Met and/or HGF, Met amplification or mutation has been reported in various cancers such as colorectal, lung, gastric, and kidney cancer and is thought to drive ligand-independent receptor activation (*2, 16*). Additionally, inducible overexpression of Met in a liver mouse model gives rise to hepatocellular carcinoma demonstrating that receptor overexpression drives ligand independent tumorigenesis (*17*). The most compelling evidence implicating Met in cancer is reported in familial and sporadic renal papillary carcinoma (RPC) patients. Mutations in the kinase domain of Met that lead to constitutive activation of the receptor were identified as germline and somatic mutations in RPC (*18*). Introduction of these mutations in transgenic mouse models leads to tumorigenesis and metastasis. (*19*).

Although the role of the Met kinase domain has been investigated in detail, and it has been theorized that increased expression levels of HGF/c-met probably underlie development of some cancers, direct evidence for a biological role for non-kinase domains of c-met has been lacking . Indeed, despite being implicated in the etiology of a variety of oncological conditions, the HGF/-c-met pathway has been a difficult pathway to target therapeutically. Efforts in this regard have been impeded in large part by a lack of understanding regarding mechanisms of action by which dysregulation of HGF/c-met causes tumorigenesis. Therefore, it is clear that the need for greater understanding of c-met-related oncogenic mechanisms of action is great. The invention provided herein meets this need and provides other benefits.

All references cited herein, including patent applications and publications, are incorporated by reference in their entirety.

SUMMARY

The disclosure is based at least in part on the novel finding that certain human tumors express a mutated c-met protein that exhibits decreased rates of down-regulation intracellularly, yet are capable of cell signaling. These "hyperstabilized" c-met proteins were found to have increased oncogenic activity compared to wild-type c-met. As shown herein, these tumors can be inhibited by anti-c-met inhibitors. Inhibition of hyperstabilized c-met activity provides numerous therapeutic advantages. For example, since these c-met mutants are particularly oncogenic, their targeted inhibition would be expected to diminish tumorigenesis driven by these mutants. Moreover, since c-met is found in many cell types, including normal cells, the ability to specifically target tumor-specific c-met mutants would be particularly beneficial, for example in reducing side-effects of c-met inhibition therapy. The disclosure provides methods and compositions based on the findings described herein, and are useful for targeting and/or treating tumors having hyperstabilized c-met.

In one aspect, there is provided use of an antagonist that inhibits c-met signaling activity of a human hyperstabilized c-met polypeptide, wherein the hyperstabilized c-met polypeptide comprises an in-frame deletion of exon 14, the deletion removing the Y1003 phosphorylation site, such that degradation of the polypeptide is diminished compared to wild type c-met, and wherein the hyperstabilized c-met polypeptide has c-met signaling activity, for the manufacture of a medicament for treating a tumor comprising hyperstabilized c-met in a subject, said medicament is to be administered to a subject, whereby the tumor is treated, wherein the antagonist is (i) an antibody that binds to human hyperstabilized c-met polypeptide; or (ii) an inhibitory RNA or antisense oligonucleotide that inhibits expression from a nucleic acid molecule encoding human hyperstabilized c-met polypeptide.

Disclosed is a substance capable of specifically binding to hyperstabilized c-met. In one embodiment, the substance comprises an inhibitory activity against biological activity associated with the hyperstabilized c-met. In another embodiment, the substance is capable of specific binding to the hyperstabilized c-met. In one embodiment, the substance binds to hyperstabilized c-met and inhibits c-met activity. In one embodiment, the substance binds to hyperstabilized c-met without substantially inhibiting c-met activity. These substances find a variety of uses, for example as molecules for targeting therapeutic agents to a cell expressing hyperstabilized c-met. Therapeutic agents include any of the agents described herein, e.g. toxins. Substances can be in any suitable form, including in the form of antibody-drug conjugations and fusion polypeptides.

Disclosed are c-met antagonists that disrupt HGF/c-met signaling associated with a hyperstabilized c-met protein. In one embodiment, the disclosure provides an antagonist that inhibits c-met signaling activity of a human hyperstabilized c-met polypeptide, wherein the hyperstabilized c-met polypeptide comprises a deletion of at least a portion of exon 14 such that its rate of degradation in a cell is diminished compared to wild type c-met, and wherein the hyperstabilized c-met polypeptide has c-met signaling activity.

An antagonist of the disclosure can be of any form capable of specifically inhibiting activity of a hyperstabilized c-met molecule as described herein. In one embodiment, an antagonist of the disclosure comprises an antibody. In one embodiment, an antibody of the disclosure specifically binds to an epitope formed by in-frame splicing of exon 13 and exon 15 of c-met. In one embodiment, at least a portion of exon 14 is deleted as a result of said in-frame splicing. In another embodiment, an antagonist of the disclosure comprises an aptamer. In one embodiment, an aptamer of the disclosure specifically binds to an epitope formed by in-frame splicing of exon 13 and exon 15 of c-met. In one embodiment, at least a portion of exon 14 is deleted as a result of said in-frame splicing. In one embodiment, an antagonist of the disclosure comprises an inhibitory RNA that preferentially/selectively inhibits expression from a nucleic acid molecule encoding a splice variant of c-met wherein exon 13 is spliced to exon 15. In one embodiment, the nucleic acid encodes a hyperstabilized c-met in which at least a portion of exon 14 is deleted as a result of variant splicing. In one embodiment, the disclosure provides an antagonist comprising an antisense oligonucleotide that preferentially/selectively inhibits a nucleic acid molecule encoding a splice variant of c-met wherein exon 13 is spliced to exon 15. In one embodiment, the nucleic acid molecule encodes a hyperstabilized c-met in which at least a portion of exon 14 is deleted as a result of variant splicing.

Inhibition of c-met activity can be effected in any of a number of ways known in the art, so long as biological activity of hyperstabilized c-met is diminished in a cell. For

example, in one embodiment, inhibition of c-met activity by an antagonist of the disclosure comprises enhancement of cellular degradation of the hyperstabilized c-met protein. In another embodiment, inhibition of c-met activity by an antagonist of the disclosure comprises inhibition of phosphorylation of the hyperstabilized c-met protein. In yet another embodiment, inhibition of c-met activity by an antagonist of the disclosure comprises inhibition of phosphorylation of a member of the HGF/c-met signaling pathway by the hyperstabilized c-met. Inhibition of c-met activity by an antagonist of the disclosure can also be effected by reduction of levels of hyperstabilized c-met polypeptide in a cell. Thus, for example, in one embodiment, inhibition of c-met activity by an antagonist of the disclosure comprises inhibition of expression of hyperstabilized c-met protein, for example transcription and/or translation from a polynucleotide encoding a hyperstabilized c-met polypeptide. In another embodiment, inhibition of c-met activity by an antagonist of the disclosure comprises cell death associatd with a cytotoxin linked to a molecule (e.g., an antibody-drug conjugate) that specifically binds to hyperstabilized c-met in a cell.

In one embodiment, an antagonist of the disclosure is a monoclonal antibody, antibody fragment, chimeric antibody, humanized antibody, human antibody, multi-specific antibody or single-chain antibody. Antagonists employed in the methods of the disclosure may optionally be conjugated to a growth inhibitory agent or cytotoxic agent such as a toxin, including, for example, a maytansinoid or calicheamicin, an antibiotic, a radioactive isotope, a nucleolytic enzyme, or the like. In some embodiments of methods of the disclosure, a chemotherapeutic agent is also administered to the subject.

In general, effective c-met antagonists include c-met inhibitors that interfere with binding of a ligand such as HGF to hyperstabilized c-met. For example, a c-met inhibitor may bind to hyperstabilized c-met such that binding of HGF to c-met is inhibited. In one embodiment, an antagonist antibody is a chimeric antibody, for example, an antibody comprising antigen binding sequences from a non-human donor grafted to a heterologous non-human, human or humanized sequence (e.g., framework and/or constant domain sequences). In one embodiment, the non-human donor is a mouse. In one embodiment, an antigen binding sequence is synthetic, e.g. obtained by mutagenesis (e.g., phage display screening, etc.). In one embodiment, a chimeric antibody of the disclosure has murine V regions and human C region. In one embodiment, the murine light chain V region is fused to a human kappa light chain. In one embodiment, the murine heavy chain V region is fused to a human IgG1 C region. In one embodiment, the antigen binding sequences comprise at least one, at least two or all three CDRs of a light and/or heavy chain. In one embodiment, the antigen binding sequences comprise a heavy chain CDR3. In one embodiment, the antigen

binding sequences comprise part or all of the CDR and/or variable domain sequences of the monoclonal antibody produced by the hybridoma cell line deposited under American Type Culture Collection Accession Number ATCC HB-11894 (hybridoma 1A3.3.13) or HB-11895 (hybridoma 5D5.11.6). In one embodiment, the antigen binding sequences comprise at least CDR3 of the heavy chain of the monoclonal antibody produced by the hybridoma cell line 1A3.3.13 or 5D5.11.6. Humanized antibodies of the disclosure include those that have amino acid substitutions in the FR and affinity maturation variants with changes in the grafted CDRs. The substituted amino acids in the CDR or FR are not limited to those present in the donor or recipient antibody. In other embodiments, the antibodies of the disclosure further comprise changes in amino acid residues in the Fc region that lead to improved effector function including enhanced CDC and/or ADCC function and B-cell killing. Other antibodies of the disclosure include those having specific changes that improve stability. Antibodies of the disclosure also include fucose deficient variants having improved ADCC function in vivo.

In one embodiment, an antibody fragment of the disclosure comprises an antigen binding arm comprising a heavy chain comprising at least one, at least two or all three of CDR sequences selected from the group consisting of SYWLH (SEQ ID NO:1), MIDPSNSDTRFNPNFKD (SEQ ID NO:2) and YGSYVSPLDY (SEQ ID NO:3). In one embodiment, the antigen binding arm comprises heavy chain CDR-H1 having amino acid sequence SYWLH. In one embodiment, the antigen binding arm comprises heavy chain CDR-H2 having amino acid sequence MIDPSNSDTRFNPNFKD. In one embodiment, the antigen binding arm comprises heavy chain CDR-H3 having amino acid sequence YGSYVSPLDY. In one embodiment, an antibody fragment of the disclosure comprises an antigen binding arm comprising a light chain comprising at least one, at least two or all three of CDR sequences selected from the group consisting of KSSQSLLYTSSQKNYLA (SEQ ID NO:4), WASTRES (SEQ ID NO:5) and QQYYAYPWT (SEQ ID NO:6). In one embodiment, the antigen binding arm comprises heavy chain CDR-L1 having amino acid sequence KSSQSLLYTSSQKNYLA. In one embodiment, the antigen binding arm comprises heavy chain CDR-L2 having amino acid sequence WASTRES. In one embodiment, the antigen binding arm comprises heavy chain CDR-L3 having amino acid sequence QQYYAYPWT. In one embodiment, an antibody fragment of the disclosure comprises an antigen binding arm comprising a heavy chain comprising at least one, at least two or all three of CDR sequences selected from the group consisting of SYWLH (SEQ ID NO:1), MIDPSNSDTRFNPNFKD (SEQ ID NO:2) and YGSYVSPLDY (SEQ ID NO:3) and a light chain comprising at least one, at least two or all three of CDR sequences selected from

the group consisting of KSSQSLLYTSSQKNYLA (SEQ ID NO:4), WASTRES (SEQ ID NO:5) and QQYYAYPWT (SEQ ID NO:6).

The disclosure provides a humanized antagonist antibody that binds human hyperstabilized c-met, or an antigen-binding fragment thereof, wherein the antibody is effective to inhibit human hyperstabilized HGF/c-met activity in vivo, the antibody comprising in the H chain Variable region (V_H) at least a CDR3 sequence of the monoclonal antibody produced by the hybridoma cell line deposited under American Type Culture Collection Accession Number ATCC HB-11894 (hybridoma 1A3.3.13) or HB-11895 (hybridoma 5D5.11.6) and substantially a human consensus sequence (e.g., substantially the human consensus framework (FR) residues of human heavy chain subgroup III (V_HIII)). In one embodiment, the antibody further comprises the H chain CDR1 sequence and/or CDR2 sequence of the monoclonal antibody produced by the hybridoma cell line deposited under American Type Culture Collection Accession Number ATCC HB-11894 (hybridoma 1A3.3.13) or HB-11895 (hybridoma 5D5.11.6). In another embodiment, the preceding antibody comprises the L chain CDR1 sequence, CDR2 sequence and/or CDR3 sequence of the monoclonal antibody produced by the hybridoma cell line deposited under American Type Culture Collection Accession Number ATCC HB-11894 (hybridoma 1A3.3.13) or HB-11895 (hybridoma 5D5.11.6) with substantially the human consensus framework (FR) residues of human light chain κ subgroup I ($V_κI$).

In one embodiment, an antibody fragment of the disclosure comprises an antigen binding arm comprising a heavy chain variable domain having the sequence:

QVQLQQSGPELVRPGASVKMSCRASGYTFTSYWLHWVKQRPGQGL EWIGMIDPSNSDTRFNPNFKDKATLNVDRSSNTAYMLLSSLTSADSA VYYCATYGSYVSPLDYWGQGTSVTVSS (SEQ ID NO:7)

In one embodiment, an antibody fragment of the disclosure comprises an antigen binding arm comprising a light chain variable domain having the sequence:

DIMMSQSPSSLTVSVGEKVTVSCKSSQSLLYTSSQKNYLAWYQQKPGQSPKL LIYWASTRESGVPDRFTGSGSGTDFTLTITSVKADDLAVYYCQQYYAYPWTFGGGTK LEIK (SEQ ID NO:8)

Yet in other instances, it may be advantageous to have a c-met antagonist that does not interfere with binding of a ligand (such as HGF) to c-met. Accordingly, in some embodiments, an antagonist of the disclosure does not bind a ligand (such as HGF) binding site on c-met. In another embodiment, an antagonist of the disclosure does not substantially inhibit ligand (e.g., HGF) binding to c-met. In one embodiment, an antagonist of the disclosure does not substantially compete with a ligand (e.g., HGF) for binding to c-met. In one example, an antagonist of the disclosure can be used in conjunction with one or more

other antagonists, wherein the antagonists are targeted at different processes and/or functions within the HGF/c-met axis. Thus, in one embodiment, a c-met antagonist of the disclosure binds to an epitope on c-met distinct from an epitope to which another c-met antagonist, such as the Fab fragment of the monoclonal antibody produced by the hybridoma cell line deposited under American Type Culture Collection Accession Number ATCC HB-11894 (hybridoma 1A3.3.13) or HB-11895 (hybridoma 5D5.11.6), binds. In another embodiment, a c-met antagonist of the disclosure is distinct from (i.e., it is not) a Fab fragment of the monoclonal antibody produced by the hybridoma cell line deposited under American Type Culture Collection Accession Number ATCC HB-11894 (hybridoma 1A3.3.13) or HB-11895 (hybridoma 5D5.11.6). In one embodiment, a c-met antagonist of the disclosure does not comprise a c-met binding sequence of an antibody produced by the hybridoma cell line deposited under American Type Culture Collection Accession Number ATCC HB-11894 (hybridoma 1A3.3.13) or HB-11895 (hybridoma 5D5.11.6). In one embodiment, an antagonist of the disclosure inhibits c-met activity but does not bind to a wild-type juxtamembrane domain of c-met.

In one embodiment of a c-met antagonist of the disclosure, binding of the antagonist to c-met inhibits c-met activation by HGF. In one embodiment of a c-met antagonist of the disclosure, binding of the antagonist to c-met in a cell inhibits proliferation, scattering, morphogenesis and/or motility of the cell. In one embodiment, a c-met antagonist of the disclosure binds to hyperstabilized c-met in a cell, resulting in cell death. For example, in one embodiment, the antagonist is linked to a toxin as described herein.

In some embodiments, a c-met antagonist of the disclosure is or comprises a peptide (e.g., an oligopeptide), antibody, antibody fragment, aptamer, oligonucleotide (e.g., antisense oligonucleotide), inhibitory RNA or a combination thereof.

In some embodiments, a c-met antagonist of the disclosure is obtained by a screening or identification method of the disclosure as described herein.

Disclosed are methods for screening for or identifying a c-met antagonist. In one example, said methods comprise contacting a candidate substance with a target molecule comprising at least a portion of hyperstabilized c-met, whereby a substance that specifically binds said target molecule is selected (as a c-met antagonist). In one embodiment, the methods further comprises determining that a selected candidate substance specifically binds to a mutated region of hyperstabilized c-met. For example, if the target molecule comprises a polypeptide, a selected candidate substance should specifically bind to an epitope comprising a mutated position (or region) of hyperstabilized c-met. In another example, if the target molecule comprises a nucleic acid encoding at least a portion of

hyperstabilized c-met, a selected candidate substance should specifically inhibit expression of hyperstabilized c-met protein from a nucleic acid encoding hyperstabilized c-met. In some embodiments, screening methods of the disclosure further comprise contacting a selected substance with a cell expressing hyperstabilized c-met, wherein inhibition of c-met activity in the cell is assessed (e.g., wherein extent of downstream c-met signaling (e.g., MAPK phosphorylation) is detected or quantitated). Inhibition of c-met signaling activity can be assayed in a variety of ways known in the art, and based on any of a variety of criteria known in the art, some of which are described in greater detail herein. For example, inhibition of c-met signaling activity may be indicated by a decrease in amount of c-met activation, which may in turn be indicated by, for instance, amount of c-met-associated cell signaling within a cell. Cell signaling can be assessed by a variety of methods and based on a variety of criteria, which are known in the art, some of which are described herein. For example, occurrence of cell signaling in the HGF/c-met pathway can manifest biologically in the form of change in phosphorylation of target molecules in the signaling pathway. Thus, e.g., amount of protein phosphorylation associated with one or more known phosphorylation targets in the HGF/c-met pathway could be measured. Examples of such phosphorylation targets include c-met itself and mitogen activated protein kinase (MAPK).

Disclosed are compositions comprising one or more antagonists of the disclosure and a carrier. In one embodiment, the carrier is pharmaceutically acceptable.

Disclosed are nucleic acids encoding a c-met antagonist of the disclosure. In one embodiment, a nucleic acid of the disclosure encodes a c-met antagonist which is or comprises a polypeptide (e.g., an oligopeptide). In one embodiment, a nucleic acid of the disclosure encodes a c-met antagonist which is or comprises an antibody or fragment thereof. In one embodiment, a nucleic acid of the disclosure is an aptamer. In one embodiment, a nucleic acid of the disclosure is an antisense oligonucleotide. In one embodiment, a nucleic acid of the disclosure is an inhibitory RNA (e.g., small interfering RNA).

Disclosed are vectors comprising a nucleic acid of the disclosure.

Disclosed are host cells comprising a nucleic acid or a vector of the disclosure. A vector can be of any type, for example a recombinant vector such as an expression vector. Any of a variety of host cells can be used. In one embodiment, a host cell is a prokaryotic cell, for example, *E. coli*. In one embodiment, a host cell is a eukaryotic cell, for example a mammalian cell such as Chinese Hamster Ovary (CHO) cell.

Disclosed are methods for making an antagonist of the disclosure. For example, the disclosure provides a method of making a c-met antagonist which is or comprises an antibody (or fragment thereof), said method comprising expressing in a suitable host cell a recombinant vector of the disclosure encoding said antibody (or fragment thereof), and recovering said antibody. In another example, the disclosure provides a method of making a c-met antagonist which is or comprises a polypeptide (such as an oligopeptide), said method comprising expressing in a suitable host cell a recombinant vector of the disclosure encoding said polypeptide (such as an oligopeptide), and recovering said polypeptide (such as an oligopeptide).

Disclosed is an article of manufacture comprising a container; and a composition contained within the container, wherein the composition comprises one or more c-met antagonists of the disclosure. In one embodiment, the composition comprises a nucleic acid of the disclosure. In one embodiment, a composition comprising antagonist further comprises a carrier, which in some embodiments is pharmaceutically acceptable. In one embodiment, an article of manufacture of the disclosure further comprises instructions for administering the composition (e.g., the antagonist) to a subject.

Disclosed is a kit comprising a first container comprising a composition comprising one or more c-met antagonists of the disclosure; and a second container comprising a buffer. In one embodiment, the buffer is pharmaceutically acceptable. In one embodiment, a composition comprising antagonist further comprises a carrier, which in some embodiments is pharmaceutically acceptable. In one embodiment, a kit further comprises instructions for administering the composition (e.g., the antagonist) to a subject.

Disclosed is use of a c-met antagonist of the disclosure in the preparation of a medicament for the therapeutic and/or prophylactic treatment of a disease, such as a cancer, a tumor, a cell proliferative disorder, an immune (such as autoimmune) disorder and/or an angiogenesis-related disorder. The c-met antagonist can be of any form described herein, including antibody, antibody fragment, polypeptide (e.g., an oligopeptide), nucleic acid (e.g., an oligonucleotide, such as an antisense oligonucleotide, inhibitory RNA), an aptamer, or combination thereof.

Disclosed is use of a nucleic acid of the invention in the preparation of a medicament for the therapeutic and/or prophylactic treatment of a disease, such as a cancer, a tumor, a cell proliferative disorder, an immune (such as autoimmune) disorder and/or an angiogenesis-related disorder.

Disclosed is use of an expression vector of the disclosure in the preparation of a medicament for the therapeutic and/or prophylactic treatment of a disease, such as a cancer, a tumor, a cell proliferative disorder, an immune (such as autoimmune) disorder and/or an angiogenesis-related disorder.

Disclosed is use of a host cell of the disclosure in the preparation of a medicament for the therapeutic and/or prophylactic treatment of a disease, such as a cancer, a tumor, a cell proliferative disorder, an immune (such as autoimmune) disorder and/or an angiogenesis-related disorder.

Disclosed is use of an article of manufacture of the disclosure in the preparation of a medicament for the therapeutic and/or prophylactic treatment of a disease, such as a cancer, a tumor, a cell proliferative disorder, an immune (such as autoimmune) disorder and/or an angiogenesis-related disorder.

Disclosed is use of a kit of the disclosure in the preparation of a medicament for the therapeutic and/or prophylactic treatment of a disease, such as a cancer, a tumor, a cell proliferative disorder, an immune (such as autoimmune) disorder and/or an angiogenesis-related disorder.

The disclosure provides methods and compositions useful for modulating disease states associated with dysregulation of the HGF/c-met signaling axis associated with delayed down-regulation of c-met. The HGF/c-met signaling pathway is involved in multiple biological and physiological functions, including, e.g., cell proliferation and angiogenesis. Thus, disclosed is a method comprising administering to a subject an antagonist that targets hyperstabilized c-met, whereby HGF/c-met signaling is modulated.

Disclosed is a method of treating a tumor in a subject, said method comprising administering an antagonist of the disclosure to a subject, whereby the tumor is treated. In one embodiment, the tumor is determined to comprise hyperstabilized c-met. In one embodiment, the tumor is determined to comprise mutant c-met comprising deletion of at least a portion of exon 14.

In one embodiment of methods of the disclosure, a c-met inhibitor of the disclosure is administered in conjunction with an agent that induces and/or enhances receptor protein degradation.

Disclosed is a method of inhibiting c-met activated cell proliferation, said method comprising contacting a cell or tissue with an effective amount of a c-met antagonist of the disclosure, whereby cell proliferation associated with c-met activation is inhibited.

Disclosed is a method of treating a pathological condition associated with dysregulation of c-met activation in a subject, said method comprising administering to the subject an effective amount of a c-met antagonist of the disclosure, whereby said condition is treated.

Disclosed is a method of inhibiting the growth of a cell that expresses c-met or hepatocyte growth factor, or both, said method comprising contacting said cell with a c-met antagonist of the disclosure thereby causing an inhibition of growth of said cell. In one embodiment, the cell is contacted by HGF expressed by a different cell (e.g., through a paracrine effect).

Disclosed is a method of therapeutically treating a mammal having a cancerous tumor comprising a cell that expresses c-met or hepatocyte growth factor, or both, said method comprising administering to said mammal an effective amount of a c-met antagonist of the disclosure, thereby effectively treating said mammal. In one embodiment, the cell is contacted by HGF expressed by a different cell (e.g., through a paracrine effect).

Disclosed is a method for treating or preventing a cell proliferative disorder associated with increased expression or activity of c-met or hepatocyte growth, or both, said method comprising administering to a subject an effective amount of a c-met antagonist of the disclosure, thereby effectively treating or preventing said cell proliferative disorder. In one embodiment, said proliferative disorder is cancer.

Disclosed is a method for inhibiting the growth of a cell, wherein growth of said cell is at least in part dependent upon a growth potentiating effect of c-met or hepatocyte growth factor, or both, said method comprising contacting said cell with an effective amount of a c-met antagonist of the disclosure, thereby inhibiting the growth of said cell. In one embodiment, the cell is contacted by HGF expressed by a different cell (e.g., through a paracrine effect).

Disclosed is a method of therapeutically treating a tumor in a mammal, wherein the growth of said tumor is at least in part dependent upon a growth potentiating effect of c-met or hepatocyte growth factor, or both, said method comprising contacting said cell with an effective amount of a c-met antagonist of the disclosure, thereby effectively treating said tumor. In one embodiment, the cell is contacted by HGF expressed by a different cell (e.g., through a paracrine effect).

Methods of the disclosure can be used to affect any suitable pathological state, for example, cells and/or tissues associated with dysregulation of the HGF/c-met signaling pathway. In one embodiment, a cell that is targeted in a method of the disclosure is a cancer

cell. For example, a cancer cell can be one selected from the group consisting of a breast cancer cell, a colorectal cancer cell, a lung cancer cell, a papillary carcinoma cell (e.g., of the thyroid gland), a colon cancer cell, a pancreatic cancer cell, an ovarian cancer cell, a cervical cancer cell, a central nervous system cancer cell, an osteogenic sarcoma cell, a renal carcinoma cell, a hepatocellular carcinoma cell, a bladder cancer cell, aprostate cancer cell, a gastric carcinoma cell, a head and neck squamous carcinoma cell, a lymphoma cell, a melanoma cell and a leukemia cell. In one embodiment, a cell that is targeted in a method of the disclosure is a hyperproliferative and/or hyperplastic cell. In one embodiment, a cell that is targeted in a method of the disclosure is a dysplastic cell. In yet another embodiment, a cell that is targeted in a method of the disclosure is a metastatic cell.

Methods of the disclosure can further comprise additional treatment steps. For example, in one embodiment, a method further comprises a step wherein a targeted cell and/or tissue (e.g., a cancer cell) is exposed to radiation treatment and/or a chemotherapeutic agent.

As described herein, c-met activation is an important biological process the dysregulation of which leads to numerous pathological conditions. Accordingly, in one embodiment of methods of the disclosure, a cell that is targeted (e.g., a cancer cell) is one in which activation of c-met is enhanced as compared to a normal cell of the same tissue origin. In one embodiment, a method of the disclosure causes the death of a targeted cell. For example, contact with an antagonist of the disclosure may result in a cell's inability to signal through the c-met pathway, which results in cell death or inhibition of cell growth. In another example, an antagonist of the disclosure targets a linked toxin to a cell expressing hyperstabilized c-met.

Dysregulation of c-met activation (and thus signaling) can result from a number of cellular changes, including, for example, overexpression of HGF (c-met's cognate ligand) and/or c-met itself (due to delayed down-regulation/degradation, increased expression levels, etc.). Accordingly, in some embodiments, a method of the disclosure comprises targeting a cell wherein c-met or hepatoctye growth factor, or both, is more abundantly expressed by said cell (e.g., a cancer cell) as compared to a normal cell of the same tissue origin. A c-met-expressing cell can be regulated by HGF from a variety of sources, i.e. in an autocrine or paracrine manner. For example, in one embodiment of methods of the disclosure, a targeted cell is contacted/bound by hepatocyte growth factor expressed in/by a different cell (e.g., via a paracrine effect). Said different cell can be of the same or of a different tissue origin relative to a targeted cell. In one embodiment, a targeted cell is contacted/bound by HGF expressed by the targeted cell itself (e.g., via an autocrine effect/loop). C-met activation and/or

signaling can also occur independent of ligand. Hence, in one embodiment of methods of the disclosure, c-met activation in a targeted cell occurs independent of ligand.

In one embodiment of methods of the disclosure, the methods further comprise a step of determining whether a tumor cell comprises hyperstabilized c-met (e.g., by detecting a polynucleotide or polypeptide mutation, as described herein).

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts illustrative intronic mutations flanking exon 14 of Met. A schematic representation of Met exon 14 showing the corresponding nucleic acid (NM_000245) deletions and/or point mutations (light grey text) with respect to the intron/exon structure. (A) H596, lung cancer cell line. (B) pat. 14, patient 14 lung tumor specimen. (C) pat. 16, patient 16 lung tumor specimen. For reference, in tumor H596, there is a point mutation from G to T at position marked +1 in (A). In tumor Pat 14, there is a deletion of the sequence from position marked -27 to -6 in (B). In tumor Pat 16, there is a deletion of the sequence from position marked 3195 to +7 in (C).

FIG. 2. Delayed down regulation of hyperstabilized c-met is associated with activation of Met and MAPK. (A) 293 cells co-transfected with Met constructs and Cbl-flag were immunoprecipitated (IP) with V5 or Cbl antibodies followed by immunoblotting (IB) with V5, flag, or P-Tyr antibodies. Lysates were probed with flag or Cbl antibodies. (B) 293 cells were transfected with Met constructs followed by IP of endogenous Cbl. Immunoblotting with V5 antibody shows that Met WT, but not MetΔEx14 co-IPs with endogenous Cbl. The membrane was stripped and reprobed with Y1003, YY1234/1235, Y1349, or Y1365 phospho-specific antibodies. (C) Lysates from transient transfection of 293 cells were immunoprecipitated with V5 antibody and immunoblotted with ubiquitin antibody to detect ubiquitinated Met. The membrane was stripped and reprobed with V5 antibody to detect the presence of Met. Lysates were probed with flag or actin antibodies to detect Cbl-flag or actin for equivalent expression. (D) 293 cells were transfected with the indicated constructs and treated with 10 μg/ml cycloheximide. Lysates were probed with V5 antibody or actin. (E) Serum-starved lung cancer cell lines were stimulated for 10 minutes with 50 ng/ml rhuHGF, then rinsed and returned to serum-free media. Lysates were collected at the indicated times and immunoblotted for P-Met (Y1230/Y1234/Y1235), Met, P-MAPK, MAPK, P-Akt, or Akt. (F) Rat 1A stable clones were serum-starved and treated for 10 minutes with an agonistic Met monoclonal antibody 3D6 (5 μg/ml), rinsed with PBS, and returned to serum-free media. At