US008404453B2

(12) **United States Patent**
Basilico et al.

(10) **Patent No.: US 8,404,453 B2**
(45) **Date of Patent: Mar. 26, 2013**

(54) **HIGH AFFINITY BINDING SITE OF HGFR AND METHODS FOR IDENTIFICATION OF ANTAGONISTS THEREOF**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 613 days.

(21) Appl. No.: **12/465,307**

(22) Filed: **May 13, 2009**

(65) **Prior Publication Data**

US 2009/0298079 A1 Dec. 3, 2009

(30) **Foreign Application Priority Data**

May 14, 2008 (EP) 08103958

(51) **Int. Cl.**

C12Q 1/48	(2006.01)
G01N 33/48	(2006.01)
G01N 33/483	(2006.01)
G01N 33/50	(2006.01)
C07K 14/71	(2006.01)

(52) **U.S. Cl.** **435/7.21**; 435/4; 435/7.1; 435/7.2; 435/7.23; 435/7.8; 435/15; 435/455; 435/325; 530/395

(58) **Field of Classification Search** 435/4, 7.1, 435/7.2, 7.21, 7.23, 7.8, 15, 455, 325; 530/395
See application file for complete search history.

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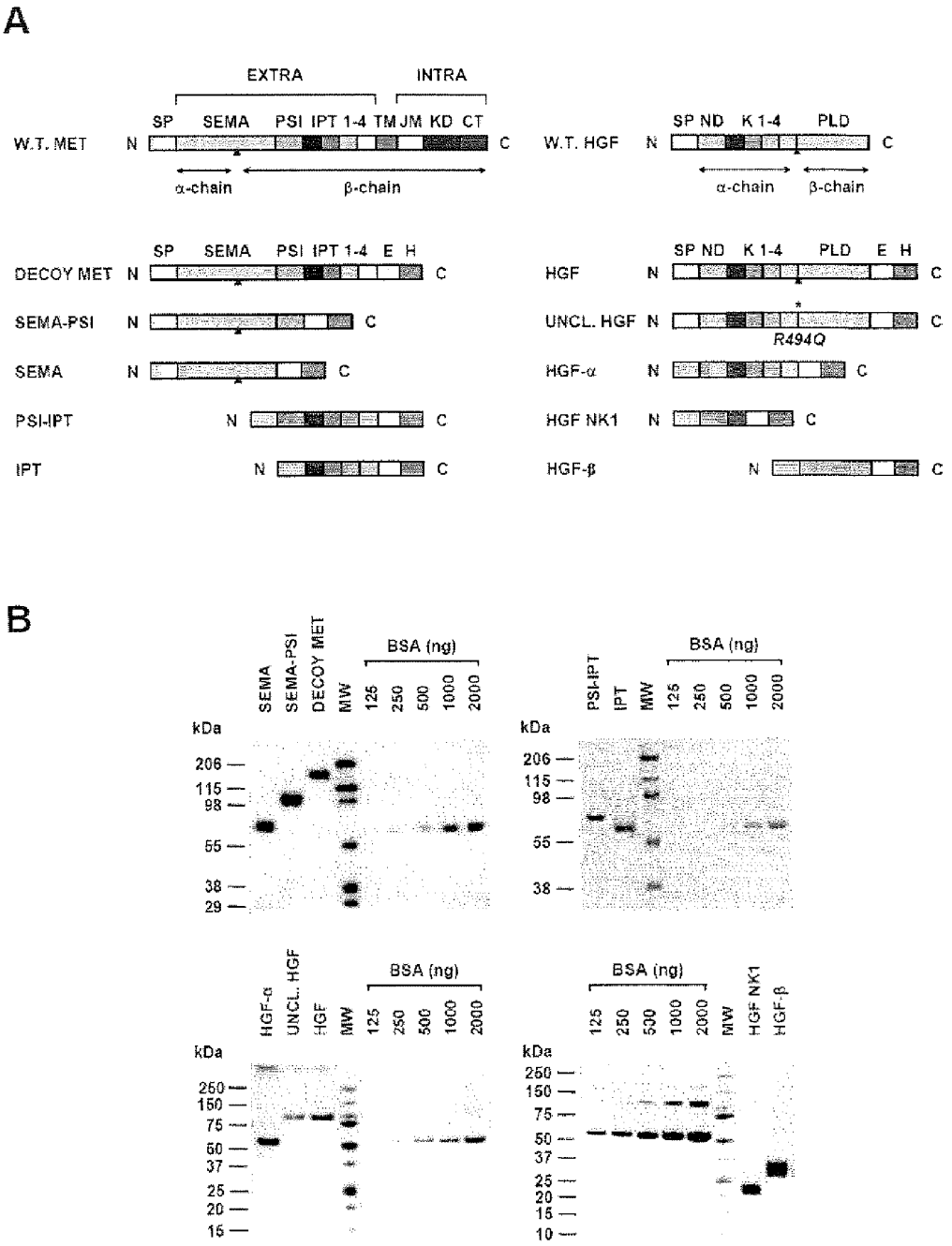
(57) **ABSTRACT**

Use of a polynucleotide encoding or a polypeptide comprising at least the extracellular IPT-3 and IPT-4 domains of hepatocyte growth factor receptor for the screening and/or development of pharmacologically active agents useful in the treatment of cancer, preferably a cancer with dysregulation of hepatocyte growth factor receptor.

13 Claims, 6 Drawing Sheets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EXTRA
INTRA
SP SEMA PSI IPT 1-4 TM JM KD CT
W.T. MET
N
C
α-chain
β-chain
SP ND K 1-4 PLD
W.T. HGF
SP SEMA PSI IPT 1-4 E H
DECOY MET
SEMA-PSI
SEMA
PSI-IPT
IPT
SP ND K 1-4 PLD E H
HGF
UNCL. HGF
R494Q
HGF-α
HGF NK1
HGF-β
B
BSA (ng)
SEMA
SEMA-PSI
DECOY MET
MW
125
250
500
1000
2000
kDa
206
115
98
55
38
29
PSI-IPT
IPT
HGF-α
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HGF
HGF NK1
HGF-β
250
150
75
50
37
25
20
15
10

Figure 1

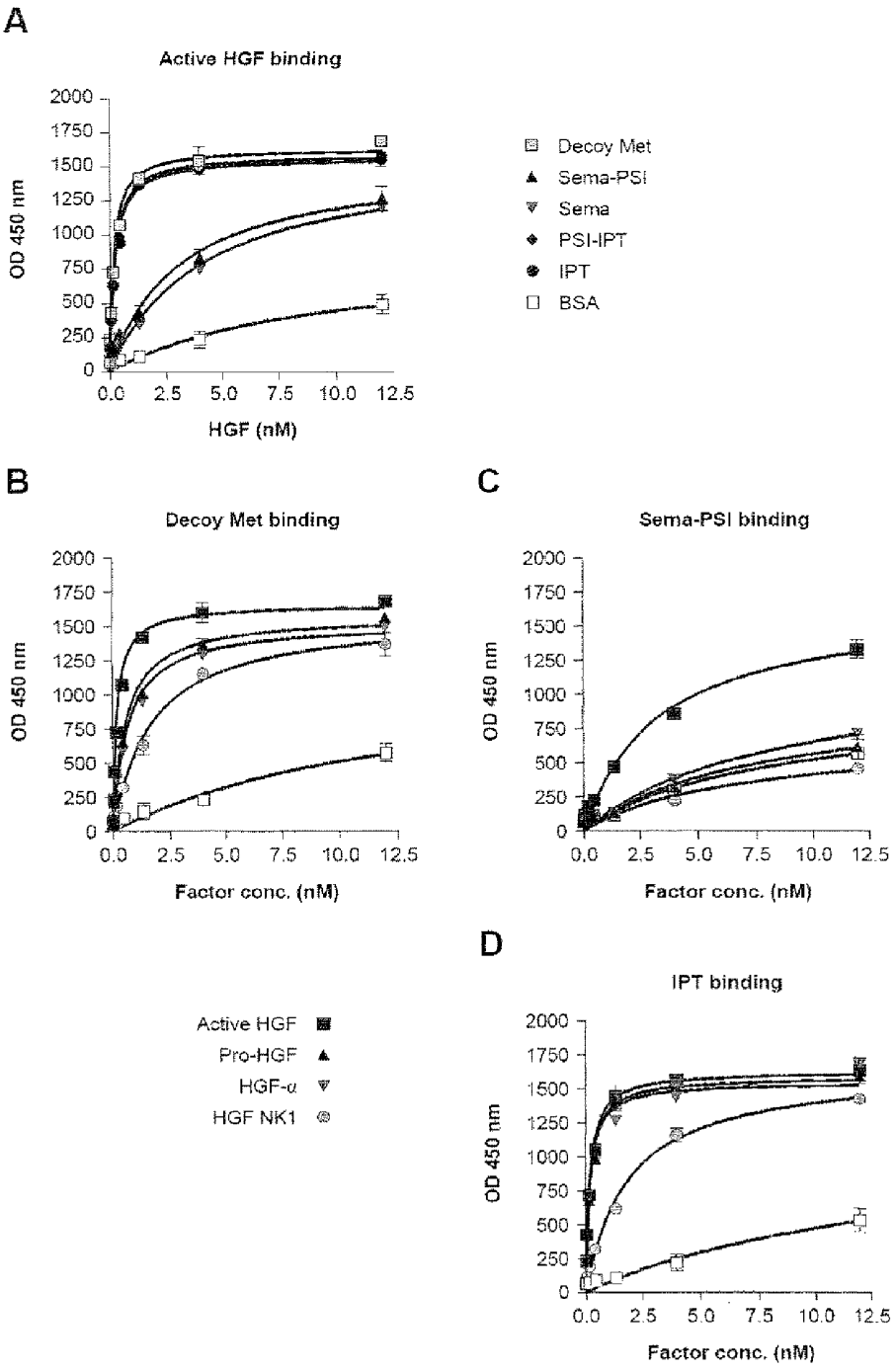
A
Active HGF binding
OD 450 nm
HGF (nM)
Decoy Met
Sema-PSI
Sema
PSI-IPT
IPT
BSA
B
Decoy Met binding
Factor conc. (nM)
C
Sema-PSI binding
Factor conc. (nM)
Active HGF
Pro-HGF
HGF-α
HGF NK1
D
IPT binding
Factor conc. (nM)

Figure 2

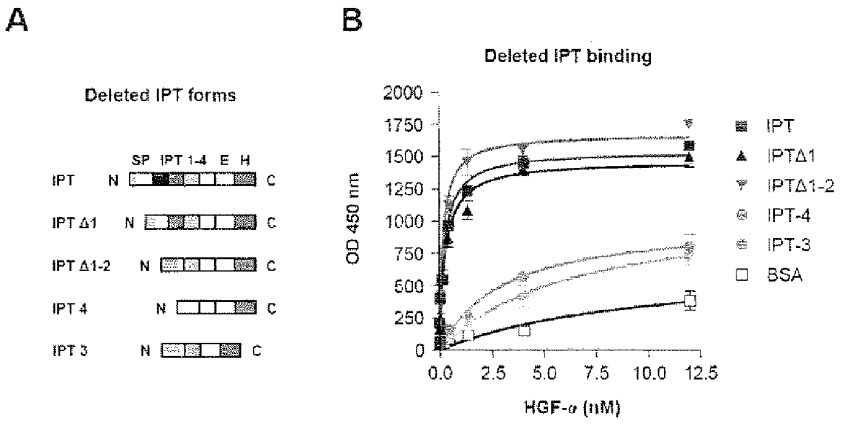
A
Deleted IPT forms
SP IPT 1-4 E H
IPT N C
IPT Δ1 N C
IPT Δ1-2 N C
IPT 4 N C
IPT 3 N C
B
Deleted IPT binding
OD 450 nm
2000
1750
1500
1250
1000
750
500
250
0
0.0 2.5 5.0 7.5 10.0 12.5
HGF-α (nM)
IPT
IPTΔ1
IPTΔ1-2
IPT-4
IPT-3
BSA

Figure 3

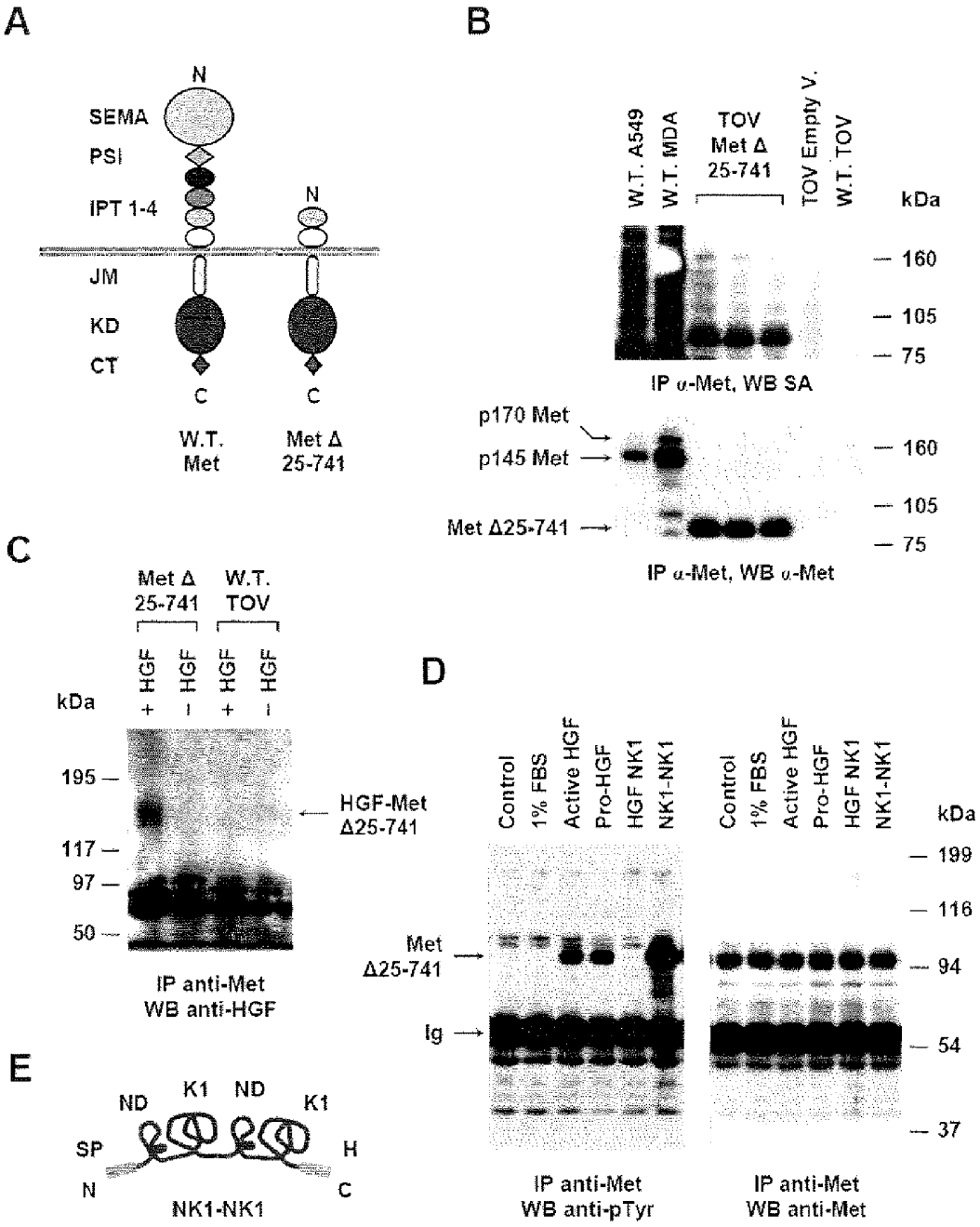
A
N
SEMA
PSI
IPT 1-4
JM
KD
CT
C
W.T. Met
Met Δ 25-741
B
W.T. A549
W.T. MDA
TOV Met Δ 25-741
TOV Empty V.
W.T. TOV
kDa
160
105
75
IP α-Met, WB SA
p170 Met
p145 Met
Met Δ25-741
IP α-Met, WB α-Met
C
Met Δ 25-741
W.T. TOV
HGF
195
117
97
50
HGF-Met Δ25-741
IP anti-Met WB anti-HGF
D
Control
1% FBS
Active HGF
Pro-HGF
HGF NK1
NK1-NK1
Met Δ25-741
Ig
199
116
94
54
37
IP anti-Met WB anti-pTyr
IP anti-Met WB anti-Met
E
ND
K1
SP
H
NK1-NK1

Figure 4

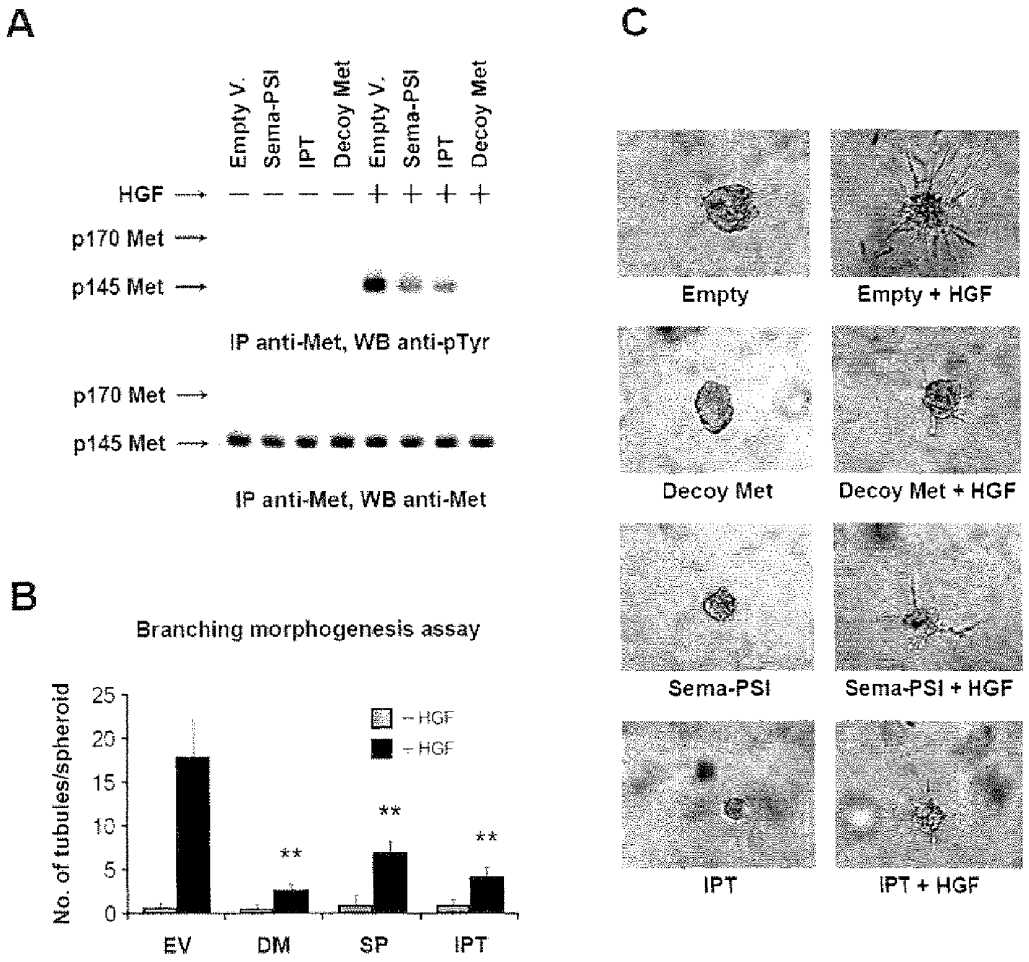
A
Empty V.
Sema-PSI
IPT
Decoy Met
Empty V.
Sema-PSI
IPT
Decoy Met
HGF
p170 Met
p145 Met
IP anti-Met, WB anti-pTyr
p170 Met
p145 Met
IP anti-Met, WB anti-Met
B
Branching morphogenesis assay
No. of tubules/spheroid
25
20
15
10
5
0
- HGF
+ HGF
**
**
**
EV
DM
SP
IPT
C
Empty
Empty + HGF
Decoy Met
Decoy Met + HGF
Sema-PSI
Sema-PSI + HGF
IPT
IPT + HGF

Figure 5

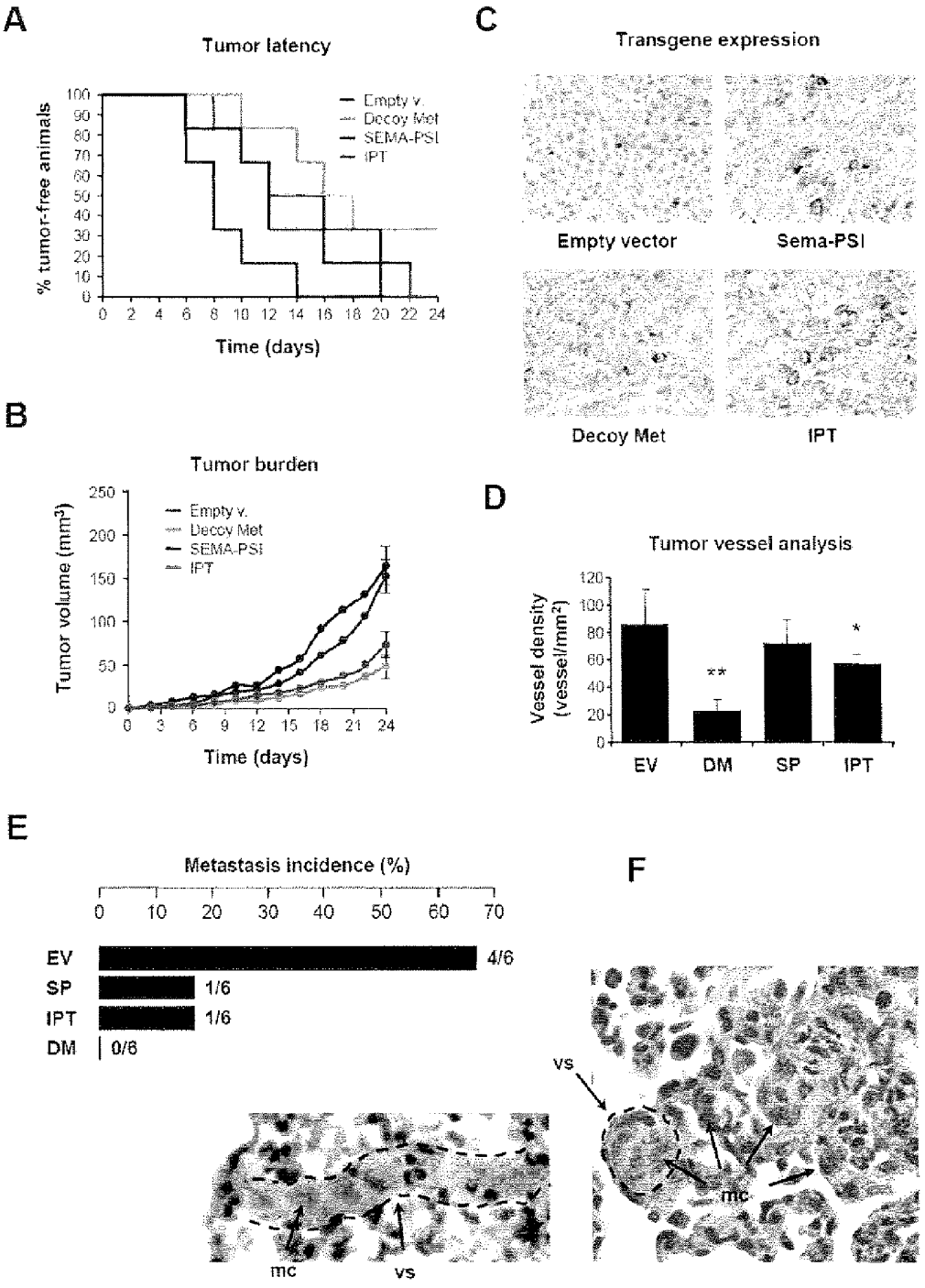
A
Tumor latency
% tumor-free animals
Time (days)
Empty v.
Decoy Met
SEMA-PSI
IPT
B
Tumor burden
Tumor volume (mm3)
Time (days)
C
Transgene expression
Empty vector
Sema-PSI
Decoy Met
IPT
D
Tumor vessel analysis
Vessel density (vessel/mm2)
EV
DM
SP
IPT
E
Metastasis incidence (%)
EV 4/6
SP 1/6
IPT 1/6
DM 0/6
F
vs
mc
mc
vs

Figure 6

HIGH AFFINITY BINDING SITE OF HGFR AND METHODS FOR IDENTIFICATION OF ANTAGONISTS THEREOF

This application claims priority to European Application No. 08103958.8, filed 14 May 2008, the entire contents of which is hereby incorporated by reference.

TECHNICAL FIELD OF THE INVENTION

The present invention relates to the area of the hepatocyte growth factor receptor (HGFR) protein. More specifically, the present invention relates to the identification of the high affinity binding site of HGFR for its ligand, the hepatocyte growth factor (HGF), and methods for the identification of antagonists of HGFR targeting the high affinity binding site of HGFR.

BACKGROUND OF THE INVENTION

The hepatocyte growth factor receptor (also known as Met) is a tyrosine kinase and is the product of the c-met proto-oncogene. It consists of a 50 kDa α-subunit and of a 145 kDa β-subunit, which are linked by a disulfide bond, the α-subunit being completely extracellular, while the β-subunit includes (from N- to C-terminus) an extracellular region, a transmembrane domain and a cytoplasmic tyrosine kinase domain. The mature α/β hetero-dimeric receptor is generated by proteolytic processing and terminal glycosilation from a 170 kDa single-chain precursor.

HGF, also known as Scatter Factor, is a heparin-binding glycoprotein with a broad spectrum of biological activities including cell proliferation, motility, survival and morphogenesis. It is synthesized and secreted as an inactive single chain precursor (pro-HGF) that is stored into the extracellular matrix due to its high affinity for proteoglycans. Pro-HGF undergoes proteolytic cleavage at residues R494-V495 to give rise to the biologically active form, a disulfide-linked α/β hetero-dimer, where the α-chain consists of an N-terminal domain followed by four kringle domains and the β-chain shares structural homology with the chymotrypsin family of serine proteases. The β-chain, however, lacks proteolytic activity since two of the three critical residues that form the catalytic triad typical of serine proteases are not conserved in HGF. Despite its inability to signal, pro-HGF binds to Met at high affinity and displaces active HGF.

Recently, a number of structure-function studies have shed some light onto the interactions between the extracellular portion of Met and HGF.

The Met extracellular region has a modular structure, which encompasses three functional domains: the Sema domain (present also in Semaphorins and plexins) which spans the first 500 residues at the N-terminus of the protein and has a seven-bladed β-propeller structure, the PST domain (also found in Plexins, Semaphorins and Integrins) which covers about 50 residues and contains four conserved disulfide bonds, and additional 400 residues which link the PSI domain to the trans-membrane helix and are occupied by four IPT domains (Immunoglobulin-related domains present in Plexins and Transcription factors).

HGF is a bivalent ligand, containing a high affinity binding site for Met in the α-chain and a low affinity binding site in the β-chain. Cooperation between the α- and the β-chain is required for the biological activity of HGF; while the α-chain, and more precisely the N-domain and the first kringle, is sufficient for Met binding, the β-chain is necessary for Met activation.

Resolution of the crystal structure of the SEMA and PSI domains of Met in complex with the β-chain of HGF (see i.a. WO-A-2005/108424) revealed that the low affinity binding site for HGF is located in blades 2-3 of the β-propeller, and that the portion of HGF-β that binds to Met is the same region that serine proteases use to bind to their substrates or inhibitors. Importantly, determination of HGF β-chain crystal structure at 2.53 Å resolution and specific mutagenesis analysis unveiled that the residues involved in Met binding in the activation pocket of HGF β-chain get exposed only following proteolytic conversion of pro-HGF, thus explaining why pro-HGF binds to Met at high affinity without activating it. While the low affinity interaction between the β-chain of HGF and the Sema domain of Met is well characterized both structurally and functionally, at the moment it is not clear what region of Met binds to the α-chain of HGF at high affinity. Thus, the main mechanism by which HGF activates Met still remains poorly understood. This is somehow surprising when considering the great biological and therapeutic importance of this pathway.

HGF-Met signaling is essential during embryogenesis and in tissue regeneration in the adult life. Importantly, deregulated HGF Met signaling plays a key role in tumorigenesis and metastasis. Inappropriate Met activation by different mechanisms including autocrine HGF stimulation, receptor overexpression, gene amplification and point mutation is described in a wide variety of human malignancies and correlates with poor prognosis. These findings resulted in a growing interest in the HGF-Met pathway as a target for cancer therapy, leading to the development of a variety of Met/HGF inhibitors. These include small molecule compounds targeting Met kinase activity, neutralizing anti Met or anti-HGF antibodies, decoy receptors and HGF-derived factors. Nevertheless, whether such molecules target the high affinity binding site for HGF and which is the exact molecular mechanism at the basis of HGF binding to Met at high affinity, remains still unknown. This may prevent from isolating more selective therapeutic agents, with increased sensitivity and fewer side effects. The exact knowledge of the Met high affinity binding site for HGF will certainly help to design highly specific antagonists of Met.

SUMMARY OF THE INVENTION

The need is therefore felt for improved solutions enabling the identification of Met antagonists with increased sensitivity and fewer side effects for the development of more effective therapeutic strategies for the treatment of cancers.

The object of this disclosure is providing such improved solutions.

According to the present invention such objects are achieved thanks to the solution having the characteristics referred to specifically in the ensuing claims. The claims form integral part of the technical teaching herein provided in relation to the present invention.

Thus, object of the present disclosure is the identification of the HGF high affinity binding site to the hepatocyte growth factor receptor (HGFR), i.e. the extracellular IPT-3 and IPT-4 domains of HGFR. A further object of the present invention is to provide means for the identification of antagonists of HGFR targeting the high affinity biding site of HGFR for HGF for the development of new therapeutic strategies for the treatment of cancers.

In an embodiment, the present invention provides for the use of a polypeptide comprising, or a polynucleotide encoding at least the extracellular IPT-3 and IPT-4 domains of hepatocyte growth factor receptor for the screening and/or

development of pharmacologically active agents useful in the treatment of cancer, in particular a cancer with dysregulation of hepatocyte growth factor receptor activity.

In an embodiment, the pharmacologically active agent is a hepatocyte growth factor receptor inhibitor and/or antagonist and can be selected among small molecule inhibitors, aptamers, antisense nucleotides, RNA-based inhibitors, siRNAs, antibodies, peptides, dominant negative factors.

In a further embodiment, the present invention concerns a method to detect the ability of a test agent to act as an antagonist/inhibitor of hepatocyte growth factor receptor useful in the treatment of cancer, preferably a cancer with dysregulation of hepatocyte growth factor receptor activity, comprising the steps of:

(a) putting in contact a test agent with at least the extracellular IPT-3 and IPT-4 domains of hepatocyte growth factor receptor, or cells expressing at least the extracellular IPT-3 and IPT-4 domains of hepatocyte growth factor receptor,

(b) measuring hepatocyte growth factor receptor activity, function, stability and/or expression, and

(c) selecting the agent that reduces hepatocyte growth factor receptor activity, function, stability and/or expression.

In a still further embodiment, the present invention concerns the use of the extracellular IPT-3 and IPT-4 domains of hepatocyte growth factor receptor as a medicament for treating cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. **1**A and **1**B show the engineering and purification of Met and HGF subdomains. (FIG. **1**A) Schematic representation of the engineered proteins used in this study. Left panel: engineered receptors. W.T. MET, wild-type Met; EXTRA, extracellular portion; INTRA, intracellular portion; SP, signal peptide; SEMA, semaphorin homology domain; PSI, plexin-semaphorin-integrin homology domain; IPT 1-4, immunoglobulin-plexin-transcription factor homology domain 1-4; TM, trans-membrane domain; JM, juxta-membrane domain; KD, kinase domain; CT, C-terminal tail; E, FLAG or MYC epitope; H, poly-histidine tag. The red triangle indicates the proteolytic cleavage site between the α- and β-chain. Right panel: engineered ligands. W.T. HGF, wild-type HGF; ND, N-domain; K 1-4, kringle 1-4; PLD, protease-like domain; UNCL. HGF, Uncleavable HGF. The asterisk indicates the R494Q amino acid substitution in the proteolytic site. (FIG. **1**B) Coomassie staining of affinity-purified receptors and ligands. Each protein group (Sema, Sema-PSI, Decoy Met; PSI-IPT, IPT; HGF-α, Uncleavable HGF, HGF; HGF NK1, HGF-β) has been resolved by SDS-PAGE in non-reducing conditions and is quantified against a standard curve of bovine serum albumin (BSA). MW, molecular weight marker; kDa, kilo-Dalton.

FIGS. **2**A-**2**D show an ELISA analysis of HGF-Met interactions. (FIG. **2**A) Binding of Met sub-domains to active HGF. Engineered receptors were immobilized in solid phase and exposed to increasing concentrations of active HGF in liquid phase. Binding was revealed using anti-HGF antibodies. Non-specific binding was measured by using BSA instead of purified receptors in solid phase. (FIGS. **2**B-**2**D) Binding of Decoy Met, Sema-PSI, and IPT to different forms of HGF. Engineered receptors were immobilized in solid phase and exposed to increasing concentrations of MYC-tagged active HGF, pro-HGF, HGF-α or HGF NK**1** in liquid phase. Binding was revealed using anti-MYC antibodies. Non-specific binding was measured by using MYC-tagged angiostatin (AS) in liquid phase.

FIGS. **3**A and **3**B show that IPT domains **3** and **4** are sufficient to binding to HGF-α at high affinity. (FIG. **3**A) Schematic representation of deleted IPT variants. Color code and legend as in FIG. **1**A. (FIG. **3**B) ELISA analysis of interactions between IPT variants and HGF-α. Engineered IPTs were immobilized in solid phase and exposed to increasing concentrations of HGF-α in liquid phase. Binding was revealed using anti-HGF antibodies.

FIGS. **4**A-**4**E show that IPT domains **3** and **4** are sufficient for binding to HGF in living cells. (FIG. **4**A) Schematic representation of the deleted MetΔ25-741 receptor. Color code and legend as in F*igure* **1**A. (FIG. **4**B) Surface biotinylation analysis. Cellular proteins were immuno-precipitated (IP) using antibodies directed against the C-terminal portion of Met and analyzed by Western blotting (WB) using horseradish peroxidase-conjugated streptavidin (SA). The same blots were re-probed with anti-Met antibodies. W.T., wild-type; A549, A549 human lung carcinoma cells; MDA, MDA-MB-435 human melanoma cells; TOV, TOV-112D human ovary carcinoma cells; Empty V., empty vector. The p170 band corresponds to unprocessed Met; p145 is the mature form of the receptor. (FIG. **4**C) Chemical cross-linking analysis. TOV-112D cells expressing $Met_{\Delta 25\text{-}741}$ (Met Δ25-741) and wild-type TOV-112D cells (W.T. TOV) were incubated with HGF and then subjected to chemical cross-linking. Cell lysates were immuno-precipitated using anti-Met antibodies and analyzed by Western blotting using anti-HGF antibodies. Arrow indicates HGF-$Met_{\Delta 25\text{-}741}$ complexes. (FIG. **4**D) Met phosphorylation analysis. TOV-112D cells expressing $Met_{\Delta 25\text{-}741}$ were stimulated with 1% FBS as a negative control and with equal amounts of HGF, pro-HGF, HGF NK1 or NK1-NK1. Receptor phosphorylation was determined by immuno-precipitation with anti-Met antibodies and Western blotting with anti-phosphotyrosine (anti-pTyr) antibodies. The same blots were re-probed using anti-Met antibodies. Arrows indicate bands corresponding to $Met_{\Delta 25\text{-}741}$ or immunoglobulins (Ig). (FIG. **4**E) Schematic representation of NK1-NK1. From N- to C-terminus: SP, signal peptide; ND, N-domain; K1, kringle 1; H, poly-histidine tag.

FIGS. **5**A-**5**C show that soluble IPT inhibits HGF-induced invasive growth in vitro. (FIG. **5**A) Lentiviral vector transduced MDA-MB-435 cells were stimulated with recombinant HGF and Met phosphorylation was determined by immuno-blotting using anti-phosphotyrosine antibodies (upper panel). The same blot was re-probed using anti-Met antibodies (lower panel). Empty V., Empty Vector. (FIG. **5**B) Branching morphogenesis assay. Pre-formed spheroids of lentiviral vector-transduced MDA-MB-435 cells were embedded in collagen and then stimulated with recombinant HGF to form branched tubules. Collagen invasion was quantified by scoring the mean number of tubules sprouting from each spheroid. EV, Empty Vector; DM, Decoy Met; SP, Sema-PSI. (FIG. **5**C) Representative images from the experiment described in B. Magnification: 200X.

FIGS. **6**A-**6**F show that soluble IPT displays anti-tumor and anti-metastatic activity in mice. CD-1 nu-/- mice were injected subcutaneously with lentiviral vector-transduced MDA-MB-435 cells, and tumor growth was monitored over time. (FIG. **6**A) Kaplan-Meier-like plots of tumor latency (X axis, time in days; Y axis, percent of tumor free-animals). Empty v., empty vector. (FIG. **6**B) Mean tumor volume over time. (FIG. **6**C) Immuno-histochemical analysis of tumor sections using anti-FLAG antibodies. Magnification: 400X. (FIG. **6**D) Tumor vessel analysis. Tumor sections were stained with anti-Von Willebrand factor antibodies. The number of vessels per square mm of tumor section was determined by microscopy. EV, Empty Vector; DM, Decoy Met; SP,

Sema-PSI. (FIG. **6**E) Metastasis incidence analysis. Upon autopsy, serial lung sections were analyzed by microscopy to determine the presence of micrometastases. Metastasis incidence -i.e. the number of mice with metastasis over the total- is indicated in both percentage (bars) and fraction (at the end of bars). (FIG. **6**F) Representative images of micrometastases from the empty vector group. Lung sections were stained with hematoxylin and eosin. Dotted lines identify the walls of blood vessels (vs). Metastatic cells (mc) can be found inside vessels as an embolus or in the parenchyma. Magnification: 400X.

DETAILED DESCRIPTION OF THE INVENTION

The present invention will now be described in detail in relation to some preferred embodiments by way of non-limiting examples with reference to the annexed drawings, wherein:

FIG. **1** shows the engineering and purification of Met and HGF subdomains. (A) Schematic representation of the engineered proteins used in this study. Left panel: engineered receptors. W.T. MET, wild-type Met; EXTRA, extracellular portion; INTRA, intracellular portion; SP, signal peptide; SEMA, semaphorin homology domain; PSI, plexin-semaphorin-integrin homology domain; IPT 1-4, immunoglobulin-plexin-transcription factor homology domain 1-4; TM, trans-membrane domain; JM, juxta-membrane domain; KD, kinase domain; CT, C-terminal tail; E, FLAG or MYC epitope; H, poly-histidine tag. The red triangle indicates the proteolytic cleavage site between the α- and β-chain. Right panel: engineered ligands. W.T. HGF, wild-type HGF; ND, N-domain, K 1-4, kringle 1-4; PLD, protease-like domain; UNCL. HGF, Uncleavable HGF. The asterisk indicates the R494Q amino acid substitution in the proteolytic site. (B) Coomassie staining of affinity-purified receptors and ligands. Each protein group (Sema, Sema-PSI, Decoy Met; PSI-IPT, IPT; HGF-α, Uncleavable HGF, HGF; HGF NK1, HGF-β) has been resolved by SDS-PAGE in non-reducing conditions and is quantified against a standard curve of bovine serum albumin (BSA). MW, molecular weight marker; kDa, kilo-Dalton.

FIG. **2** shows an ELISA analysis of HGF-Met interactions. (A) Binding of Met sub-domains to active HGF. Engineered receptors were immobilized in solid phase and exposed to increasing concentrations of active HGF in liquid phase. Binding was revealed using anti-HGF antibodies. Non-specific binding was measured by using BSA instead of purified receptors in solid phase. (B, C, D) Binding of Decoy Met, Sema-PSI, and IPT to different forms of HGF. Engineered receptors were immobilized in solid phase and exposed to increasing concentrations of MYC-tagged active HGF, pro-HGF, HGF-α or HGF NK1 in liquid phase. Binding was revealed using anti-MYC antibodies. Non-specific binding was measured by using MYC-tagged angiostatin (AS) in liquid phase.

FIG. **3** shows that IPT domains 3 and 4 are sufficient to binding to HGF-a at high affinity. (A) Schematic representation of deleted IPT variants. Color code and legend as in FIG. **1**A. (B) ELISA analysis of interactions between IPT variants and HGF-α. Engineered IPTs were immobilized in solid phase and exposed to increasing concentrations of HGF-α in liquid phase. Binding was revealed using anti-HGF antibodies.

FIG. **4** shows that IPT domains 3 and 4 are sufficient for binding to HGF in living cells. (A) Schematic representation of the deleted MetΔ25-741 receptor. Color code and legend as in FIG. **1**A. (B) Surface biotinylation analysis. Cellular proteins were immuno-precipitated (IP) using antibodies directed against the C-terminal portion of Met and analyzed by Western blotting (WB) using horseradish peroxidase-conjugated streptavidin (SA). The same blots were re-probed with anti-Met antibodies. W.T., wild-type, A549, A549 human lung carcinoma cells; MDA, MDA-MB-435 human melanoma cells; TOV, TOV-112D human ovary carcinoma cells; Empty V., empty vector. The p170 band corresponds to unprocessed Met; p145 is the mature form of the receptor. (C) Chemical cross-linking analysis. TOV-112D cells expressing $Met_{\Delta 25\text{-}741}$ (Met Δ25-741) and wild-type TOV-112D cells (W.T. TOV) were incubated with HGF and then subjected to chemical cross-linking. Cell lysates were immuno-precipitated using anti-Met antibodies and analyzed by Western blotting using anti-HGF antibodies. Arrow indicates HGF-$Met_{\Delta 25\text{-}741}$ complexes. (D) Met phosphorylation analysis. TOV-112D cells expressing $Met_{\Delta 25\text{-}741}$ were stimulated with 1% FBS as a negative control and with equal amounts of HGF, pro-HGF, HGF NK1 or NK1-NK1. Receptor phosphorylation was determined by immuno-precipitation with anti-Met antibodies and Western blotting with anti-phosphotyrosine (anti-pTyr) antibodies. The same blots were re-probed using anti-Met antibodies. Arrows indicate bands corresponding to $Met_{\Delta 25\text{-}741}$ or immunoglobulins (Ig). (F) Schematic representation of NK1-NK1. From N- to C-terminus: SP, signal peptide; ND, N-domain; K1, kringle 1, H, poly-histidine tag.

FIG. **5** shows that soluble IPT inhibits HGF-induced invasive growth in vitro. (A) Lentiviral vector transduced MDA-MB-435 cells were stimulated with recombinant HGF and Met phosphorylation was determined by immuno-blotting using anti-phosphotyrosine antibodies (upper panel). The same blot was re-probed using anti-Met antibodies (lower panel). Empty V., Empty Vector. (B) Branching morphogenesis assay. Pre-formed spheroids of lentiviral vector-transduced MDA-MB-435 cells were embedded in collagen and then stimulated with recombinant HGF to form branched tubules. Collagen invasion was quantified by scoring the mean number of tubules sprouting from each spheroid. EV, Empty Vector; DM, Decoy Met; SP, Sema-PSI. (C) Representative images from the experiment described in B. Magnification: 200×.

FIG. **6** shows that soluble IPT displays anti-tumor and anti-metastatic activity in mice. CD-1 nu−/− mice were injected subcutaneously with lentiviral vector-transduced MDA-MB-435 cells, and tumor growth was monitored over time. (A) Kaplan-Meier-like plots of tumor latency (X axis, time in days; Y axis, percent of tumor free-animals). Empty v., empty vector. (B) Mean tumor volume over time. (C) Immuno-histochemical analysis of tumor sections using anti-FLAG antibodies. Magnification: 400×. (D) Tumor vessel analysis. Tumor sections were stained with anti-Von Willebrand factor antibodies. The number of vessels per square mm of tumor section was determined by microscopy. EV, Empty Vector; DM, Decoy Met; SP, Sema-PSI. (E) Metastasis incidence analysis. Upon autopsy, serial lung sections were analyzed by microscopy to determine the presence of micrometastases. Metastasis incidence—i.e. the number of mice with metastasis over the total—is indicated in both percentage (bars) and fraction (at the end of bars). (F) Representative images of micrometastases from the empty vector group. Lung sections were stained with hematoxylin and eosin. Dotted lines identify the walls of blood vessels (vs). Metastatic cells (mc) can be found inside vessels as an embolus or in the parenchyma. Magnification: 400×.

The data presented in the present disclosure suggest that the α-chain of HGF binds to the IPT region of Met at high affinity, and that it does so independently of proteolytic pro-

cessing of the ligand. They also suggest that HGF binding to IPT in the context of a trans-membrane Met lacking the Sema domain is sufficient for transmitting the signal for receptor activation to the cytoplasmic kinase domain, although without distinction between the inactive and active form of the ligand. Finally, they provide evidence that engineered proteins derived from the IPT region and Sema domain of Met are capable of neutralizing the pro-invasive activity of HGF both in vitro and in vivo.

It has been known for long time that HGF is a bivalent factor. Early protein engineering studies identified a high affinity Met-binding site in the N domain and first kringle of HGF. Subsequently, combined biochemical and biological analysis demonstrated that the HGF serine protease-like domain (β-chain), while not necessary for binding, plays a key role in mediating receptor activation. More recently, detailed crystallographic and mutagenesis data have thoroughly characterized both structurally and functionally the low affinity Met-binding site on the β-chain of HGF and its interaction with the Sema domain of Met. The interface between the α-chain of HGF and Met had remained however elusive. Small angle X-ray scattering and cryo-electron microscopy studies suggested the presence of contacts among the N-terminal and first kringle domain of HGF and the Sema domain of Met. However, plasmon resonance analysis revealed that this interaction has a very low affinity (about 2 times lower than that of HGF-β for Sema and 100 times lower than that of HGF-α for the intact receptor). Since this weak interaction cannot account per se for the tight bond between HGF and Met, the high affinity HGF-binding site on Met had still to be identified.

The results presented here contribute to fill this gap and suggest that this long sought-after HGF-binding site lies in the IPT region of Met and more precisely in the last two immunoglobulin like domains close to the cell membrane. Several distinct experimental evidences provided in the present disclosure suggest that this is the case. Firstly, a soluble, deleted Met receptor containing nothing but the four IPT domains (IPT) binds to HGF with substantially the same affinity as the entire extra-cellular portion of Met. Conversely, Sema displays very low affinity towards HGF. Secondly, IPT binds to active HGF, pro-HGF or HGF-A with unchanged strength. Thirdly, deletion of IPT 1 and IPT 2 does not affect the affinity of IPT for any form of HGF. Fourthly, an engineered Met receptor carrying a large deletion in its ectodomain corresponding to the Sema domain, the PSI module and the first two immunoglobulin-like domains ($Met_{\Delta 25\text{-}741}$) retains the ability to bind to HGF and to transduce the signal for kinase activation to the inside of the cell, although it cannot distinguish between active HGF and Pro-HGF. Finally, a dimeric form of HGF NK1, which is known to contain the minimal Met binding domain of HGF-α, is capable of eliciting activation of $Met_{\Delta 25\text{-}741}$ as efficiently as if not more powerfully than HGF, thus identifying in IPT 3-4 the HGF NK1-binding site.

While these data point at a key role of IPT in HGF binding, it is noteworthy that two previous structure/function studies on the extracellular portion of met failed to identify any ligand binding site in this region. A first draft of the Met ectodomain map suggested that the Sema domain is necessary and sufficient for HGF binding based on ELISA assays. A second study analyzed the role of the Sema domain in receptor dimerization and suggested that an engineered form of the Met extracellular portion containing a deletion in the Sema domain was not capable of co-precipitating HGF.

The present disclosure further demonstrates that cooperation between Sema and IPT is observed also when the extracellular portion of met is used as a biotechnological tool to inhibit HGF-induced invasive growth. In the in vitro analysis and in mouse xenografts both the IPT and Sema-PSI soluble proteins displayed a significant inhibitory effect. However, none of them could achieve the powerful inhibition displayed by the full Met ectodomain, which contains both the low affinity and high affinity HGF-binding site. This implies that both of these interactions contribute to controlling Met activity. While the HGF-β-Sema contact had already been identified as a target for therapy, the results presented here unveil a second interface that offers opportunities for pharmacological intervention. Recombinant proteins or antibodies that bind to the IPT region in place of bona fide HGF have an application as highly competitive inhibitors of Met for the treatment of HGF/Met-dependent cancers.

Materials and Methods

Protein Engineering

Soluble or trans-membrane receptors and engineered ligands described in this work have been generated by standard PCR and genetic engineering techniques. All factors conserve the leader sequence of their parental protein at the N-terminus. The amino acid (aa) sequences of soluble Met proteins (Gene Bank N. X54559) correspond to aa 1-24 (signal peptide) plus: Decoy Met, aa 25-932; Sema, aa 25-515; Sema-PSI, aa 25-562; PSIIPT, aa 516-932; IPT, aa 563-932; IPT Δ1, aa 657-932; IPT Δ1-2, aa 742-932; IPT-3, aa 742-838; IPT-4, aa 839-932. At the C-terminus of each molecule a double FLAG (SDYKDDDDK—SEQ ID No.:19) or single MYC (EQKLISEEDLN—SEQ ID No.:20) epitope sequence and a poly-histidine tag (HHHHHHH—SEQ ID No.:21) were added for protein detection and purification. The trans-membrane engineered MetΔ25-741 is identical to wild-type Met except for the deleted region (aa 25-741). The amino acid sequences of engineered HGF proteins (Gene Bank N. M73239) correspond to aa 1-31 (signal peptide) plus: HGF, aa 32-728; HGF-α, aa 32-473; HGF NK1, aa 32-205; HGF-β, aa 495-728. The above MYC or FLAG epitope and poly-histidine tag were added at the C-terminus. Uncleavable HGF has been described before (Mazzone, M. et al. (2004) *J Clin Invesn.* 114(10), 1418-1432). NK1-NK1 is a dimeric form of HEGF NK1 consisting of the same N-terminal region HGF repeated in tandem (aa 1-205 directly linked to aa 32-205 without spacer). The cDNAs encoding all engineered proteins were subcloned into the lentiviral transfer vector pRRLsin.PPT.CMV.eGFP.Wpre (SEQ ID No.:1) in place of the gfp cDNA as disclosed in Follenzi, A. et al. (2000) *Nat Genet.* 25(2), 217-222. The GFP coding sequence was replaced by the following cDNAs: decoy met FLAG.his (SEQ ID No.:2), Sema FLAG.his (SEQ ID No.:3), Sema-PSI FLAG.his (SEQ ID No.:4), PST-IPT FLAG.his (SEQ ID No.:5), IPT FLAG.his (SEQ ID No.:6), IPT Δ1 FLAG.his (SEQ ID No.:7), IPT Δ1-2 FLAG.his (SEQ ID No.:8), IPT 3 FLAG.his (SEQ ID No.:9), IPT 4 FLAG.his (SEQ ID No.:10), Met Δ25-741 (SEQ ID No.:11), HGF MYC his (SEQ ID No.:12), HGF-α MYC his (SEQ ID No.:13), HGF-NK1 MYC his (SEQ ID No.:14), HGF-β MYC his (SEQ ID No.:15), uncleavable HGF MYC his (SEQ ID No.:16), NK1-NK1 his (SEQ ID No.:17), angiostatin NYC his (SEQ ID No.:18).

Enzyme-Linked Immunosorbant Assays

All engineered receptors and factors were collected from the conditioned medium of lentiviral vector-transduced MDA-MB-345 human melanoma cells in the absence of serum. Factor purification was performed by immobilized-metal affinity chromatography as previously described in Michieli P. et al. (2002) *Nat Biotechnol.* 20(5), 488-495. Conversion of pro-HGF into active HGF was performed by incubating purified pro-HGF (maximal concentration 100

ng/μl) with 2-10% FBS (Sigma, St. Louis, Mo.) at 37° C. for 24 hours. Factor conversion was analyzed by Western blotting using anti-HGF antibodies (R&D Systems, Minneapolis, Minn.). Uncleavable HGF subjected to the same incubation with FBS was used as pro-HGF in all assays that compared active HGF with unprocessed HGF. Binding of engineered ligands to soluble receptors was measured by ELISA using FLAG-tagged soluble receptors in solid phase and MYC-tagged engineered ligands in liquid phase. A fixed concentration of purified soluble receptor (100 ng/well) was adsorbed to 96-well ELISA plates. Protein-coated plates were incubated with increasing concentrations of engineered ligands, and binding was revealed using biotinylated anti-HGF antibodies (R&D Systems, Minneapolis, Minn.) or anti-MYC antibodies (Santa Cruz Biotechnology, Santa Cruz, Calif.). Binding data were analyzed and fit using Prism software (Graph Pad Software, San Diego, Calif.).

Cell Culture

MDA-MB-435 human melanoma cells were purchased from the Georgetown University Tissue Culture Shared Resource (Washington, District of Columbia). Cells were maintained in DMEM supplemented with 10% FBS (Sigma). TOV-112D human ovarian carcinoma cells were obtained from ATCC (Rockville, Md.; ATCC N. CRL-11731) and were cultured using a 1:1 mixture of MCDB 105 Medium and Medium 199 supplemented with 15% BBS (all from Sigma). A549 human lung carcinoma cells were also obtained from ATCC (ATCC N. CCL-185) and maintained in RPMI supplemented with 10% FBS.

Lentiviral Vectors

Vector stocks were produced by transient transfection of 293T cells as previously described in Follenzi, A. et al. (2000) *Nat Genet.* 25 (2), 217-222. Briefly, the plasmid DNA mix for transfection was prepared as follows: ENV plasmid (VSV-G), 9 μg; PACKAGING plasmid pMDLg/pRRE 16.2 μg; REV plasmid, 6.25 μg; TRANSFER VECTOR (plasmid #2-18), 37.5 μg. The plasmids were diluted in a solution of TE/$CaCl_2$, to which a HBS solution was added while vortexing at maximum speed. The DNA/$CaCl_2$/HBS mix was immediately added drop-wise to the cell plates that were then incubated at 37° C. After 14-16 hours the culture medium was replaced with a fresh one. Cell culture supernatants containing vector particles were collected about 36 hours after medium changing. After collection, the supernatants were filtered through 0.2 μm pore membranes and stored at −80° C. Viral p24 antigen concentration was determined by the HIV-1 p24 core profile ELISA kit (NEN Life Science Products, Boston, Mass.) according to the manufacturer's instructions. Cells were transduced in six-well plates (10^5 cells/well in 2 ml of medium) using 40 ng/ml of p24 in the presence of 8 μg/ml polybrene (Sigma) as described in Vigna, E. and Naldini, L. (2000) *J Gene Med.* 2(5), 308-316. Medium was changed about 18 hours after transduction. Cell growth and protein production was monitored over time. Transduced cell lines were then seeded in 15 cm plates, grown to 80% confluence and incubated in medium without serum. After 72 hours, the supernatants containing the recombinant soluble proteins were collected, filtered and purified by affinity chromatography or stored at −30° C.

Immuno-Precipitation and Western Blot Analysis

Cell lysis, immuno-precipitation and Western blot analysis were performed using extraction buffer (EB) as described in Longati, P. et al. (1994) *Oncogene* 9(1), 49-57. Signal was detected using ECL system (Amersham Biosciences, Piscataway, N.J.) according to the manufacturer's instructions. Anti-Met antibodies for immunoprecipitation have been described by Ruco, L. P. et al. (1996) *J Pathol.* 180(3), 266-270 and were purchased from UBI (Lake Placid, N.Y.). Anti-Met antibodies for Western blot were purchased from Santa Cruz. Anti-FLAG antibodies were obtained from Sigma. Met phosphorylation analysis in lentiviral vector transduced MDA-MB-435 cells was performed as previously described in Michieli, P. et al. (2004) *Cancer Cell* 6(1), 61-73.

HGF Cross-Linking and Met Activation Analysis

Lentiviral vector-transduced TOV-112D cells expressing $Met_{\Delta 25\text{-}741}$ were subjected to surface biotinylation analysis using an ECL™ Surface Biotinylation Module kit (Amersham Biosciences) according to the manufacturer's instructions. Chemical cross-linking was performed as previously described in Mazzone, M. et al. (2004) *J Clin Invest.* 114(10), 1418-1432. Briefly, cells were deprived of serum growth factors for 3 days and then incubated with 1 μM HGF for 3 hours. Cell lysates were immuno-precipitated using antibodies directed against the C-terminal portion of Met as disclosed in Ruco, L. P. et al. (1996) *J Pathol.* 180(3), 266-270, resolved by SDS-PAGE using a 3-10% polyacrylamide gradient and analyzed by Western blotting using anti-HGF antibodies (R&D). For receptor activation analysis, TOV-112D cells expressing $Met_{\Delta 25\text{-}741}$ were deprived of serum growth factors for 3 days and then stimulated with 1 nM HGF, Uncleavable HGF, HGF Nk1 or NK1-NK1 for 10 minutes. Cells were lysed using EB as described in Longati, P. et al. (1994) *Oncogene* 9(1), 49-57. Cellular proteins were immuno-precipitated with anti-met antibodies as above and analyzed by Western blotting using anti-phosphotyrosine antibodies (UBI). The same blots were re-probed with anti-Met antibodies (Ruco, L. P. et al. (1996) *J Pathol.* 180(3), 266-270).

Biological Assays

Collagen invasion assays using MDA MB-435 cells were performed using preformed spheroids as described in Michieli, P. et al. (2004) *Cancer Cell* 6(1), 61-73, Briefly, spheroids were generated by incubating cells overnight (700 cells/well) in non-adherent 96-well plates (Greiner, Frickenhausen, Germany) in the presence of 0.24 g/ml methylcellulose (Sigma). Spheroids were embedded into a collagen matrix containing 1.3 mg/ml type I collagen from rat tail (BD Biosciences, Bedford, Mass.) and 10% FBS using 96-well plates (40 spheroids/well), Embedded spheroids were cultured at 37° C. for 24 hours, and then stimulated with 30 ng/ml HGF (R&D) or no factor for additional 24 hours. The number of tubules sprouting from each spheroid was scored by microscopy. At least 12 spheroids per experimental point were analyzed.

Tumorigenesis Assays

Lentiviral vector-transduced MDA-MB-435 tumor cells ($3 \cdot 10^6$ cells/mouse) in 0.2 ml of DMEM were injected subcutaneously into the right posterior flank of six-week old immunodeficient nu−/− female mice on Swiss CD-1 background (Charles River Laboratories, Calco, Italy). Tumor size was evaluated every 2 days using a caliper. Tumor volume was calculated using the formula $V=4/3\pi x2y/2$ where x is the minor tumor axis and y the major tumor axis. A mass of 15 mm^3-corresponding approximately to the initial volume occupied by injected cells was chosen as threshold for tumor positivity. Mice whose tumors were below this threshold were considered tumor-free. After approximately 4 weeks, mice were euthanized and tumors were extracted for analysis. Animals were subjected to autopsy. Tumors and lungs were embedded in paraffin and processed for histology. Micrometastasis analysis was performed by microscopy on serial lung sections stained with hematoxylin and eosin. Tumor sections were stained with hematoxylin and eosin and analyzed by an independent pathologist not informed of sample identity. Transgene expression was determined on tumor sections by

immuno-histochemistry using anti-FLAG antibodies (Sigma). Sections were counterstained with Meyer hematoxylin (Sigma). Tumor angiogenesis was analyzed by immuno-histochemistry using anti-Von Willebrand factor antibodies (DAKO, Glostrup, Denmark). Sections were counterstained as above. Vessel density was assessed by microscopy. At least 12 fields per animal were analyzed. All animal procedures were approved by the Ethical Commission of the University of Turin, Italy, and by the Italian Ministry of Health.

Statistical Analysis

Statistical significance was determined using a two-tail homoscedastic Student's t-Test (array 1, control group; array 2, experimental group). For all data analyzed, a significance threshold of $p<0.05$ was assumed, In all figures, values are expressed as mean±standard deviation, and statistical significance is indicated by a single ($p<0.05$) or double ($p<0.01$) asterisk.

Results

Engineering of HGF/Met Functional Domains

A schematic representation of the functional domains contained in Met and HGF is shown in FIG. **1**A. The extracellular portion of Met includes a Sema domain, a PSI hinge, and four IPT modules (left panel). HGF is composed of an α- and a β-chain joined by a disulphide bridge in the mature protein. The α-chain in turn comprises an N-terminal domain and four kringles (right panel). To analyze the interactions between Met and HGF, the inventors expressed all these functional domains as individual, soluble proteins. Functional domains were engineered to contain the signal peptide of the parental protein at their N-terminus, so that they could be properly secreted. At the C-terminus, an exogenous epitope (FLAG or MYC) for antibody recognition and a poly-histidine tag for protein purification were added. All cDNAs encoding the engineered factors were subcloned into the lentiviral vector pRRLsin.PPT.CMV.Wpre, and recombinant lentiviral particles were produced as described in Materials and Methods. Recombinant proteins were collected from the conditioned medium of lentiviral vector-transduced MDA-MB-435 human melanoma cells and purified to homogeneity by affinity chromatography. Purified proteins were quantified against standards by SDS-PAGE (FIG. **1**B).

ELISA Analysis of Met-HGF Interactions

The ability of Met ectodomains to interact with HGF was tested in ELISA binding assays. Soluble receptors (Decoy Met, Sema-PSI, Sema, PSI-IPT, IPT) were immobilized in solid-phase and exposed to increasing concentrations of active HGF. Binding was revealed using biotinylated anti-HGF antibodies. Non-specific HGF binding was determined using bovine serum albumin (BSA) in solid phase instead of soluble Met domains Binding affinity was determined by nonlinear regression analysis as described in Materials and Methods. In these conditions, decoy Met bound to HGF with a K_D of approximately 0.2-0.3 nM. Consistent with previous measurements, Sema-PSI and Sema bound to HGF with an affinity at least one log lower compared to decoy Met. Surprisingly, both PSI-IPT and IPT bound to HGF very efficiently, with almost the same affinity as decoy Met (FIG. **2**A). The presence or absence of the PSI domain did not affect the binding affinity for HGF of either Sema or IPT. Since almost all Sema domains found so far in nature have a PSI module at their C-terminus, the inventors therefore continued the binding analysis using decoy Met, Sema-PST, and IPT.

To determine the affinity of each Met module for pro-HGF, HGF-α, HGF NK1 and HGF-β and to compare it with that for active HGF, engineered receptors were immobilized in solid phase and exposed to increasing concentrations of MYC-tagged ligands. Binding was revealed using anti-MYC antibodies. Non-specific binding was determined using the kringle-containing protein angiostatin (AS) also tagged with a MYC epitope—in liquid phase. Pro-HGF, HGF α-chain and HGF NK1, which represents the minimal Met-binding module of HGF α-chain, bound to Decoy Met with a 3-, 4- and 10-time reduced affinity compared to active HGF, respectively (FIG. **2**B). Binding of HGF-β to decoy Met (or to any other Met domain) was too low to be detected in this kind of assay. Sema-PSI bound at a significant affinity to active HGF only, while binding to pro-HGF, HGF-α or HGF NK1 was undistinguishable from non-specific binding (FIG. **2**C). In contrast, IPT bound to active HGF, pro-HGF and HGF-α with the same high affinity (FIG. **2**D). HGF NK1 bound to IPT 10 times less tightly than active HGF, i.e. with the same affinity as it bound to Decoy Met. These data suggest that the IPT region of Met binds to the α-chain of HGF at high affinity independently of proteolytic processing of the ligand.

The α-Chain of HGF Binds to IPT Domains 3 and 4 with High Affinity

The IPT region of Met extends for about 400 amino acids and contains four IPT domains. To more finely map the IPT-HGF interface, a series of IPT variants that were deleted in one or more domains were engineered (FIG. **3**A). IPT Δ1 and IPT Δ1-2 are two N-terminal deleted forms of IPT lacking the first or the first two immunoglobulin-like domains, respectively. IPT-3 and IPT-4 correspond to the two C-terminal immunoglobulin-like domains expressed as single proteins. Protein production and purification were performed as described above. The ability of the engineered IPTs to interact with HGF α-chain was investigated in ELISA binding assays using the whole IPT region as a control. IPT, IPT Δ1, IPT Δ1-2, IPT-3 and IPT-4 were immobilized in solid phase and exposed to increasing concentrations of HGF-α. Binding was revealed using anti-HGF antibodies. Non-specific binding was measured using BSA as above. As shown in FIG. **3**B, deletion of the first two immunoglobulin-like domains did not substantially affect HGF binding.

In fact, IPT Δ1-2, a protein corresponding to the last two immunoglobulin-like domains of Met, bound to the α-chain of HGF with equal if not higher strength than IPT. However, further deletion of either the third or fourth immunoglobulin-like domain did almost completely impair HGF-α binding. Similar results were obtained using active HGF or pro-HGF instead of HGF-α. These data suggest that the last two immunoglobulin-like domains of Met, that lie close to the transmembrane helix in the context of a bona fide Met, are sufficient for binding the α-chain of HGF at high affinity.

IPT Domains 3 and 4 are Sufficient for Binding to HGF in Living Cells.

To determine whether HGF could bind to IPT 3 and 4 in the context of a membrane-anchored receptor, a Met protein carrying a large deletion in the extracellular region was engineered. Amino acids 25-741, corresponding to the Sema domain (aa 25-515), the PSI domain (aa 516-562) and the first two IPT domains (IPT 1 and 2, aa 563-741) were deleted, generating a recombinant receptor TO containing IPT domains 3 and 4, the transmembrane helix and the full cytoplasmic region (FIG. **4**A). The cDNA encoding the engineered receptor $Met_{\Delta 25\text{-}741}$ was subcloned into the same lentiviral vector described above. Recombinant lentiviral particles were used to transduce the human ovary carcinoma cell line TOV-112D, which lacks endogenous Met expression as determined by RT-PCR analysis (Michieli, P., et al. (2004) *Cancer Cell* 6(1), 61-73). Surface biotinylation analysis revealed that $Met_{\Delta 25\text{-}741}$ was properly expressed and exposed on the membrane of TOV-112D cells (FIG. **4**B).

To examine whether $Met_{\Delta 25\text{-}741}$ could bind to HGF, lentiviral vector-transduced cells were incubated in the presence or absence of recombinant HGF and subsequently treated them with the cross-linking agent BS3. Cell lysates were immuno-precipitated with an antibody raised against the C-terminal portion of Met, resolved by SDS PAGE and analyzed by western blotting using anti-HGF biotinylated antibodies. As a control, the same analysis was performed on wildtype TOV-112D cells. Immunoblots showed a distinct band with a molecular weight of approximately 180 kD in the lane corresponding to cells expressing $Met_{\Delta 25\text{-}741}$ treated with HGF but not in lanes corresponding to the same cells without HGF or to wild-type TOV-112D cells, either in the presence or absence of the ligand (FIG. **4**C). Considering that both $Met_{\Delta 25\text{-}741}$ and HGF have a molecular weight of approximately 90 kDa, the immuno-precipitated cross linked protein is compatible with a complex formed by HGF plus $Met_{\Delta 25\text{-}741}$.

HGF Binding to IPT Domains 3 and 4 Results in Met Activation in Living Cells.

The inventors next tested whether HGF binding to $Met_{\Delta 25\text{-}741}$ could induce Met kinase activation. To this end, lentiviral vector transduced TOV-112D cells were stimulated with pro-HGF or active HGF, and cell lysates were immunoprecipitated with anti-Met antibodies as above. Receptor activation was determined by Western blot analysis using anti-phosphotyrosine antibodies. The same blots were re-probed with anti-Met antibodies to normalize the amount of receptor immuno-precipitated. Remarkably, both pro-HGF and active HGF were capable of inducing robust phosphorylation of $Met_{\Delta 25\text{-}741}$ (FIG. **4**D). Since pro-HGF binding to full-size Met does not induce kinase activation, this suggests that the Sema domain somehow exerts an auto-inhibitory effect on Met catalytic activity that is released upon binding to active HGF. Receptor stimulation was also performed using HGF NK1 and an engineered dimeric ligand consisting of two NK1 fragments repeated in tandem (NK1-NK1; FIG. **4**E). As shown in FIG. **4**D, NK1-NK1 stimulation of lentiviral vector-transduced TOV-112D cells resulted in potent phosphorylation of $Met_{\Delta 25\text{-}741}$, while stimulation with monomeric NK1 had no effect. These results suggest that the two C-terminal IPT domains of Met (IPT 3 and 4) are sufficient to bind to HGF (and more precisely to HGF NK1 that represents the minimal met-binding module in the α-chain of HGF) and to transmit the signal for receptor activation to the cytoplasmic kinase domain, presumably following ligand-induced receptor dimerization. However, they also suggest that IPT 3 and 4 alone are not sufficient for distinguishing the biologically active form of HGF from its inactive precursor, pro-HGF.

Soluble IPT Inhibits HGF-Induced Invasive Growth In Vitro.

In a previous study, it has been demonstrated that the extracellular portion of Met expressed as a soluble protein (Decoy Met) inhibits HGF-induced invasive growth both in vitro and in mouse models of cancer (Michieli P. et al. (2004) *Cancer Cell* 6(1), 61-73). Recombinant soluble Sema-PSI was also shown to inhibit both ligand-dependent and -independent Met phosphorylation (Kong-Beltran M. et al. (2004) *Cancer Cell* 6 (1), 75-84). Based on these results, it has been tested whether soluble IPT displayed HGF/Met antagonistic activity in living cells. MDA-MB-435 human melanoma cells, which express Met and are an established model system for analysis of HGF-mediated invasive growth, were transduced with lentiviral vectors encoding soluble Decoy Met, Sema-PSI or IPT. Cells transduced with an empty vector were used as control. Lentiviral vector-transduced cells secreting comparable levels of soluble factors (approximately 50 pmol/10^6 cells/24 hours) were serum-starved for several days, allowing the recombinant factors to accumulate in the medium, and then stimulated with recombinant HGF. Met tyrosine phosphorylation was determined by immuno-blotting with anti-phosphotyrosine antibodies as described above. As shown in FIG. **5**A, both IPT and Sema-PSI partially inhibited HGF-induced Met phosphorylation, while Decoy Met completely neutralized the ability of HGF to induce Met activation. Re-probing of the same immuno-blots with antibodies directed against the C-terminal tail of Met revealed no substantial difference in the amounts of immuno-precipitated protein.

To test the inhibitory potential of Met ectodomains in a more biological setting, the same cells were employed to perform an HGF-dependent branching morphogenesis assay. Preformed cell spheroids were seeded in a three dimensional collagen matrix and then stimulated with recombinant HGF to form tubular structures. Branching was quantified by scoring the mean number of tubules sprouting from each colony. As shown in FIG. **5**B, both soluble IPT and Sema-PSI inhibited HGF-induced colony branching (Empty vector, 17.5 tubules/colony; IPT, 4.0 tubules/colony, Sema-PSI, 6.7 tubules/colony). However, consistent with the results obtained in phosphorylation experiments, decoy Met was a more potent HGF-inhibitor than either of its subdomain (2.5 tubules/colony). Representative images of colony morphology are shown in FIG. **5**C.

Soluble IPT Displays Anti-Tumor and Anti-Metastatic Activity in Mice.

The above results prompted the present inventors to explore the therapeutic potential of soluble IPT in mouse models of cancer. Lentiviral vector-transduced MDA-MB-435 melanoma cells were injected subcutaneosly into CD-1 nu−/− mice, and tumor growth was monitored over time. After approximately three weeks, tumors were extracted for analysis, and mice were subjected to autopsy. In a Kaplan-Meier-like analysis, where the percentage of tumor-free animals is plotted against time and tumor latency is quantified calculating the median in days, all engineered soluble receptors delayed the appearance of experimental tumors. However, IPT was slightly more effective than Sema-PSI and decoy Met was more potent than either IPT or Sema-PSI (FIG. **6**A). Analysis of tumor burden over time revealed that IPT was only slightly less effective than decoy Met, while Sema-PSI inhibited neoplastic growth only during the very early stages of the experiment (FIG. **6**B). Immuno-histochemical analysis of transgene expression showed that Decoy Met, Sema-PSI and IPT reached similar levels and distribution in tumors (FIG. **6**C).

As HGF is a potent pro-angiogenic factor, it has been determined whether inhibition of HGF/Met in tumors resulted in impairment of angiogenesis. Tumor sections were analyzed by immuno-histochemistry using antibodies against Von Willebrand factor, and vessel density was assessed by microscopy (FIG. **6**D). IPT decreased tumor vessel density by 1.5 times, while decoy Met achieved a much stronger inhibition (approximately 4 times); Sema-PSI did not significantly affect tumor angiogenesis. Upon autopsy, lungs from the mice described above were extracted and processed for histology. Serial lung sections were stained with hematoxylin and eosin, and analyzed by microscopy to determine the presence of micrometastases. The results are shown in FIG. **6**A. In the control group, 4 out of 6 mice (67%) were bearing micrometastases. In the IPT and Sema PSI group, micrometastases could be found in only 1 out of 6 mice (17%), while no metastasis could be found in the decoy Met group. Meta-

static lesions were both parenchymal (extravascular) and embolic (intravascular; see FIG. **6**F for representative images).

The identification of the high affinity HGF binding site on the HGFR provided in the present disclosure allows designing of novel procedures leading to the generation of more specific inhibitors/antagonists of HGF and of HGFR. The following are non-limiting examples of novel methods for the identification of inhibitors/antagonists of HGF/HGFR targeting the high affinity binding site of HGFR or utilizing the high affinity binding site of HGFR as a tool to generate novel inhibitors/antagonists.

Development of a Monoclonal Antibody that Binds to Extracellular IPT-3 and IPT-4 Domains of HGF Preventing HGF Binding

Given that interaction of HGF with the extracellular IPT-3 and IPT-4 domains is essential for high affinity HGF binding, one can generate specific monoclonal antibodies that bind to IPT-3 and IPT-4 and compete with HGF for HGFR binding. This can be achieved by several strategies.

(A) A recombinant protein or peptide derived from IPT-3 and IPT-4 is generated by standard genetic engineering technology or chemical synthesis. This protein or peptide is injected into an appropriate laboratory animal (usually a mouse or a rat) to give rise to an immune reaction. Splenocytes are then isolated from the immunized animal and fused to a myeloma cell line, and antibody-producing hybridoma clones are selected by standard monoclonal antibody technology. Antibodies directed against IPT-3 and IPT-4 are then screened by an ELISA method similar to the ones described in this disclosure that utilizes recombinant IPT-3 and IPT-4 in solid phase and hybridoma-produced antibodies in liquid phase. Binding is revealed using anti-mouse immuno globulin antibodies that are available commercially. Alternatively, antibodies are screened for their ability to displace recombinant HGF (in liquid phase) from IPT-3 and IPT-4 (in solid phase), or for their ability to immuno-precipitate recombinant IPT-3 and IPT-4 proteins.

(B) A polynucleotide sequence coding for IPT-3 and IPT-4 inserted in an appropriate expression vector is injected directly into a laboratory animal to give rise to an immune response against the gene product. Antibodies directed against IPT-3 and IPT-4 are then isolated and screened as described above.

(C) A polynucleotide sequence coding for IPT-3 and IPT-4 inserted in an appropriate expression vector is transferred into a mammalian cell line not expressing HGFR to obtain expression of IPT-3 and IPT-4 on the cell surface. Cells expressing IPT-3 and IPT-4 are then injected into a laboratory animal to give rise to an immune response, and antibodies directed against IPT-3 and IPT-4 are isolated and screened as described above.

(D) A library of native antibodies generated by standard genetic engineering techniques (for example by the technology known as phage display) from mammalian lymphocytes (preferably human, for example from lymphocytes infiltrating a tumor expressing HGFR) is screened using recombinant IPT-3 and IPT 4 proteins. Positive clones (i.e. those clones that bind to IPT-3 and IPT-4 at high affinity) are then isolated, expanded, and the antibody characterized biochemically.

(E) Human memory B cells are isolated from the peripheral blood of a patient harboring a tumor expressing HGFR as disclosed by several studies including Traggiai E. et al. (2004) *Nat Med.* 10(8), 871-875. Once cultures of immortalized memory B cells are established, a skilled artisan in the field can screen for cells secreting an antibody directed against IPT-3 and IPT-4 using the methods described here above in (A). Having identified such antibody-producing cells, the desired antibody can be cloned by polymerase chain reaction and standard genetic engineering procedures.

Identification of a Test Compound that Binds to Extracellular IPT-3 and IPT-4 Domains of HGFR Inhibiting HGFR Activity

With a different approach, it is possible to isolate test compounds of diverse origin that bind to the high affinity HGF binding site of HGFR and interfere with HGF-induced HGFR activation. This can be achieved by several strategies.

(A) Using ELISA assays similar to those described in this disclosure, a skilled artisan in the field can screen a compound library (including but not limited to a synthetic chemical library, a natural compound library, a small molecule library, a peptide library) for agents that displace HGF interaction with IPT-3 and IPT-4. In this kind of assays, recombinant IPT 3 and IPT-4 protein is immobilized in solid phase and incubated with a fixed amount of HGF in liquid phase. Following exposure to library compounds, HGF binding is measured with commercially available anti-HGF antibodies.

(B) Using a cell line expressing an engineered form of the HGFR containing nothing but the IPT-3 and IPT-4 domains in the extracellular part similar to that described in this disclosure ($Met_{\Delta 25\text{-}741}$), a skilled artisan in the field can screen a compound library (including but not limited to a synthetic chemical library, a natural compound library, a small molecule library, a peptide library) for agents that displace HGF interaction with IPT-3 and IPT-4 or inhibit HGF-induced HGFR activation. This can be achieved by putting said engineered cells in contact with the library and then measuring HGF-induced HGFR phosphorylation as described in the present study, or by other means that reveal HGFR activation including a scatter assay, a reconstituted matrix invasion assay, a branching morphogenesis assay, a cell survival assay or other in vitro biological tests as described in Michieli, P. et al. (2004) *Cancer Cell* 6(1), 61-73.

(C) Using the engineered cell line described above, a skilled artisan in the field can screen a genetic library (including but not limited to a cDNA expression library, a short hairpin RNA library, an antisense DNA library, a random nucleotide library) for polynucleotides or gene products that displace HGF interaction with IPT-3 and IPT 4 or inhibit HGF-induced HGFR activation. This can be achieved by transfecting, transducing or anyway introducing the nucleotide library into said cells and then testing the ability of HGF to activate the deleted form of HGFR expressed by the same cells. HGFR activation is measured as described in (B).

Exemplary Functional Assays that Measure the Biological Activity of Compounds that Bind to Extracellular IPT-3 and IPT-4 Domains of HGFR

Whatever strategy is employed to generate anti-IPT antibodies or IPT-binding compounds, the final product (i.e. monoclonal antibodies directed against IPT-3 and IPT-4 or natural or synthetic compounds that bind to IPT-3 and IPT-4) is then subjected to biological assays aimed at determining whether these agents have the ability to interfere with HGFR activity. These assays can be performed in vitro using cultured mammalian cells or in vivo using laboratory animals.

(A) Scatter assay. Epithelial cells growing in compact colonies in a Petri dish and expressing HGFR are induced to 'scatter' by stimulation with HGF. As a result of HGF stimulation, cells in the Petri dish appear more separated and dispersed. This assay can be performed in the presence of several test compounds. Among the compounds tested, an HGF/HGFR inhibitor/antagonist can be identified by the absence of a scattered phenotype in response to HGF stimulation.

(B) Cell migration assay. Cells expressing HGFR have the ability to migrate towards an HGF gradient. In other words, cells are attracted by chemotaxis towards higher concentrations of HGF. This ability can be exploited to screen for HGF inhibitors in a Boyden chamber assay. Cells are seeded in the first chamber and HGF is applied in the second chamber that is separated from the first by a porous membrane. Cells migrate through the membrane and reach the second chamber were HGF is more concentrated. Agents that inhibit this process are identified as HGF/HGFR inhibitors/antagonists.

(C) Transwell™ migration assay or reconstituted matrix invasion assay. This is a variation of the assay described in (B) in which the porous membrane is covered with a layer of collagen, Matrigel™, or other reconstituted organic matrices. Cells have to digest the organic matrix in order to migrate through it. This is a more rigorous assay that measures invasion rather than simple migration of cells.

(D) Collagen invasion assay or branching morphogenesis assay. In this assay, mammalian cells expressing HGFR (preferably epithelial cells or carcinoma cells) are seeded in a tridimensional layer of collagen and then allowed to grow until they form spheroids of approximately 1,000 cells each. Alternatively, spheroids can be pre-formed ahead by incubating cells overnight in non-adherent 96-well plates in the presence of methylcellulose as disclosed in Michieli, P. et al. (2004) *Cancer Cell* 6(1), 61-73. Once spheroids are embedded in the collagen layer, they are stimulated with HGF and incubated at 37° C. This results in the sprouting of hollow tubules from the spheroids; each tubule is formed by several cells organized in a tubular structure and polarized so that there is a side of the cell that sees the lumen and a second side that sees the medium outside. As the assay goes on tubules tend to branch and to form a more complex architecture. This assay is highly specific for HGF. Agents that inhibit this process have a high probability to represent very specific HGF/HGFR antagonists.

(E) Mitogenic assay. HGF has the ability to induce DNA replication and cell division in some cell expressing HGFR. The most responsive cells are certainly primary hepatocytes, usually mouse or rat. To test HGF-induced DNA replication, cells are deprived of serum growth factors and then stimulated with increasing concentrations of HGF. Shortly after, radioactive thymidine is added and the cells are incubated at 37° C. for approximately one day. Following extensive washing and fixation, radioactive thymidine incorporated into cell DNA is measured by liquid scintillation counting or other standard methods that allow radiation quantification.

(F) Survival assay. HGF has the ability to protect HGFR-expressing cells against apoptosis or programmed cell death. This can be exploited to measure HGFR activity in a survival assay, Cells are preincubated with HGF and the test compound (potential HGFR inhibitor), and then subjected to an apoptotic stimulus such as a toxic drug, absence of adherence, hypoxia, heat shock, radiation, or DNA damage. After an appropriate time interval, cell death is measured by standard methods including TUNEL (Terminal deoxynucleotidyl transferase biotin-dUTP Nick End Labeling), nucleosomes, DNA ladder, caspase activity, vital dye staining or any variation thereof.

(G) Mouse tumorigenesis assay. In this kind of assay, the activity of a potential HGF/HGFR inhibitor is tested directly in a laboratory animal, preferably a mouse or a rat. There are several methods for obtaining a tumor in a mouse. The most utilized strategy is to create a xenograft, i.e. to transplant tumor cells (usually of human origin) into an animal recipient (usually an immunodeficient mouse). Cells can be implanted subcutaneously (a quick and simple method to obtain an experimental tumor) or orthotopically, i.e. in the same organ where the tumor cell has been isolated (e.g. a breast carcinoma into the mammary fat pad, a colon carcinoma into the intestine mucosa, a hepatocarcinoma into the liver parenchyma and so on). Whatever the method employed is, injection of tumor cells into a laboratory animal gives rise to an experimental tumor. This tumor-bearing animal can now be used to evaluate the anti-tumor potential of test compounds. Anti-HGF/HGFR antibodies or compounds can be delivered to an animal carrying a tumor lesion with dysregulated HGF/HGFR signaling by the most appropriate existing method including intravenous injection, intraperitoneal injection, osmotic pump, oral administration, suppository, gene therapy protocol, local administration and so on. After an appropriate treatment period, the animal is euthanized and its tumor and organs are explanted for analysis.

(H) Mouse metastogenesis assay. Experimental metastases can be induced in a mouse by systemic injection of tumor cells. These get entrapped in the lung capillaries and subsequently extravasate to give rise to pulmonary metastases. These can be measured upon autopsy by several approaches including microscopy, histological analysis, immuno-histochemical methods, whole-body imaging. The test compounds are delivered as described in (G).

(I) Gene therapy protocol. In case the HGF/HGFR inhibitor is an antibody, a recombinant protein, a peptide or a small interfering RNA, delivery to the tumor-bearing animal can be achieved by a gene therapy approach. This consists in introducing the desired polynucleotide into an appropriate delivery vector that can be chosen among lentiviral vectors, adenoviral vectors, retroviral vectors, naked DNA, or any variation thereof. The vector preparation can be delivered sytemically or locally to the tumor depending on the tumor site, on the vector or on the tumor histotype. The biological effects of gene therapy are analyzed as described for other compounds in (G).

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gttgctgagt acaagactgg gcctgtgctg gaacacccag attgtttccc atgtcaggac 300

tgcagcagca aagccaattt atcaggaggt gtttggaaag ataacatcaa catggctcta 360

gttgtcgaca cctactatga tgatcaactc attagctgtg gcagcgtcaa cagagggacc 420

tgccagcgac atgtctttcc ccacaatcat actgctgaca tacagtcgga ggttcactgc 480

atattctccc cacagataga agagcccagc cagtgtcctg actgtgtggt gagcgccctg 540

ggagccaaag tcctttcatc tgtaaaggac cggttcatca acttctttgt aggcaatacc 600

ataaattctt cttatttccc agatcatcca ttgcattcga tatcagtgag aaggctaaag 660

gaaacgaaag atggttttat gtttttgacg gaccagtcct acattgatgt tttacctgag 720

ttcagagatt cttaccccat taagtatgtc catgcctttg aaagcaacaa ttttatttac 780

ttcttgacgg tccaaaggga aactctagat gctcagactt ttcacacaag aataatcagg 840

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acagaaaaga gaaaaaagag atccacaaag aaggaagtgt ttaatatact tcaggctgcg 960

tatgtcagca agcctggggc ccagcttgct agacaaatag gagccagcct gaatgatgac 1020

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gccatgtgtg cattccctat caaatatgtc aacgacttct tcaacaagat cgtcaacaaa 1140

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acacttctga gaaattcatc aggctgtgaa gcgcgccgtg atgaatatcg aacagagttt 1260

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agacatttcc agtcctgcag tcaatgcctc tctgccccac cctttgttca gtgtggctgg 1620

tgccacgaca aatgtgtgcg atcggaggaa tgcctgagcg ggacatggac tcaacagatc 1680

tgtctgcctg caatctacaa ggttttccca aatagtgcac cccttgaagg agggacaagg 1740

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actagagttc tccttggaaa tgagagctgc accttgactt taagtgagag cacgatgaat 1860

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acattgaaat gcacagttgg tcctgccatg aataagcatt tcaatatgtc cataattatt 1920

tcaaatggcc acgggacaac acaatacagt acattctcct atgtggatcc tgtaataaca 1980

agtatttcgc cgaaatacgg tcctatggct ggtggcactt tacttacttt aactggaaat 2040

tacctaaaca gtgggaattc tagacacatt tcaattggtg gaaaaacatg tactttaaaa 2100

agtgtgtcaa acagtattct tgaatgttat accccagccc aaaccatttc aactgagttt 2160

gctgttaaat tgaaaattga cttagccaac cgagagacaa gcatcttcag ttaccgtgaa 2220

gatcccattg tctatgaaat tcatccaacc aaatctttta ttagtggtgg gagcacaata 2280

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aagggaaatg atattgaccc tgaagcagtt aaaggtgaag tgttaaaagt tggaaataag 2640

agctgtgaga atatacactt acattctgaa gccgttttat gcacggtccc caatgacctg 2700

ctgaaattga acagcgagct aaatatagag tggaagcaag caatttcttc aaccgtcctt 2760

ggaaaagtaa tagttcaacc agatcagaat ttcacagcta gctctgacta caaggacgac 2820

gatgacaaga gcgattacaa agacgatgat gataagctgc agcatcacca ccatcatcac 2880

cattga 2886

<210> SEQ ID NO 3
<211> LENGTH: 1590
<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: Coding sequence of Sema_FLAGhis

<400> SEQUENCE: 3

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tatcagcttc ccaacttcac cgcggaaaca cccatccaga atgtcattct acatgagcat 180

cacattttcc ttggtgccac taactacatt tatgttttaa atgaggaaga ccttcagaag 240

gttgctgagt acaagactgg gcctgtgctg gaacacccag attgtttccc atgtcaggac 300

tgcagcagca aagccaattt atcaggaggt gtttggaaag ataacatcaa catggctcta 360

gttgtcgaca cctactatga tgatcaactc attagctgtg gcagcgtcaa cagagggacc 420

tgccagcgac atgtctttcc ccacaatcat actgctgaca tacagtcgga ggttcactgc 480

atattctccc cacagataga agagcccagc cagtgtcctg actgtgtggt gagcgccctg 540

ggagccaaag tcctttcatc tgtaaaggac cggttcatca acttctttgt aggcaatacc 600

ataaattctt cttatttccc agatcatcca ttgcattcga tatcagtgag aaggctaaag 660

gaaacgaaag atggttttat gtttttgacg gaccagtcct acattgatgt tttacctgag 720

ttcagagatt cttaccccat taagtatgtc catgcctttg aaagcaacaa ttttatttac 780

ttcttgacgg tccaaaggga aactctagat gctcagactt ttcacacaag aataatcagg 840

ttctgttcca taaactctgg attgcattcc tacatggaaa tgcctctgga gtgtattctc 900

acagaaaaga gaaaaaagag atccacaaag aaggaagtgt ttaatatact tcaggctgcg 960

tatgtcagca agcctggggc ccagcttgct agacaaatag gagccagcct gaatgatgac 1020

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attcttttcg gggtgttcgc acaaagcaag ccagattctg ccgaaccaat ggatcgatct 1080

gccatgtgtg cattccctat caaatatgtc aacgacttct tcaacaagat cgtcaacaaa 1140

aacaatgtga gatgtctcca gcatttttac ggacccaatc atgagcactg ctttaatagg 1200

acacttctga gaaattcatc aggctgtgaa gcgcgccgtg atgaatatcg aacagagttt 1260

accacagctt tgcagcgcgt tgacttattc atgggtcaat tcagcgaagt cctcttaaca 1320

tctatatcca ccttcattaa aggagacctc accatagcta atcttgggac atcagagggt 1380

cgcttcatgc aggttgtggt ttctcgatca ggaccatcaa cccctcatgt gaattttctc 1440

ctggactccc atccagtgtc tccagaagtg attgtggagc atacattaaa ccaaaatggc 1500

gctagctctg actacaagga cgacgatgac aagagcgatt acaaagacga tgatgataag 1560

ctgcagcatc accaccatca tcaccattag 1590

<210> SEQ ID NO 4
<211> LENGTH: 1779
<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: Coding sequence of Sema-PSI_FLAGhis

<400> SEQUENCE: 4

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tatcagcttc ccaacttcac cgcggaaaca cccatccaga atgtcattct acatgagcat 180

cacatttcc ttggtgccac taactacatt tatgttttaa atgaggaaga ccttcagaag 240

gttgctgagt acaagactgg gcctgtgctg gaacacccag attgtttccc atgtcaggac 300

tgcagcagca aagccaattt atcaggaggt gtttggaaag ataacatcaa catggctcta 360

gttgtcgaca cctactatga tgatcaactc attagctgtg gcagcgtcaa cagagggacc 420

tgccagcgac atgtctttcc ccacaatcat actgctgaca tacagtcgga ggttcactgc 480

atattctccc cacagataga agagcccagc cagtgtcctg actgtgtggt gagcgccctg 540

ggagccaaag tcctttcatc tgtaaaggac cggttcatca acttctttgt aggcaatacc 600

ataaattctt cttatttccc agatcatcca ttgcattcga tatcagtgag aaggctaaag 660

gaaacgaaag atggttttat gtttttgacg gaccagtcct acattgatgt tttacctgag 720

ttcagagatt cttaccccat taagtatgtc catgcctttg aaagcaacaa ttttatttac 780

ttcttgacgg tccaaaggga aactctagat gctcagactt ttcacacaag aataatcagg 840

ttctgttcca taaactctgg attgcattcc tacatggaaa tgcctctgga gtgtattctc 900

acagaaaaga gaaaaaagag atccacaaag aaggaagtgt ttaatatact tcaggctgcg 960

tatgtcagca agcctggggc ccagcttgct agacaaatag gagccagcct gaatgatgac 1020

attcttttcg gggtgttcgc acaaagcaag ccagattctg ccgaaccaat ggatcgatct 1080

gccatgtgtg cattccctat caaatatgtc aacgacttct tcaacaagat cgtcaacaaa 1140

aacaatgtga gatgtctcca gcatttttac ggacccaatc atgagcactg ctttaatagg 1200

acacttctga gaaattcatc aggctgtgaa gcgcgccgtg atgaatatcg aacagagttt 1260

accacagctt tgcagcgcgt tgacttattc atgggtcaat tcagcgaagt cctcttaaca 1320

tctatatcca ccttcattaa aggagacctc accatagcta atcttgggac atcagagggt 1380

cgcttcatgc aggttgtggt ttctcgatca ggaccatcaa cccctcatgt gaattttctc 1440

ctggactccc atccagtgtc tccagaagtg attgtggagc atacattaaa ccaaaatggc 1500

tacacactgg ttatcactgg gaagaagatc acgaagatcc cattgaatgg cttgggctgc 1560

agacatttcc agtcctgcag tcaatgcctc tctgccccac cctttgttca gtgtggctgg 1620

tgccacgaca aatgtgtgcg atcggaggaa tgcctgagcg ggacatggac tcaacagatc 1680

tgtctgggag ctagctctga ctacaaggac gacgatgaca agagcgatta caaagacgat 1740

gatgataagc tgcagcatca ccaccatcat caccattga 1779

<210> SEQ ID NO 5
<211> LENGTH: 1458
<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: Coding sequence of PSI-IPT_FLAGhis

<400> SEQUENCE: 5

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aggagcaatg ggtacacact ggttatcact gggaagaaga tcacgaagat cccattgaat 120

ggcttgggct gcagacattt ccagtcctgc agtcaatgcc tctctgcccc acccctttgtt 180

cagtgtggct ggtgccacga caaatgtgtg cgatcggagg aatgcctgag cgggacatgg 240

actcaacaga tctgtctgcc tgcaatctac aaggttttcc caaatagtgc acccccttgaa 300

ggagggacaa ggctgaccat atgtggctgg gactttggat ttcggaggaa taataaattt 360

gatttaaaga aaactagagt tctccttgga aatgagagct gcaccttgac tttaagtgag 420

agcacgatga atacattgaa atgcacagtt ggtcctgcca tgaataagca tttcaatatg 480

tccataatta tttcaaatgg ccacgggaca acacaataca gtacattctc ctatgtggat 540

cctgtaataa caagtatttc gccgaaatac ggtcctatgg ctggtggcac tttacttact 600

ttaactggaa attacctaaa cagtgggaat tctagacaca tttcaattgg tggaaaaaca 660

tgtactttaa aaagtgtgtc aaacagtatt cttgaatgtt ataccccagc ccaaaccatt 720

tcaactgagt ttgctgttaa attgaaaatt gacttagcca accgagagac aagcatcttc 780

agttaccgtg aagatcccat tgtctatgaa attcatccaa ccaaatcttt tattagtggt 840

gggagcacaa taacaggtgt tgggaaaaac ctgaattcag ttagtgtccc gagaatggtc 900

ataaatgtgc atgaagcagg aaggaacttt acagtggcat gtcaacatcg ctctaattca 960

gagataatct gttgtaccac tccttccctg caacagctga atctgcaact ccccctgaaa 1020

accaaagcct ttttcatgtt agatgggatc ctttccaaat actttgatct catttatgta 1080

cataatcctg tgtttaagcc ttttgaaaag ccagtgatga tctcaatggg caatgaaaat 1140

gtactggaaa ttaagggaaa tgatattgac cctgaagcag ttaaaggtga agtgttaaaa 1200

gttggaaata agagctgtga gaatatacac ttacattctg aagccgtttt atgcacggtc 1260

cccaatgacc tgctgaaatt gaacagcgag ctaaatatag agtggaagca agcaatttct 1320

tcaaccgtcc ttggaaaagt aatagttcaa ccagatcaga atttcacagc tagctctgac 1380

tacaaggacg acgatgacaa gagcgattac aaagacgatg atgataagct gcagcatcac 1440

caccatcatc accattag 1458

<210> SEQ ID NO 6
<211> LENGTH: 1290
<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: Coding sequence of IPT_FLAGhis

<400> SEQUENCE: 6

atgaaggccc ccgctgtgct tgcacctggc atcctcgtgc tcctgtttac cttggtgcag 60

aggagcaatg gggaggggg cggtggatcc cctgcaatct acaaggtttt cccaaatagt 120

gcacccctg aaggagggac aaggctgacc atatgtggct gggactttgg atttcggagg 180

aataataaat ttgatttaaa gaaaactaga gttctccttg gaaatgagag ctgcaccttg 240

actttaagtg agagcacgat gaatacattg aaatgcacag ttggtcctgc catgaataag 300

catttcaata tgtccataat tatttcaaat ggccacggga caacacaata cagtacattc 360

tcctatgtgg atcctgtaat aacaagtatt tcgccgaaat acggtcctat ggctggtggc 420

actttactta ctttaactgg aaattaccta aacagtggga attctagaca catttcaatt 480

ggtggaaaaa catgtacttt aaaaagtgtg tcaaacagta ttcttgaatg ttatacccca 540

gcccaaacca tttcaactga gtttgctgtt aaattgaaaa ttgacttagc caaccgagag 600

acaagcatct tcagttaccg tgaagatccc attgtctatg aaattcatcc aaccaaatct 660

tttattagtg gtgggagcac aataacaggt gttgggaaaa acctgaattc agttagtgtc 720

ccgagaatgg tcataaatgt gcatgaagca ggaaggaact ttacagtggc atgtcaacat 780

cgctctaatt cagagataat ctgttgtacc actccttccc tgcaacagct gaatctgcaa 840

ctccccctga aaaccaaagc ctttttcatg ttagatggga tcctttccaa atactttgat 900

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ggcaatgaaa atgtactgga aattaaggga aatgatattg accctgaagc agttaaaggt 1020

gaagtgttaa aagttggaaa taagagctgt gagaatatac acttacattc tgaagccgtt 1080

ttatgcacgg tccccaatga cctgctgaaa ttgaacagcg agctaaatat agagtggaag 1140

caagcaattt cttcaaccgt ccttggaaaa gtaatagttc aaccagatca gaatttcaca 1200

gctagctctg actacaagga cgacgatgac aagagcgatt acaaagacga tgatgataag 1260

ctgcagcatc accaccatca tcaccattga 1290

<210> SEQ ID NO 7
<211> LENGTH: 1008
<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: Coding sequence of IPTdelta1_FLAGhis

<400> SEQUENCE: 7

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aggagcaatg gggaggggg cggtggatct cctgtaataa caagtatttc gccgaaatac 120

ggtcctatgg ctggtggcac tttacttact ttaactggaa attacctaaa cagtgggaat 180

tctagacaca tttcaattgg tggaaaaaca tgtactttaa aaagtgtgtc aaacagtatt 240

cttgaatgtt ataccccagc ccaaaccatt tcaactgagt ttgctgttaa attgaaaatt 300

gacttagcca accgagagac aagcatcttc agttaccgtg aagatcccat tgtctatgaa 360

attcatccaa ccaaatcttt tattagtggt gggagcacaa taacaggtgt tgggaaaaac 420

ctgaattcag ttagtgtccc gagaatggtc ataaatgtgc atgaagcagg aaggaacttt 480

acagtggcat gtcaacatcg ctctaattca gagataatct gttgtaccac tccttccctg 540

caacagctga atctgcaact ccccctgaaa accaaagcct ttttcatgtt agatgggatc 600

ctttccaaat actttgatct catttatgta cataatcctg tgtttaagcc ttttgaaaag 660

ccagtgatga tctcaatggg caatgaaaat gtactggaaa ttaagggaaa tgatattgac 720

cctgaagcag ttaaaggtga agtgttaaaa gttggaaata agagctgtga gaatatacac 780

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ttacattctg aagccgtttt atgcacggtc cccaatgacc tgctgaaatt gaacagcgag 840

ctaaatatag agtggaagca agcaatttct tcaaccgtcc ttggaaaagt aatagttcaa 900

ccagatcaga atttcacagc tagctctgac tacaaggacg acgatgacaa gagcgattac 960

aaagacgatg atgataagct gcagcatcac caccatcatc accattga 1008

<210> SEQ ID NO 8
<211> LENGTH: 753
<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: Coding sequence of IPTdelta1-2_FLAGhis

<400> SEQUENCE: 8

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aggagcaatg gggaggggg cggtggatct cccattgtct atgaaattca tccaaccaaa 120

tcttttatta gtggtgggag cacaataaca ggtgttggga aaaacctgaa ttcagttagt 180

gtcccgagaa tggtcataaa tgtgcatgaa gcaggaagga actttacagt ggcatgtcaa 240

catcgctcta attcagagat aatctgttgt accactcctt ccctgcaaca gctgaatctg 300

caactccccc tgaaaaccaa agccttttc atgttagatg ggatcctttc caaatacttt 360

gatctcattt atgtacataa tcctgtgttt aagccttttg aaaagccagt gatgatctca 420

atgggcaatg aaaatgtact ggaaattaag ggaaatgata ttgaccctga agcagttaaa 480

ggtgaagtgt taaaagttgg aaataagagc tgtgagaata tacacttaca ttctgaagcc 540

gttttatgca cggtccccaa tgacctgctg aaattgaaca gcgagctaaa tatagagtgg 600

aagcaagcaa tttcttcaac cgtccttgga aaagtaatag ttcaaccaga tcagaatttc 660

acagctagct ctgactacaa ggacgacgat gacaagagcg attacaaaga cgatgatgat 720

aagctgcagc atcaccacca tcatcaccat tga 753

<210> SEQ ID NO 9
<211> LENGTH: 471
<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: Coding sequence of IPT3_FLAGhis

<400> SEQUENCE: 9

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aggagcaatg gggaggggg cggtggatct cccattgtct atgaaattca tccaaccaaa 120

tcttttatta gtggtgggag cacaataaca ggtgttggga aaaacctgaa ttcagttagt 180

gtcccgagaa tggtcataaa tgtgcatgaa gcaggaagga actttacagt ggcatgtcaa 240

catcgctcta attcagagat aatctgttgt accactcctt ccctgcaaca gctgaatctg 300

caactccccc tgaaaaccaa agccttttc atgttagatg ggatcctttc caaatacttt 360

gatctcattt atgtacataa tgctagctct gactacaagg acgacgatga caagagcgat 420

tacaaagacg atgatgataa gctgcagcat caccaccatc atcaccattg a 471

<210> SEQ ID NO 10
<211> LENGTH: 462
<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: Coding sequence of IPT4_FLAGhis

<400> SEQUENCE: 10

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atgaaggccc ccgctgtgct tgcacctggc atcctcgtgc tcctgtttac cttggtgcag 60

aggagcaatg gggaggggggg cggtggatct cctgtgttta agccttttga aaagccagtg 120

atgatctcaa tgggcaatga aaatgtactg gaaattaagg gaaatgatat tgaccctgaa 180

gcagttaaag gtgaagtgtt aaaagttgga aataagagct gtgagaatat acacttacat 240

tctgaagccg ttttatgcac ggtccccaat gacctgctga aattgaacag cgagctaaat 300

atagagtgga agcaagcaat ttcttcaacc gtccttggaa aagtaatagt tcaaccagat 360

cagaatttca cagctagctc tgactacaag gacgacgatg acaagagcga ttacaaagac 420

gatgatgata agctgcagca tcaccaccat catcaccatt ga 462

<210> SEQ ID NO 11
<211> LENGTH: 2040
<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: Coding sequence for Met delta 25-741

<400> SEQUENCE: 11

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aggagcaatg gggaggggggg cggtggatct cccattgtct atgaaattca tccaaccaaa 120

tcttttatta gtggtgggag cacaataaca ggtgttggga aaaacctgaa ttcagttagt 180

gtcccgagaa tggtcataaa tgtgcatgaa gcaggaagga actttacagt ggcatgtcaa 240

catcgctcta attcagagat aatctgttgt accactcctt ccctgcaaca gctgaatctg 300

caactccccc tgaaaaccaa agccttttttc atgttagatg ggatcctttc caaatacttt 360

gatctcattt atgtacataa tcctgtgttt aagccttttg aaaagccagt gatgatctca 420

atgggcaatg aaaatgtact ggaaattaag ggaaatgata ttgaccctga agcagttaaa 480

ggtgaagtgt taaaagttgg aaataagagc tgtgagaata tacacttaca ttctgaagcc 540

gttttatgca cggtccccaa tgacctgctg aaattgaaca gcgagctaaa tatagagtgg 600

aagcaagcaa tttcttcaac cgtccttgga aaagtaatag ttcaaccaga tcagaatttc 660

acaggattga ttgctggtgt tgtctcaata tcaacagcac tgttattact acttgggttt 720

ttcctgtggc tgaaaaagag aaagcaaatt aaagatctgg gcagtgaatt agttcgctac 780

gatgcaagag tacacactcc tcatttggat aggcttgtaa gtgcccgaag tgtaagccca 840

actacagaaa tggtttcaaa tgaatctgta gactaccgag ctacttttcc agaagatcag 900

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ttgaacagaa tcactgacat aggagaagtt tcccaatttc tgaccgaggg aatcatcatg 1260

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gagactcata atccaactgt aaaagatctt attggctttg gtcttcaagt agccaaaggc 1440

atgaaatatc ttgcaagcaa aaagtttgtc cacagagact tggctgcaag aaactgtatg 1500

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gaactggtgt cccggatatc agcaatcttc tctactttca ttggggagca ctatgtccat 1920

gtgaacgcta cttatgtgaa cgtaaaatgt gtcgctccat atccttctct gttgtcatca 1980

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<210> SEQ ID NO 12
<211> LENGTH: 2259
<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: Coding sequence for HGF_MYChis

<400> SEQUENCE: 12

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ctgctcccca tcgccatccc ctatgcagag ggacaaagga aaagaagaaa tacaattcat 120

gaattcaaaa aatcagcaaa gactacccta atcaaaatag atccagcact gaagataaaa 180

accaaaaaag tgaatactgc agaccaatgt gctaatagat gtactaggaa taaaggactt 240

ccattcactt gcaaggcttt tgtttttgat aaagcaagaa aacaatgcct ctggttcccc 300

ttcaatagca tgtcaagtgg agtgaaaaaa gaatttggcc atgaatttga cctctatgaa 360

aacaaagact acattagaaa ctgcatcatt ggtaaaggac gcagctacaa gggaacagta 420

tctatcacta agagtggcat caaatgtcag ccctggagtt ccatgatacc acacgaacac 480

agctttttgc cttcgagcta tcggggtaaa gacctacagg aaaactactg tcgaaatcct 540

cgaggggaag aaggggacc ctggtgtttc acaagcaatc cagaggtacg ctacgaagtc 600

tgtgacattc ctcagtgttc agaagttgaa tgcatgacct gcaatgggga gagttatcga 660

ggtctcatgg atcatacaga atcaggcaag atttgtcagc gctgggatca tcagacacca 720

caccggcaca aattcttgcc tgaaagatat cccgacaagg gctttgatga taattattgc 780

cgcaatcccg atggccagcc gaggccatgg tgctatactc ttgaccctca cacccgctgg 840

gagtactgtg caattaaaac atgcgctgac aatactatga atgacactga tgttcctttg 900

gaaacaactg aatgcatcca aggtcaagga gaaggctaca ggggcactgt caataccatt 960

tggaatggaa ttccatgtca gcgttgggat tctcagtatc ctcacgagca tgacatgact 1020

cctgaaaatt tcaagtgcaa ggacctacga gaaaattact gccgaaatcc agatgggtct 1080

gaatcaccct ggtgttttac cactgatcca aacatccgag ttggctactg ctcccaaatt 1140

ccaaactgtg atatgtcaca tggacaagat tgttatcgtg ggaatggcaa aaattatatg 1200

ggcaacttat cccaaacaag atctggacta acatgttcaa tgtgggacaa gaacatggaa 1260

gacttacatc gtcatatctt ctgggaacca gatgcaagta agctgaatga gaattactgc 1320

cgaaatccag atgatgatgc tcatggaccc tggtgctaca cgggaaatcc actcattcct 1380

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caacataaaa tgagaatggt tcttggtgtc attgttcctg gtcgtggatg tgccattcca 2100

aatcgtcctg gtatttttgt ccgagtagca tattatgcaa aatggataca caaaattatt 2160

ttaacatata aggtaccaca gtcagctagc gaacaaaaac tcatctcaga agaggatctg 2220

aatggagggc tcgagcatca ccaccatcac catcattga 2259

<210> SEQ ID NO 13
<211> LENGTH: 1494
<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: Coding sequence of HGF-alfa_MYChis

<400> SEQUENCE: 13

atgtgggtga ccaaactcct gccagccctg ctgctgcagc atgtcctcct gcatctcctc 60

ctgctcccca tcgccatccc ctatgcagag ggacaaagga aaagaagaaa tacaattcat 120

gaattcaaaa aatcagcaaa gactacccta atcaaaatag atccagcact gaagataaaa 180

accaaaaaag tgaatactgc agaccaatgt gctaatagat gtactaggaa taaaggactt 240

ccattcactt gcaaggcttt tgtttttgat aaagcaagaa aacaatgcct ctggttcccc 300

ttcaatagca tgtcaagtgg agtgaaaaaa gaatttggcc atgaatttga cctctatgaa 360

aacaaagact acattagaaa ctgcatcatt ggtaaaggac gcagctacaa gggaacagta 420

tctatcacta agagtggcat caaatgtcag ccctggagtt ccatgatacc acacgaacac 480

agctttttgc cttcgagcta tcggggtaaa gacctacagg aaaactactg tcgaaatcct 540

cgaggggaag aaggggacc ctggtgtttc acaagcaatc cagaggtacg ctacgaagtc 600

tgtgacattc ctcagtgttc agaagttgaa tgcatgacct gcaatgggga gagttatcga 660

ggtctcatgg atcatacaga atcaggcaag atttgtcagc gctgggatca tcagacacca 720

caccggcaca aattcttgcc tgaaagatat cccgacaagg gctttgatga taattattgc 780

cgcaatcccg atggccagcc gaggccatgg tgctatactc ttgaccctca cacccgctgg 840

gagtactgtg caattaaaac atgcgctgac aatactatga atgacactga tgttcctttg 900

gaaacaactg aatgcatcca aggtcaagga gaaggctaca ggggcactgt caataccatt 960

tggaatggaa ttccatgtca gcgttgggat tctcagtatc ctcacgagca tgacatgact 1020

cctgaaaatt tcaagtgcaa ggacctacga gaaaattact gccgaaatcc agatgggtct 1080

gaatcaccct ggtgttttac cactgatcca aacatccgag ttggctactg ctcccaaatt 1140

ccaaactgtg atatgtcaca tggacaagat tgttatcgtg ggaatggcaa aaattatatg 1200

ggcaacttat cccaaacaag atctggacta acatgttcaa tgtgggacaa gaacatggaa 1260

gacttacatc gtcatatctt ctgggaacca gatgcaagta agctgaatga gaattactgc 1320

cgaaatccag atgatgacgc tcatggaccc tggtgctaca cgggaaatcc actcattcct 1380

tgggattatt gccctatttc tcgttgtgaa ggtgataccg ctagcgaaca aaaactcatc 1440

tcagaagagg atctgaatgg agggctcgag catcaccacc atcaccatca ttga 1494

<210> SEQ ID NO 14
<211> LENGTH: 705
<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: Coding sequence of HGF NK1_MYChis

<400> SEQUENCE: 14

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gaattcaaaa aatcagcaaa gactacccta atcaaaatag atccagcact gaagataaaa 180

accaaaaaag tgaatactgc agaccaatgt gctaatagat gtactaggaa taaaggactt 240

ccattcactt gcaaggcttt tgtttttgat aaagcaagaa aacaatgcct ctggttcccc 300

ttcaatagca tgtcaagtgg agtgaaaaaa gaatttggcc atgaatttga cctctatgaa 360

aacaaagact acattagaaa ctgcatcatt ggtaaaggac gcagctacaa gggaacagta 420

tctatcacta agagtggcat caaatgtcag ccctggagtt ccatgatacc acacgaacac 480

agctttttgc cttcgagcta tcggggtaaa gacctacagg aaaactactg tcgaaatcct 540

cgaggggaag aaggggacc ctggtgtttc acaagcaatc cagaggtacg ctacgaagtc 600

tgtgacattc ctcagtgttc agaagttgaa gctagcgaac aaaaactcat ctcagaagag 660

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<210> SEQ ID NO 15
<211> LENGTH: 903
<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: Coding sequence of HGF-beta_MYChis

<400> SEQUENCE: 15

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gaattcgttg taaatgggat tccaacacga acaaacatag gatggatggt tagtttgaga 180

tacagaaata aacatatctg cggaggatca ttgataaagg agagttgggt tcttactgca 240

cgacagtgtt tcccttctcg agacttgaaa gattatgaag cttggcttgg aattcatgat 300

gtccacggaa gaggagatga gaaatgcaaa caggttctca atgtttccca gctggtatat 360

ggccctgaag gatcagatct ggttttaatg aagcttgcca ggcctgctgt cctggatgat 420

tttgttagta cgattgattt acctaattat ggagccacaa ttcctgaaaa gaccagttgc 480

agtgtttatg gctggggcta cactggattg atcaactatg atggcctatt acgagtggca 540

catctctata taatgggaaa tgagaaatgc agccagcatc atcgagggaa ggtgactctg 600

aatgagtctg aaatatgtgc tggggctgaa aagattggat caggaccatg tgaggggat 660

tatggtggcc cacttgtttg tgagcaacat aaaatgagaa tggttcttgg tgtcattgtt 720

cctggtcgtg gatgtgccat tccaaatcgt cctggtattt ttgtccgagt agcatattat 780

gcaaaatgga tacacaaaat tattttaaca tataaggtac cacagtcagc tagcgaacaa 840

aaactcatct cagaagagga tctgaatgga gggctcgagc atcaccacca tcaccatcat 900

tga 903

<210> SEQ ID NO 16
<211> LENGTH: 2259

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<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: Coding sequence of Uncleavable HGF_MYChis

<400> SEQUENCE: 16

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gaattcaaaa aatcagcaaa gactacccta atcaaaatag atccagcact gaagataaaa 180

accaaaaaag tgaatactgc agaccaatgt gctaatagat gtactaggaa taaaggactt 240

ccattcactt gcaaggcttt tgtttttgat aaagcaagaa aacaatgcct ctggttcccc 300

ttcaatagca tgtcaagtgg agtgaaaaaa gaatttggcc atgaatttga cctctatgaa 360

aacaaagact acattagaaa ctgcatcatt ggtaaaggac gcagctacaa gggaacagta 420

tctatcacta agagtggcat caaatgtcag ccctggagtt ccatgatacc acacgaacac 480

agctttttgc cttcgagcta tcggggtaaa gacctacagg aaaactactg tcgaaatcct 540

cgaggggaag aaggggacc ctggtgtttc acaagcaatc cagaggtacg ctacgaagtc 600

tgtgacattc ctcagtgttc agaagttgaa tgcatgacct gcaatgggga gagttatcga 660

ggtctcatgg atcatacaga atcaggcaag atttgtcagc gctgggatca tcagacacca 720

caccggcaca aattcttgcc tgaaagatat cccgacaagg gctttgatga taattattgc 780

cgcaatcccg atggccagcc gaggccatgg tgctatactc ttgaccctca cacccgctgg 840

gagtactgtg caattaaaac atgcgctgac aatactatga atgacactga tgttcctttg 900

gaaacaactg aatgcatcca aggtcaagga gaaggctaca ggggcactgt caataccatt 960

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gaatcaccct ggtgttttac cactgatcca aacatccgag ttggctactg ctcccaaatt 1140

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ggcaacttat cccaaacaag atctggacta acatgttcaa tgtgggacaa gaacatggaa 1260

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gctgaaaaga ttggatcagg accatgtgag ggggattatg gtggcccact tgtttgtgag 2040

caacataaaa tgagaatggt tcttggtgtc attgttcctg gtcgtggatg tgccattcca 2100

aatcgtcctg gtatttttgt ccgagtagca tattatgcaa aatggataca caaaattatt 2160

ttaacatata aggtaccaca gtcagctagc gaacaaaaac tcatctcaga agaggatctg 2220

aatggagggc tcgagcatca ccaccatcac catcattga 2259

<210> SEQ ID NO 17
<211> LENGTH: 1197
<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: Coding sequence of NK1-NK1_his

<400> SEQUENCE: 17

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gaattcaaaa aatcagcaaa gactacccta atcaaaatag atccagcact gaagataaaa 180

accaaaaaag tgaatactgc agaccaatgt gctaatagat gtactaggaa taaaggactt 240

ccattcactt gcaaggcttt tgtttttgat aaagcaagaa aacaatgcct ctggttcccc 300

ttcaatagca tgtcaagtgg agtgaaaaaa gaatttggcc atgaatttga cctctatgaa 360

aacaaagact acattagaaa ctgcatcatt ggtaaaggac gcagctacaa gggaacagta 420

tctatcacta agagtggcat caaatgtcag ccctggagtt ccatgatacc acacgaacac 480

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cgaggggaag aaggggacc ctggtgtttc acaagcaatc cagaggtacg ctacgaagtc 600

tgtgacattc ctcagtgttc agaagttgaa gctagcgaat tcaaaaaatc agcaaagact 660

accctaatca aaatagatcc agcactgaag ataaaaacca aaaaagtgaa tactgcagac 720

caatgtgcta atagatgtac taggaataaa ggacttccat tcacttgcaa ggcttttgtt 780

tttgataaag caagaaaaca atgcctctgg ttccccttca atagcatgtc aagtggagtg 840

aaaaaagaat ttggccatga atttgacctc tatgaaaaca aagactacat tagaaactgc 900

atcattggta aaggacgcag ctacaaggga acagtatcta tcactaagag tggcatcaaa 960

tgtcagccct ggagttccat gataccacac gaacacagct ttttgccttc gagctatcgg 1020

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tgtttcacaa gcaatccaga ggtacgctac gaagtctgtg acattcctca gtgttcagaa 1140

gttgaagcta gtgggggtgg tagtggagga gggcatcacc atcatcacca tcactga 1197

<210> SEQ ID NO 18
<211> LENGTH: 1218
<212> TYPE: DNA
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: Coding sequence of hAS_MYC

<400> SEQUENCE: 18

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tatctctcag agtgcaagac tgggaatgga aagaactaca gagggacgat gtccaaaaca 120

aaaaatggca tcacctgtca aaaatggagt tccacttctc cccacagacc tagattctca 180

cctgctacac acccctcaga gggactggag gagaactact gcaggaatcc agacaacgat 240

ccgcaggggc cctggtgcta tactactgat ccagaaaaga gatatgacta ctgcgacatt 300

cttgagtgtg aagaggaatg tatgcattgc agtggagaaa actatgacgg caaaatttcc 360

aagaccatgt ctggactgga atgccaggcc tgggactctc agagcccaca cgctcatgga 420

tacattcctt ccaaatttcc aaacaagaac ctgaagaaga attactgtcg taaccccgat 480

agggagctgc ggccttggtg tttcaccacc gaccccaaca agcgctggga actttgcgac 540

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acaggtgaaa actatcgcgg gaatgtggct gttaccgttt ccgggcacac ctgtcagcac 660

tggagtgcac agacccctca cacacataac aggacaccag aaaacttccc ctgcaaaaat 720

ttggatgaaa actactgccg caatcctgac ggaaaaaggg ccccatggtg ccatacaacc 780

aacagccaag tgcggtggga gtactgtaag ataccgtcct gtgactcctc cccagtatcc 840

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accacagacc ccagcgtcag gtgggagtac tgcaacctga aaaaatgctc aggaacagaa 1140

gcggctagcg aacaaaaact catctcagaa gaggatctga atggagggct cgagcatcac 1200

caccatcacc atcattga 1218

<210> SEQ ID NO 19
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: Flag at the C terminus

<400> SEQUENCE: 19

Ser Asp Tyr Lys Asp Asp Asp Asp Lys
1 5

<210> SEQ ID NO 20
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: Single Myc at the C terminus

<400> SEQUENCE: 20

Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn
1 5 10

<210> SEQ ID NO 21
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: poly-histidine tag

<400> SEQUENCE: 21

His His His His His His His
1 5

The invention claimed is:

1. A method to detect the ability of a test agent to act as an antagonist/inhibitor of hepatocyte growth factor receptor useful in the treatment of cancer, comprising the steps of:

(a) putting in contact a test agent with

i) a polypeptide consisting of the extra-cellular IPT-3 and IPT-4 domains of hepatocyte growth factor receptor, or

ii) cells expressing extra-cellular domains of hepatocyte growth factor receptor, wherein said extra-cellular domains consist of the IPT-3 and IPT-4 domains,

(b) measuring hepatocyte growth factor receptor activity, function and/or stability, and

(c) selecting the agent that reduces hepatocyte growth factor receptor activity, function and/or stability.

2. The method according to claim **1**, wherein said selected agent interferes with catalytic activity, function and/or stability of hepatocyte growth factor receptor.

3. The method according to claim **1**, wherein said selected agent down-regulates catalytic activity, function and/or stability of hepatocyte growth factor receptor.

4. The method according to claim **1**, wherein said selected agent is a hepatocyte growth factor receptor inhibitor and/or antagonist.

5. The method according to claim 1, wherein said selected agent is selected from the group consisting of small molecule inhibitors, aptamers, antibodies, peptides and dominant negative factors.

6. The method according to claim 1, wherein the cancer is a cancer with dysregulation of hepatocyte growth factor receptor activity.

7. A method of identifying a pharmaceutically active agent suitable for use in the treatment of cancer, comprising the steps of:

(a) putting in contact a test agent with

ii) a polypeptide consisting of the extra-cellular IPT-3 and IPT-4 domains of hepatocyte growth factor receptor, or

ii) cells expressing extra-cellular domains of hepatocyte growth factor receptor, wherein said extra-cellular domains consist of the IPT-3 and IPT-4 domains, and expressing the transmembrane helix and the full cytoplasmic region of hepatocyte growth factor receptor,

(b) measuring hepatocyte growth factor receptor activity, function and/or stability, in the presence and absence of said test agent, and

(c) selecting a test agent that reduces hepatocyte growth factor receptor activity, function and/or stability as said pharmaceutically active agent suitable for use in the treatment of cancer.

8. The method according to claim 7, wherein said measuring in step b) comprises measuring cell signalling, cell survival, and cell proliferation.

9. The method according to claim 7 wherein said polypeptide is expressed on a cell surface.

10. The method according to claim 7 wherein said polypeptide is expressed in a cell comprising a polynucleotide encoding said polypeptide.

11. The method according to claim 7 wherein said cancer is a cancer with dysregulation of hepatocyte growth factor receptor activity.

12. The method according to claim 7, wherein said active agent is selected from the group consisting of small molecule inhibitors, aptamers, antibodies, and dominant negative factors.

13. The method according to claim 7 wherein said active agent is a peptide.

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