids represent the Flis tag.

Human c-Met ECD (SEQ ID NO:75)

MKAPAVLAPGILVLLFTLVQRSNGECKEALAKSEMNVNMKYQLPNFTAETPIQN
VILHEHHIFLGATNYIYVLNEEDLQKVAEYKTGPVLEHPDCFPCQDCSSKANLSG
GVWKDNINMALVVDTYYDDQLISCGSVNRGTCQRHVFPHNHTADIQSEVHCIFS
PQIEEPSQCPDCVVSALGAKVLSSVKDRFINFFVGNTINSSYFPDHPLHSISVRRLK
ETKDGFMFLTDQSYIDVLPEFRDSYPIKYVHAFESNNFIYFLTVQRETLDAQTFHT
RIIRFCSINSGLHSYMEMPLECILTEKRKKRSTKKEVFNILQAAYVSKPGAQLARQI
GASLNDDILFGVFAQSKPDSAEPMDRSAMCAFPIKYVNDFFNKIVNKNNVRCLQ
HFYGPNHEHCFNRTLLRNSSGCEARRDEYRTEFTTALQRVDLFMGQFSEVLLTSI
STFIKGDLTIANLGTSEGRFMQVVVSRSGPSTPHVNFLLDSHPVSPEVIVEHTLNQ
NGYTLVITGKKITKIPLNGLGCRHFQSCSQCLSAPPFVQCGWCHDKCVRSEECLS
GTWTQQICLPAIYKVFPNSAPLEGGTRLTICGWDFGFRRNNKFDLKKTRVLLGNE
SCTLTLSESTMNTLKCTVGPAMNKHFNMSIIISNGHGTTQYSTFSYVDPVITSISPK
YGPMAGGTLLTLTGNYLNSGNSRHISIGGKTCTLKSVSNSILECYTPAQTISTEFA
VKLKIDLANRETSIFSYREDPIVYEIHPTKSFISGGSTITGVGKNLNSVSVPRMVINV
HEAGRNFTVACQHRSNSEIICCTTPSLQQLNLQLPLKTKAFFMLDGILSKYFDLIY
VHNPVFKPFEKPVMISMGNENVLEIKGNDIDPEAVKGEVLKVGNKSCENIHLHSE
AVLCTVPNDLLKLNSELNIEWKQAISSTVLGKVIVQPDQNFT

Bold, italicized amino acids represent the signal sequence.

Human c-Met Sema Domain with Flis tag (SEQ ID NO:76)

MKAPAVLAPGILVLLFTLVQRSNGECKEALAKSEMNVNMKYQLPNFTAETPIQN
VILHEHHIFLGATNYIYVLNEEDLQKVAEYKTGPVLEHPDCFPCQDCSSKANLSG
GVWKDNINMALVVDTYYDDQLISCGSVNRGTCQRHVFPHNHTADIQSEVHCIFS
PQIEEPSQCPDCVVSALGAKVLSSVKDRFINFFVGNTINSSYFPDHPLHSISVRRLK
ETKDGFMFLTDQSYIDVLPEFRDSYPIKYVHAFESNNFIYFLTVQRETLDAQTFHT
RIIRFCSINSGLHSYMEMPLECILTEKRKKRSTKKEVFNILQAAYVSKPGAQLARQI
GASLNDDILFGVFAQSKPDSAEPMDRSAMCAFPIKYVNDFFNKIVNKNNVRCLQ
HFYGPNHEHCFNRTLLRNSSGCEARRDEYRTEFTTALQRVDLFMGQFSEVLLTSI
STFIKGDLTIANLGTSEGRFMQVVVSRSGPSTPHVNFLLDSHPVSPEVIVEHTLNQ
NGYTLVITGKKITKIPLNGLGCRHFQSCSQCLSAPPFVQCGWCHDKCVRSEECLS
GTWTQQICL**<u>DYKDDDDKHVHHHHHH</u>**

Bold, italicized amino acids represent the signal sequence; bold underlined amino acids represent the Flis tag

C8- Antibody Epitopes in the Human c-Met Extracellular Domain

$_{121}$VVDTYYDDQL$_{130}$ (SEQ ID NO:77)

$_{131}$ISCGSVNRGTCQRHVFPHNHTADIQS$_{156}$ (SEQ ID NO:78)

$_{179}$ALGAKVLSSVKDRFINF$_{195}$ (SEQ ID NO:79)

$_{216}$VRRLKETKDGFM$_{227}$ (SEQ ID NO:80)

$_{123}$DTYYDD$_{128}$ (SEQ ID NO:81)

$_{144}$HVFPHNHTADIQS$_{156}$ (SEQ ID NO: 82)

$_{192}$FINF$_{195}$ (SEQ ID NO:83)

$_{220}$KETKDGFM$_{227}$ (SEQ ID NO:84)

D11- Antibody Epitopes in the Human c-Met Extracellular Domain

$_{84}$YKTGPVLEHPDCFPCQDCSSKANL$_{95}$ (SEQ ID NO:85)

$_{95}$CFPCQDCSSKA$_{105}$ (SEQ ID NO:86)

Consensus CDR Sequences

D11- Antibody Light Chain CDR2

GTSX$_1$LX$_2$S (SEQ ID NO:87), wherein X$_1$ is Y or R, and X$_2$ is A or R;

C8- Antibody Light Chain CDR1

SVSSSVX$_3$SIYLH (SEQ ID NO:88), wherein X$_3$ is S or R;

C8- Antibody Light Chain CDR3

X$_4$X$_5$YX$_6$GYPLT (SEQ ID NO:89), wherein X$_4$ is I or Q, X$_5$ is Q or V, and X$_6$ is S or R;

D11- Heavy Chain Antibody CDR2

WIYPVTGDTYYX$_7$EX$_8$FKG (SEQ ID NO:90), wherein X$_7$ is N, I, or R, and X$_8$ is K or P;

D11- Antibody Heavy Chain CDR 3

GYGAFX$_9$Y (SEQ ID NO:91), wherein X$_9$ is Y or F;

C8- Antibody Heavy Chain CDR 2

RVNPX$_{10}$RX$_{11}$X$_{12}$TTYNQKFEG (SEQ ID NO:92), wherein X$_{10}$ is N or Y, X$_{11}$ is G or R, and X$_{12}$ is G or S;

C8- Antibody Heavy Chain CDR3

X$_{13}$NX$_{14}$LDY (SEQ ID NO:93), wherein X$_{13}$ is T or A, and X$_{14}$ is W or I;

Consensus Light Chain Variable Region Sequences

D11- Antibody Light ChainVariable Region Consensus Sequence (SEQ ID NO:94)

EIVLTQSPGTLSLSPGERATLSCSVSSSISSTNLHWYQQKPGQAPRLLIY

GTSX$_1$LX$_2$SGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQWSSYPYSFG QGTKLEIK

wherein X$_1$ is Y or R, and X$_2$ is A or R;

C8- Antibody Light Chain Variable Region Consensus Sequence (SEQ ID NO:95)

DIQMTQSPSSLSASVGDRVTITCSVSSSVX$_3$SIYLHWYQQKPGKAPKLLIY STSNLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCX$_4$X$_5$YX$_6$GYPLTFG GGTKVEIK

wherein X$_3$ is S or R, X$_4$ is I or Q, X$_5$ is Q, or V, and X$_6$ is S or R;

Consensus Heavy Chain Variable Region Sequences

D11- Antibody Heavy Chain Variable Region Consensus Sequence (SEQ ID NO:96)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSRYIHWVRQAPGQGLEWMGW IYPVTGDTYYX$_7$EX$_8$FKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARGY GAFX$_9$YWGQGTLVTVS

wherein X$_7$ is N, I, or R, X$_8$ is K or P, and X$_9$ is Y or F;

C8- Antibody Heavy Chain Variable Region Consensus Sequence (SEQ ID NO:97)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYMHWVRQAPGQGLEWMGR VNPX$_{10}$RX$_{11}$X$_{12}$TTYNQKFEGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARX$_{13}$ NX$_{14}$LDYWGQGTTVTVS

wherein X$_{10}$ is Y or N, X$_{11}$ is G or R, X$_{12}$ is S or G, X$_{13}$ is A or T, and X$_{14}$ is I or W.

We Claim:

1. A monoclonal antibody that:
   a) binds an epitope within the α-chain of human c-Met,
   b) induces internalization of cell surface human c-Met.

2. The monoclonal antibody or antigen-binding fragment thereof of claim 1, wherein the antibody or antigen-binding fragment thereof induces hepatocyte-growth factor-independent internalization of cell surface human c-Met.

3. The monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-2, which induces internalization of human c-Met in cells comprising a human c-Met variant containing a gain of function mutation selected from the group consisting of c-Met kinase domain mutation M1149T and juxtamembrane domain mutation R988C.

4. The monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-3, wherein the antibody or antigen-binding fragment thereof binds within an amino acid sequence selected from the group consisting of:

   a) $_{121}$VVDTYYDDQL$_{130}$ (SEQ ID NO:77),
   b) $_{131}$ISCGSVNRGTCQRHVFPHNHTADIQS$_{156}$ (SEQ ID NO:78),
   c) $_{179}$ALGAKVLSSVKDRFINF$_{195}$ (SEQ ID NO:79), and
   d) $_{216}$VRRLKETKDGFM$_{227}$ (SEQ ID NO:80).

5. The monoclonal antibody of any one of claims 1-4, wherein the antibody or antigen-binding fragment thereof binds within an amino acid sequence selected from the group consisting of:

   a) $_{123}$DTYYDD$_{128}$ (SEQ ID NO:81),
   b) $_{144}$HVFPHNHTADIQS$_{156}$ (SEQ ID NO: 82),

c) $_{192}$FINF$_{195}$ (SEQ ID NO:83), and

d) $_{220}$KETKDGFM$_{227}$ (SEQ ID NO:84).

6. The monoclonal antibody or antigen binding fragment thereof of any one of claims 1-5, wherein the antibody or antigen-binding fragment thereof binds an amino acid sequence within the conformational epitope characterized by $_{123}$DTYYDD$_{128}$ (SEQ ID NO:81), $_{144}$HVFPHNHTADIQS$_{156}$ (SEQ ID NO: 82), $_{192}$FINF$_{195}$ (SEQ ID NO:83), and $_{220}$KETKDGFM$_{227}$ (SEQ ID NO:84) inclusive.

7. The monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-3, wherein the antibody or antigen-binding fragment thereof binds within an amino acid sequence $_{84}$YKTGPVLEHPDCFPCQDCSSKANL$_{107}$ (SEQ ID NO:85) inclusive.

8. The monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-3, wherein the antibody or antigen-binding fragment thereof binds within an amino acid sequence $_{95}$CFPCQDCSSKA$_{105}$ (SEQ ID NO:86) inclusive.

9. The monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-8, wherein the antibody or antigen-binding fragment thereof comprises three light chain complementarity determining regions (LCDRs) and three heavy chain complementarity determining regions (HCDRs), wherein said three LCDRs and said three HCDRs are selected from the group consisting of:

a) LCDR1 comprising the amino acid sequence SVSSSISSTNLH (SEQ ID NO:49);

LCDR2 comprising the amino acid sequence GTSX$_1$LX$_2$S (SEQ ID NO:87), wherein X$_1$ is Y or R, and X$_2$ is A or R;

LCDR3 comprising the amino acid sequence QQWSSYPYS (SEQ ID NO:51);

HCDR1 comprising the amino acid sequence GYTFTSRYIH (SEQ ID NO:59);

HCDR2 comprising the amino acid sequence WIYPVTGDTYYX$_7$EX$_8$FKG (SEQ ID NO:90), wherein X$_7$ is N, I, or R, and X$_8$ is K or P; and

HCDR3 comprising the amino acid sequence GYGAFX$_9$Y (SEQ ID NO:91), wherein X$_9$ is Y or F; and

b) LCDR1 comprising the amino acid sequence SVSSSVX$_3$SIYLH (SEQ ID NO:88), wherein X$_3$ is S or R;

LCDR2 comprising the amino acid sequence STSNLAS (SEQ ID NO:54),

LCDR3 comprising the amino acid sequence X$_4$X$_5$YX$_6$GYPLT (SEQ ID NO:89), wherein X$_4$ is I or Q, X$_5$ is Q or V, and X$_6$ is S or R;

HCDR1 comprising the amino acid sequence GYTFTDYYMH (SEQ ID NO:65),

HCDR2 comprising the amino acid sequence RVNPX$_{10}$RX$_{11}$X$_{12}$TTYNQKFEG (SEQ ID NO:92), wherein X$_{10}$ is N or Y, X$_{11}$ is G or R, and X$_{12}$ is G or S; and

HCDR3 comprising the amino acid sequence X$_{13}$NX$_{14}$LDY (SEQ ID NO:93), wherein X$_{13}$ is T or A, and X$_{14}$ is W or I.

10. The monoclonal antibody, or antigen-binding fragment thereof of any one of claims 1, 3, 7 and 9, wherein the antibody or antigen-binding fragment thereof comprises three light chain complementarity determining regions (LCDRs) and three heavy chain complementarity determining regions (HCDRs), wherein

LCDR1 comprises the amino acid sequence SVSSSISSTNLH (SEQ ID NO:49),

LCDR2 comprises the amino acid sequence GTSX$_1$LX$_2$S (SEQ ID NO:87), wherein X$_1$ is Y or R, and X$_2$ is A or R,

LCDR3 comprises the amino acid sequence QQWSSYPYS (SEQ ID NO:51),

HCDR1 comprises the amino acid sequence GYTFTSRYIH (SEQ ID NO:59),

HCDR2 comprises the amino acid sequence WIYPVTGDTYYX$_7$EX$_8$FKG (SEQ ID NO:90), wherein X$_7$ is N, I, or R, and X$_8$ is K or P, and

HCDR3 comprises the amino acid sequence GYGAFX$_9$Y (SEQ ID NO:91), wherein X$_9$ is Y or F.

11. The monoclonal antibody, or antigen-binding fragment thereof of any one of claims 1-6, 8 and 9, wherein the antibody or antigen-binding fragment thereof comprises three light chain complementarity determining regions (LCDRs) and three heavy chain complementarity determining regions (HCDRs), wherein LCDR1 comprises the amino acid sequence SVSSSVX$_3$SIYLH (SEQ ID NO:88), wherein X$_3$ is S or R;
LCDR2 comprises the amino acid sequence STSNLAS (SEQ ID NO:54),
LCDR3 comprises the amino acid sequence X$_4$X$_5$YX$_6$GYPLT (SEQ ID NO:89), wherein X$_4$ is I or Q, X$_5$ is Q or V, and X$_6$ is S or R,
HCDR1 comprises the amino acid sequence GYTFTDYYMH (SEQ ID NO:65),
HCDR2 comprises the amino acid sequence RVNPX$_{10}$RX$_{11}$X$_{12}$TTYNQKFEG (SEQ ID NO:92), wherein X$_{10}$ is N or Y, X$_{11}$ is G or R, and X$_{12}$ is G or S, and
HCDR3 comprises the amino acid sequence X$_{13}$NX$_{14}$LDY (SEQ ID NO:93), wherein X$_{13}$ is T or A, and X$_{14}$ is W or I.

12. The monoclonal antibody, or antigen-binding fragment thereof of any one of claims 1-11, comprising three light chain complementarity determining regions (LCDRs) and three heavy chain complementarity determining regions (HCDRs), wherein said three LCDRs and said three HCDRs are selected from the group consisting of:

a) LCDR1 comprising the amino acid sequence SVSSSISSTNLH (SEQ ID NO:49),
LCDR2 comprising the amino acid sequence GTSYLAS (SEQ ID NO:50),
LCDR3 comprising the amino acid sequence QQWSSYPYS (SEQ ID NO:51),
HCDR1 comprising the amino acid sequence GYTFTSRYIH (SEQ ID NO:59),
HCDR2 comprising the amino acid sequence WIYPVTGDTYYNEKFKG (SEQ ID NO:60), and

HCDR3 comprising the amino acid sequence GYGAFYY (SEQ ID NO:61);

b) LCDR1 comprising the amino acid sequence SVSSSISSTNLH (SEQ ID NO:49),

LCDR2 comprising the amino acid sequence GTSYLAS (SEQ ID NO:50),

LCDR3 comprising the amino acid sequence QQWSSYPYS (SEQ ID NO:51),

HCDR1 comprising the amino acid sequence GYTFTSRYIH (SEQ ID NO:59)

HCDR2 comprising the amino acid sequence WIYPVTGDTYYIEKFKG (SEQ ID NO:62), and

HCDR3 comprising the amino acid sequence GYGAFFY (SEQ ID NO:63);

c) LCDR1 comprising the amino acid sequence SVSSSISSTNLH (SEQ ID NO:49),

LCDR2 comprising the amino acid sequence GTSRLRS (SEQ ID NO:52),

LCDR3 comprising the amino acid sequence QQWSSYPYS (SEQ ID NO:51),

HCDR1 comprising the amino acid sequence GYTFTSRYIH (SEQ ID NO:59),

HCDR2 comprising the amino acid sequence WIYPVTGDTYYREPFKG (SEQ ID NO:64), and

HCDR3 comprising the amino acid sequence GYGAFYY (SEQ ID NO:61);

d) LCDR1 comprising the amino acid sequence SVSSSVSSIYLH (SEQ ID NO:53),

LCDR2 comprising the amino acid sequence STSNLAS (SEQ ID NO:54),

LCDR3 comprising the amino acid sequence IQYSGYPLT (SEQ ID NO:55),

HCDR1 comprising the amino acid sequence GYTFTDYYMH (SEQ ID NO:65),

HCDR2 comprising the amino acid sequence RVNPNRGGTTYNQKFEG (SEQ ID NO:66), and

HCDR3 comprising the amino acid sequence TNWLDY (SEQ ID NO:67);

e) LCDR1 comprising the amino acid sequence SVSSSVSSIYLH (SEQ ID NO:53),

LCDR2 comprising the amino acid sequence STSNLAS (SEQ ID NO:54),

LCDR3 comprising the amino acid sequence QVYSGYPLT (SEQ ID NO:56),

HCDR1 comprising the amino acid sequence GYTFTDYYMH (SEQ ID NO:65),

HCDR2 comprising the amino acid sequence RVNPNRRGTTYNQKFEG (SEQ ID NO:68), and

HCDR3 comprising the amino acid sequence ANWLDY (SEQ ID NO:69), and

f) LCDR1 comprising the amino acid sequence SVSSSVRSIYLH (SEQ ID NO:57),

LCDR2 comprising the amino acid sequence STSNLAS (SEQ ID NO:54),

LCDR3 comprising the amino acid sequence QVYRGYPLT (SEQ ID NO:58),

HCDR1 comprising the amino acid sequence GYTFTDYYMH (SEQ ID NO:65),

HCDR2 comprising the amino acid sequence RVNPYRGSTTYNQKFEG (SEQ ID NO:70), and

HCDR3 comprising the amino acid sequence ANILDY (SEQ ID NO:71).

13. The monoclonal antibody, or antigen-binding fragment thereof of any one of claims 1-3, 7, 9, 10, and 12 comprising three light chain complementarity determining regions (LCDRs) and three heavy chain complementarity determining regions (HCDRs), wherein said three LCDRs and said three HCDRs are selected from the group consisting of :

a) LCDR1 comprising the amino acid sequence SVSSSISSTNLH (SEQ ID NO:49),

LCDR2 comprising the amino acid sequence GTSYLAS (SEQ ID NO:50),

LCDR3 comprising the amino acid sequence QQWSSYPYS (SEQ ID NO:51),

HCDR1 comprising the amino acid sequence GYTFTSRYIH (SEQ ID NO:59),

HCDR2 comprising the amino acid sequence WIYPVTGDTYYNEKFKG (SEQ ID NO:60), and

HCDR3 comprising the amino acid sequence GYGAFYY (SEQ ID NO:61);

b) LCDR1 comprising the amino acid sequence SVSSSISSTNLH (SEQ ID NO:49),

LCDR2 comprising the amino acid sequence GTSYLAS (SEQ ID NO:50),

LCDR3 comprising the amino acid sequence QQWSSYPYS (SEQ ID NO:51),

HCDR1 comprising the amino acid sequence GYTFTSRYIH (SEQ ID NO:59)

HCDR2 comprising the amino acid sequence WIYPVTGDTYYIEKFKG (SEQ ID NO:62), and

HCDR3 comprising the amino acid sequence GYGAFFY (SEQ ID NO:63);

c) LCDR1 comprising the amino acid sequence SVSSSISSTNLH (SEQ ID NO:49),
LCDR2 comprising the amino acid sequence GTSRLRS (SEQ ID NO:52),
LCDR3 comprising the amino acid sequence QQWSSYPYS (SEQ ID NO:51),
HCDR1 comprising the amino acid sequence GYTFTSRYIH (SEQ ID NO:59),
HCDR2 comprising the amino acid sequence WIYPVTGDTYYREPFKG (SEQ ID NO:64), and
HCDR3 comprising the amino acid sequence GYGAFYY (SEQ ID NO:61).

14. The monoclonal antibody, or antigen-binding fragment thereof of any one of claims 1-6, 8, 9, 11 and 12, comprising three light chain complementarity determining regions (LCDRs) and three heavy chain complementarity determining regions (HCDRs), wherein said three LCDRs and said three HCDRs are selected from the group consisting of:

a) LCDR1 comprising the amino acid sequence SVSSSVSSIYLH (SEQ ID NO:53),
LCDR2 comprising the amino acid sequence STSNLAS (SEQ ID NO:54),
LCDR3 comprising the amino acid sequence IQYSGYPLT (SEQ ID NO:55),
HCDR1 comprising the amino acid sequence GYTFTDYYMH (SEQ ID NO:65),
HCDR2 comprising the amino acid sequence RVNPNRGGTTYNQKFEG (SEQ ID NO:66), and

HCDR3 comprising the amino acid sequence TNWLDY (SEQ ID NO:67);

b) LCDR1 comprising the amino acid sequence SVSSSVSSIYLH (SEQ ID NO:53),

LCDR2 comprising the amino acid sequence STSNLAS (SEQ ID NO:54),

LCDR3 comprising the amino acid sequence QVYSGYPLT (SEQ ID NO:56),

HCDR1 comprising the amino acid sequence GYTFTDYYMH (SEQ ID NO:65),

HCDR2 comprising the amino acid sequence RVNPNRRGTTYNQKFEG (SEQ ID NO:68), and

HCDR3 comprising the amino acid sequence ANWLDY (SEQ ID NO:69), and

c) LCDR1 comprising the amino acid sequence SVSSSVRSIYLH (SEQ ID NO:57),

LCDR2 comprising the amino acid sequence STSNLAS (SEQ ID NO:54),

LCDR3 comprising the amino acid sequence QVYRGYPLT (SEQ ID NO:58),

HCDR1 comprising the amino acid sequence GYTFTDYYMH (SEQ ID NO:65),

HCDR2 comprising the amino acid sequence RVNPYRGSTTYNQKFEG (SEQ ID NO:70), and

HCDR3 comprising the amino acid sequence ANILDY (SEQ ID NO:71).

15. The monoclonal antibody, or antigen-binding fragment thereof of any one of claims 1-3, 8, 9, 11, 12, and 14, which comprises three light chain complementarity

determining regions (LCDRs) and three heavy chain complementarity determining regions (HCDRs), wherein

LCDR1 comprising the amino acid sequence SVSSSVSSIYLH (SEQ ID NO:53),

LCDR2 comprising the amino acid sequence STSNLAS (SEQ ID NO:54),

LCDR3 comprising the amino acid sequence IQYSGYPLT (SEQ ID NO:55),

HCDR1 comprising the amino acid sequence GYTFTDYYMH (SEQ ID NO:65),

HCDR2 comprising the amino acid sequence RVNPNRGGTTYNQKFEG (SEQ ID NO:66), and

HCDR3 comprising the amino acid sequence TNWLDY (SEQ ID NO:67);

16. The monoclonal antibody, or antigen-binding fragment thereof, of claims 1-6, 9, 11, 12, and 14, which comprises three light chain complementarity determining regions (LCDRs) and three heavy chain complementarity determining regions (HCDRs), wherein

LCDR1 comprises the amino acid sequence SVSSSVSSIYLH (SEQ ID NO:53),

LCDR2 comprises the amino acid sequence STSNLAS (SEQ ID NO:54),

LCDR3 comprises the amino acid sequence QVYSGYPLT (SEQ ID NO:56),

HCDR1 comprises the amino acid sequence GYTFTDYYMH (SEQ ID NO:65),

HCDR2 comprises the amino acid sequence RVNPNRRGTTYNQKFEG (SEQ ID NO:68), and

HCDR3 comprises the amino acid sequence ANWLDY (SEQ ID NO:69).

17. The monoclonal antibody, or antigen-binding fragment thereof of any one of claims 1-16, comprising a light chain variable region (LCVR) and a heavy chain variable region (HCVR), wherein said LCVR and said HCVR, respectively, comprise amino acid sequences selected from the group consisting of:

a) SEQ ID NO:94 and SEQ ID NO:96; and

b) SEQ ID NO:95 and SEQ ID NO:97.

18. The monoclonal antibody, or antigen-binding fragment thereof of any one of claims 1- 3, 7, 9, 10, 12, 13 and 17 comprising a light chain variable region (LCVR) and a heavy chain variable region (HCVR), wherein said LCVR comprises SEQ ID NO:94 and said HCVR comprises SEQ ID NO:96.

19. The monoclonal antibody, or antigen-binding fragment thereof of any one of claims 1-6, 8, 9, 11, 12, and 14-17 comprising a light chain variable region (LCVR) and a heavy chain variable region (HCVR), wherein said LCVR comprises SEQ ID NO:95 and said HCVR comprises SEQ ID NO:97.

20. The monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-19, wherein said LCVR and said HCVR comprise amino acid sequences selected from the group consisting of:

a) LCVR is SEQ ID NO:1 and HCVR is SEQ ID NO:13;

b) LCVR is SEQ ID NO:2 and HCVR is SEQ ID NO:14;

c) LCVR is SEQ ID NO:3 and HCVR is SEQ ID NO:15;

d) LCVR is SEQ ID NO:4 and HCVR is SEQ ID NO:16;

e) LCVR is SEQ ID NO:5 and HCVR is SEQ ID NO:17; and

f) LCVR is SEQ ID NO:6 and HCVR is SEQ ID NO:18.

21. The monoclonal antibody or antigen-binding fragment thereof of anyone of claims 1-3, 7, 9, 10, 12, 13, 17, 18 and 20, wherein said LCVR and said HCVR, respectively, comprise amino acid sequences selected from the group consisting of:

a) LCVR is SEQ ID NO:1 and HCVR is SEQ ID NO:13;

b) LCVR is SEQ ID NO:2 and HCVR is SEQ ID NO:14; and

c) LCVR is SEQ ID NO:3 and HCVR is SEQ ID NO:15.

22. The preceding monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-6, 8, 9, 11, 12, 14-17, 19, and 20 wherein said LCVR and said HCVR, comprise amino acid sequences selected from the group consisting of:

a) LCVR is SEQ ID NO:4 and HCVR is SEQ ID NO:16;

b) LCVR is SEQ ID NO:5 and HCVR is SEQ ID NO:17; and

c) LCVR is SEQ ID NO:6 and HCVR is SEQ ID NO:18.

23. The monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-3, 8, 9, 11, 12, 14, 15, 17, 19, 20, and 22, wherein said LCVR comprises the amino acid sequence of SEQ ID NO: 4 and said HCVR comprises the amino acid sequence of SEQ ID NO: 16.

24. The monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-6, 9, 11, 12, 14, 16, 17, 19, 20, and 22, wherein said LCVR comprises the amino acid sequence of SEQ ID NO: 5 and said HCVR comprises the amino acid sequence of SEQ ID NO: 17.

25. The monoclonal antibody of any one of claims 1-24, wherein said antibody comprises a light chain and a heavy chain, wherein the light chain and the heavy chain comprise amino acid sequences which are selected from the group consisting of:

a) light chain is SEQ ID NO:25 and heavy chain is SEQ ID NO:37;

b) light chain is SEQ ID NO:26 and heavy chain is SEQ ID NO:38;

g) light chain is SEQ ID NO:27 and heavy chain is SEQ ID NO:39;

h) light chain is SEQ ID NO:28 and heavy chain is SEQ ID NO:40;

i) light chain is SEQ ID NO:29 and heavy chain is SEQ ID NO:41; and

j) light chain is SEQ ID NO:30 and heavy chain is SEQ ID NO:42.

26. The monoclonal antibody of any one of claims 1-3, 7, 9, 10, 12, 13, 17, 18, 20, 21, and 25, wherein said antibody comprises a light chain and a heavy chain, wherein the

light chain and the heavy chain comprise amino acid sequences which are selected from the group consisting of:

a) light chain is SEQ ID NO:25 and heavy chain is SEQ ID NO:37,

b) light chain is SEQ ID NO:26 and heavy chain is SEQ ID NO:38, and

d) light chain is SEQ ID NO:27 and heavy chain is SEQ ID NO:39.

27. The monoclonal antibody of any one of claims 1-6, 8, 9, 11, 12, 14-17, 19, 20, and 22-25, wherein said antibody comprises a light chain and a heavy chain, wherein the light chain and the heavy chain comprise amino acid sequences which are selected from the group consisting of:

a) light chain is SEQ ID NO:28 and heavy chain is SEQ ID NO:40;

b) light chain is SEQ ID NO:29 and heavy chain is SEQ ID NO:41; and

c) light chain is SEQ ID NO:30 and heavy chain is SEQ ID NO:42.

28. The monoclonal antibody of any one of claims 1-3, 8, 9, 11, 12, 14, 15, 17, 19, 20, 22, 23, 25, and 27, wherein said light chain comprises the amino acid sequence of SEQ ID NO: 28 and said heavy chain comprises the amino acid sequence of SEQ ID NO: 40.

29. The monoclonal antibody of any one of claims 1-6, 9, 11, 12, 14, 16, 17, 19, 20, 22, 24, 25, and 27, wherein said light chain comprises the amino acid sequence of SEQ ID NO: 29 and said heavy chain comprises the amino acid sequence of SEQ ID NO: 41.

30. The monoclonal antibody of any one of claims 1-3, 8, 9, 11, 12, 14, 15, 17, 19, 20, 22, 23, 25, 27, and 28, wherein said antibody comprises two light chains and two heavy chains, wherein each light chain comprises the amino acid sequence of SEQ ID NO: 28 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 40.

31. The monoclonal antibody of any one of claims 1-6, 9, 11, 12, 14, 16, 17, 19, 20, 22, 24, 25, 27, and 29, wherein said antibody comprises two light chains and two

heavy chains, wherein each light chain comprises the amino acid sequence of SEQ ID NO: 29 and each heavy chain comprises the amino acid sequence of SEQ ID NO: 41.

32. A monoclonal antibody or antigen-binding fragment thereof that competes with the c-Met monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-31.

33. A monoclonal antibody or antigen-binding fragment thereof that binds to the same epitope of c-Met as the c-Met monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-32.

34. A pharmaceutical composition, comprising the monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-33, and a pharmaceutically acceptable carrier, diluent, or excipient.

35. A monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-34 for use in therapy.

36. A monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-35 for use in treating a cancer in a human.