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(54) Title: NEW ANTIBODY FRAGMENTS, COMPOSITIONS AND USES THEREOF

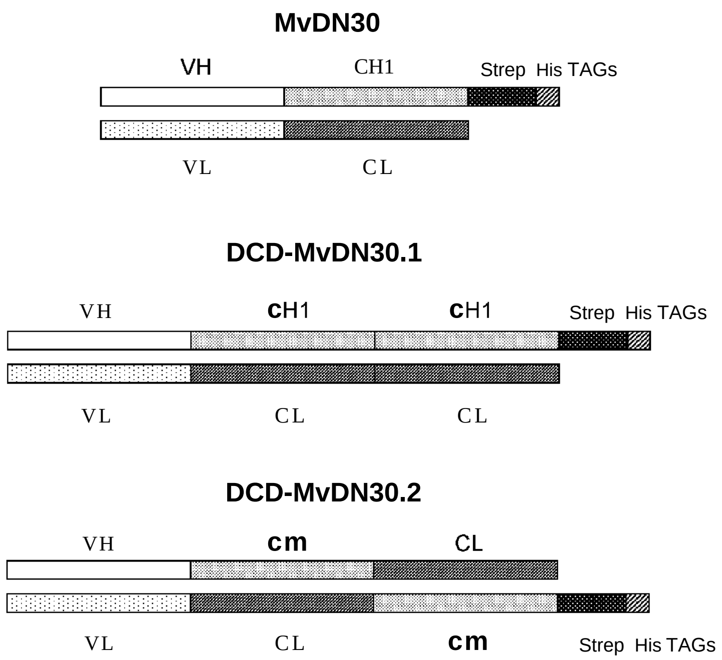

Figure 3

(57) Abstract: Antibody fragment comprising a first polypeptide comprising a light chain variable domain and two constant domains and a second polypeptide comprising a heavy chain variable domain and two constant domains, wherein two chain constant domains are light chain constant domains and two constant domains are CHI heavy chain constant domains.

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"New antibody fragments, compositions and uses thereof"

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Field of the invention

The present disclosure concerns new antibody fragments with improved *in vivo* stability.

Background

Targeted therapy, the new frontier of cancer treatment, employs pharmacological tools (drugs or antibodies) specifically blocking crucial gene products that sustain the transformed phenotype. Currently, cancer targeted therapy is employed in the clinic for the treatment of chronic myelogenous leukemias (CML), addicted to the tyrosine kinase molecule ABL, for the treatment of a subset of Non-Small Cell Lung Cancers (NSCLC) and Colon-Rectum Carcinomas (CRC) relying on Epidermal Growth Factor Receptor (EGFR/HER-1) activation and for the treatment of BRAF-dependent melanomas .

Receptors with tyrosine kinase activity (RTKs) are interesting candidates for targeted therapy as they are often hyper-activated in several types of tumors. They can be inhibited by different types of targeting molecules, such as antibodies, that upon interaction with the extracellular part of the receptor are able to perturb the receptor-induced intracellular signaling, and chemically-synthesized small molecules that interfere with the receptor catalytic activity.

Among the different RTKs, the product of the *c-met* proto-oncogene, the Hepatocyte Growth Factor Receptor (HGFR/Met) , is emerging as one of the most important activated oncogene in cancer. Met controls a genetic program known as 'invasive growth' that includes pro-mitogenic, pro- invasive and anti-apotoptic cues. Through these physiological signals, Met provides with a better fitness the tumor, helping it to overcome selective barriers in cancer progression. Moreover Met sustains tumor growth by its ability to promote tumor angiogenesis . In the last years, Met also resulted responsible for the aggressiveness developed by tumors treated with anti-angiogenic agents and for resistance to conventional radiotherapy. Additionally, *MET* gene alteration can be a primary cause of transformation, in all of those cases in which it has been genetically selected for the long term maintenance of the primary transformed phenotype.

All the above listed findings have prompted the development of several molecules suitable to inhibit Met signaling, including competitive inhibitors of HGF, chemical Met kinase inhibitors, anti-HGF and anti-Met antibodies. Some of these molecules, until now, have been tested only for research purpose. Clinical trials are currently ongoing with neutralizing anti-HGF antibodies, anti-Met antibodies and several small molecules .

From several view-points, an anti-Met antibody able to inhibit Met signaling would be preferable. Antibodies are highly specific, stable and, thank to their natural design, they are generally well tolerated by the host. In the last years, several efforts have been put to generate therapeutic anti-Met antibodies. However, a lot of failures have been registered, as the majority of the anti-Met antibodies behave as agonists, mimicking the HGF action. This is mostly due to the fact that, thanks to their bivalent structure, antibodies can stabilize receptor dimers, allowing trans-phosphorylation of Met, with its consequent activation. In one case, an agonist anti-Met antibody (5D5) has been engineered and converted in a monovalent form (One Armed-5D5) that, competing with HGF binding, is endowed with therapeutic potential (1,2). This molecule has been recently entered a phase III clinical trial for the treatment of a subset of Non Small Cell Lung Cancer patients, characterized by high level of Met expression in the tumors, in combination with erlotinib (3).

The monoclonal antibody DN30 is a mouse IgG2A directed against the extracellular moiety of the human Met receptor (4). It binds with sub-nanomolar affinity the fourth IPT domain of the Met receptor extracellular region. At the beginning, it was characterized as a partial agonist of Met, able to promote some, but not all, of the Met-mediated biological cell responses. Later it has been demonstrated that it can act as an inhibitor of tumor growth and metastasis through a mechanism of receptor Shedding' (5). Receptor shedding is a physiologic cellular mechanism of protein degradation acting on diverse growth factors, cytokines, receptors and adhesion molecules. Met shedding is articulated in two steps: first a metalloprotease, the ADAM-10, cleaves the extracellular domain of Met recognizing a specific sequence localized immediately upstream to the trans-membrane region; then the remaining transmembrane fragment becomes substrate of a second protease (γ-secretase) that detaches the kinase-containing portion from the membrane and rapidly addresses it towards the proteasome degradation pathway (6,7). The enhancement of this mechanism exerted by the DN30 leads to a reduction in the number of Met receptors exposed at the cell surface. At the same time, it releases a soluble, 'decoy' ecto-domain in the extracellular space. The latter competes with the intact trans-membrane receptor for ligand binding and inhibits receptor homo-dimerization by forming hetero-dimeric complexes with *bona fide* Met. All these actions strongly impair Met-mediated signaling and result in prevention of the downstream biological effects.

Recently the present inventors demonstrated that the monovalent Fab fragment of the DN30 anti-Met monoclonal antibody (DN30 Fab) is cleared of any agonistic activity and maintains the ability to induce shedding, thus resulting in a potent Met inhibitor (8). Induction of Met shedding by DN30-Fab is dependent on the selective antibody-antigen interaction but is independent from receptor activation. This mechanism of action, based on the simple elimination of Met from the cell surface, gives to the DN30-Fab a strong advantage over other inhibitors, as it can be effective against all the forms of Met activation, whether HGF-dependent or not, induced by overexpression, mutation or gene amplification .

While the recombinant DN30-Fab is very attractive for clinical applications, the short Fab plasma half-life - mostly due to renal clearance - severely limits its use for patient treatment.

Currently, the most consolidated technique to improve the pharmacological properties of a Fab fragment is to increase its molecular weight by conjugation with Poly Ethylen Glycol (PEG). Fab PEGylation is a route pursued in most of the cases employing Fab in the clinic. The covalent attachment of the polymer chains to the antibody fragment, obtained efficiently and without loss of antigen binding properties, is not an obvious process and requires a strong effort of setting up.

Another technique used to improve the pharmacological properties of a Fab fragment is the one disclosed in EP-A-1 718 677. Such a procedure, used to generate the One Armed form of monoclonal antibody 5D5 commented above, is the production - on recombinant basis - of three different antibody chains in the same cell, the light chain (VL-CL), the heavy chain (VH-CH1-CH2-CH3) and the Fc portion of the heavy chain (CH2-CH3). The CH2-CH3 domains are not wild type: mutations, giving rise to specific tridimensional structures, are included. In one polypeptide, the CH2-CH3 region incorporates a sequence forming a protuberance, while in the other polypeptide the CH2-CH3 region contains a sequence forming a cavity, in which the protuberance can be inserted (Knob into hole structure). The presence of these tridimensional structures allows the preferable formation of heterodimers in which the heavy chain forms disulfide bonds with the Fc fragment, but does not exclude at all the formation of homodimers (i.e. two heavy chains linked together and two Fc linked together). Purification allowing the separation of the unwanted homodimers from the wanted heterodimers is mandatory. Thus the "One Armed procedure", although very elegant, is cumbersome as it requires additional steps in the overall process that complicate the manufacturing and reduce the yield of the recombinant antibody.

It is therefore felt the necessity of a different solution to increase Fab plasma half-life for *in vivo* therapeutic use.

Summary of the invention

The object of this disclosure is to provide an antibody fragment with improved *in vivo* stability.

According to the invention, the above object is achieved thanks to the subject matter recalled specifically in the ensuing claims, which are understood as forming an integral part of this disclosure .

An embodiment of the present disclosure provides an antibody fragment comprising a first polypeptide comprising a light chain variable domain and two constant domains and a second polypeptide comprising a heavy chain variable domain and two constant domains, wherein two chain constant domains are light chain constant domains and two constant domains are heavy chain CHI constant domains, fused in different combinations to the variable domains.

A further embodiment of the present disclosure concerns an antibody fragment as defined above that is more stable *in vivo* than the Fab molecule comprising the light and heavy chain variable domains.

A still further embodiment concerns an antibody fragment as defined above that specifically binds the hepatocyte growth factor receptor (HGFR/Met) .

Brief description of the drawings

The invention will now be described in detail, purely by way of an illustrative and non-limiting example and, with reference to the accompanying drawings, wherein:

- **FIGURE 1 :** ***Met shedding and down-regulation in Met-addicted cells treated by chimeric MvDN30 or murine DN30 Fab.*** **A** SNU-5 a human gastric carcinoma cell line; **B** H1993-NC1 a non small cell lung carcinoma cell line. Cells were incubated for 48 hrs in serum free medium with the indicated concentrations of the two antibody fragments derived from DN30 mAb . Total Met levels were determined by Western blot analysis of cell extracts using anti-Met antibodies. The two Met bands correspond to the unprocessed (pl90 Met) and mature (pl45 Met) forms of the receptor. Met shedding was determined by Western blot analysis of conditioned medium using anti-Met antibodies. Both molecules efficiently induce Met-shedding/down-regulation.

- **FIGURE 2** : ***Growth assay of Met-addlcted cells treated by chimeric MvDN30 or murine DN30 Fab.*** **A** EBC-1 a non small cell lung carcinoma cell line; **B** Hs746T a human gastric carcinoma cell line. Cells were plated in 96 well dishes (1000/well) in 10% FCS medium. After 24 hrs cells were treated with increasing concentrations of antibodies for further 72 hrs. Number of cells was evaluated by Cell titer-glo (Perkin Elmer) . Each point is the mean of triplicate values; bars represent standard deviation. Both molecules efficiently inhibit cell growth of Met-addicted cells.

- **FIGURE 3** : ***Schematic representation of the new DN30 derived molecules.*** Top: chimerized DN30 Fab (MvDN30); middle: Double Constant Domain Fab with the duplicated constant domains in tandem (DCD-MvDN30 .1); bottom: Double Constant Domain Fab with the duplicated constant domains swapped reciprocally (DCD-MvDN30 .2). VH: variable domain of the DN30 heavy chain. VL: variable domain of the DN30 light chain. CHI: constant domain 1 derived from human IgGl heavy chain. CL: constant domain derived from human kappa light chain. Strep and His Tag: sequences included to allow protein purification and immuno-detection .

- **FIGURE 4** : ***Analysis of the new DN-30 derived molecules .*** The indicated purified proteins were subjected to SDS-PAGE under reducing condition. Gel was stained with Gel Code blue (Pierce) . All the molecules show two bands with the expected molecular weight.

– **FIGURE 5** : ***Binding to Met of DCD-MvDN30 molecules.*** ELISA binding analysis of MvDN30, DCD-

MvDN30.1 and DCD-MvDN30.2 (liquid phase) to a Met-Fc chimera (solid phase) . Binding was revealed using anti-strepTAG antibodies. O.D.: Optical Density; A.U. : arbitrary units. Each point is the mean of triplicate values; bars represent standard deviation. The new molecules bind to Fc-Met with the same high affinity.

– **FIGURE 6** : ***Agonistic activity of DCD-MvDN30 molecules.*** A549 cells were starved for 24 hrs and then stimulated for lOmin at 37°C with the different molecules at the indicated concentrations. Met activation was determined by immuno-precipitation with anti-Met antibodies followed by Western blotting with anti-Met antibodies specific for the phosphorylated Tyr 1234/1235 Met residues, the major phosphorylation site (Top) . The same blot was re-probed with anti-Met antibodies (Bottom) . The new molecules do not significantly activate the Met receptor.

– **FIGURE 7** : ***Agonistic activity of DCD-MvDN30 molecules.*** A549 cells were starved for 24 hrs and then stimulated for lOmin at 37°C with the different molecules at the indicated concentrations. Activation of AKT and ERK-1,2 was determined by Western blotting with anti-AKT or anti-ERK antibodies specific for the phosphorylated form. The same blot was re-probed with anti-Vinculin antibodies (Bottom) to control protein loading. The new molecules do not significantly activate the Met- dependent signaling.

- **FIGURE 8** : ***Met shedding and down-regulation in cells treated by DCD-MvDN30 molecules.*** A549 cells were incubated for 72 hrs in serum free medium with the indicated molecules (500 nM) . Total Met levels were determined by Western blot analysis of cell extracts using anti-Met antibodies. The two Met bands correspond to the unprocessed (pl90 Met) and mature (pl45 Met) forms of the receptor. As a loading control, the filter was probed with an unrelated protein (actin) . Met shedding was determined by Western blot analysis of conditioned medium using anti-Met antibodies. The new molecules efficiently induce Met shedding.

- **FIGURE 9**: ***Inhibition of HGF-induced Met-activation by DCD-MvDN30 molecules.*** A549 cells were incubated for 24 hrs in serum free medium plus the indicated molecules (IOOOηM) and then stimulated for 10 min with HGF (lOOng/ml) . Met activation was determined in total cell lysates by Western blotting with anti-Met antibodies specific for the phosphorylated Tyr 1234/1235 Met residues, the major phosphorylation site. The same blot was re-probed with anti-Met antibodies. Activation of AKT and ERK-1,2 was determined by Western blotting with anti-phosphoAKT or anti-phosphoERK antibodies. The same blot was re-probed with anti-AKT or ERK-1,2 antibodies. To control protein loading the filter was also probed with anti-Vinculin antibodies. The new molecules strongly inhibit HGF-induced Met-activation and Met-dependent signaling.

- **FIGURE 10**: ***Anchorage-dependent growth of Met-addicted cells treated with DCD-MvDN30. 1 or DCDMvDN30.2 or MvDN30.*** **A**, **B**, **C**, **D** human gastric carcinoma cell lines; **E**, **F** non small cell lung carcinoma cell lines. Cells were plated in 96 well costar (1000/well) in 5% FCS medium. After 24 hrs cells were treated with increasing concentrations of the different molecules for further 72 hrs. Number of cells was evaluated by Cell titer-glo (Promega) . The plots represent the percentage of alive cells respect to untreated control. Each point is the mean of triplicate values. The new molecules efficiently inhibit cell growth of Met-addicted cells.

- **FIGURE 11**: ***Anchorage -independent growth of cells treated with DCD-MvDN30. 1 or DCD-MvDN30.2 or MvDN30.***

A549 cells were plated in semi-solid medium (5% agarose) with or without HGF (50 ng/ml) in the presence of 1.5 mM of DCD-MvDN30.1, or DCD-MvDN30.2 or MvDN30. After 21 days colonies were stained with tetrazolium salt. Colonies were quantified by counting pixel in each well area with MetaMorphOffline Software. Each point is the mean of triplicate values. The new molecules efficiently inhibit HGF-dependent anchorage-independent cell growth.

- **FIGURE 12;** ***Pharmakokinetic profile in vivo of DCD-MvDN30.1, DCD-MvDN30.2 and MvDN30.*** Immunodeficient mice were injected intraperitoneous with a single dose (100 μq) of DCD-MvDN30.1, or DCD-MvDN30.2 or MvDN30. Peripheral blood was collected at different time points. Serum concentrations of the therapeutic molecules were measured by ELISA. Graph represents the amount of circulating molecules in function of time. Samples are in triplicate, bars represent standard deviations.

- **FIGURE 13:** ***Nucleotide and amino acid sequences of a first embodiment of a first polypeptide of an antibody fragment according to the present disclosure.*** The sequences correspond to the polypeptide derived from the light chain, VL-CL-CL. The CDR regions are underlined both in the nucleotide and amino acid sequences.

- **FIGURE 14:** ***Nucleotide and amino acid sequences of a first embodiment of a second polypeptide of an antibody fragment according to the present disclosure.*** The sequences correspond to the polypeptide derived from the heavy chain, VH-CHl-CHl-TAGs. The CDR regions are underlined both in the nucleotide and amino acid sequences. Strep and Histidine TAGs in capital italic letters.

- **FIGURE 15:** ***Nucleotide and amino acid sequences of a second embodiment of a first polypeptide of an antibody fragment according to the present disclosure.*** The sequences correspond to the polypeptide derived from the light chain, VL-CL-CHl-TAGs . The CDR regions are underlined both in the nucleotide and amino acid sequences. Strep and Histidine TAGs in capital italic letters .
- **FIGURE 16:** ***Nucleotide and amino acid sequences of a second embodiment of a second polypeptide of an antibody fragment according to the present disclosure.*** The sequences correspond to the polypeptide derived from the heavy chain, VH-CH1-CL. The CDR regions are underlined both in the nucleotide and amino acid sequences.
- **FIGURE 17:** ***Nucleotide and amino acid sequences of DN30 light chain variable domain.*** The CDR regions are underlined both in the nucleotide and amino acid sequences .
- **FIGURE 18:** ***Nucleotide and amino acid sequences of DN30 heavy chain variable domain.*** The CDR regions are underlined both in the nucleotide and amino acid sequences .

Detailed description of the invention

The invention will now be described in detail, by way of non limiting example, with reference to antibody fragments able to specifically bind hepatocyte growth factor receptor.

It is clear that the scope of this description is in no way limited to such target antigen, since the antibody fragments described herein can be characterized by specifically binding other target antigens .

In the following description, numerous specific details are given to provide a thorough understanding of embodiments. The embodiments can be practiced without one or more of the specific details, or with other methods, components, materials, etc. In other instances, well-known structures, materials, or operations are not shown or described in detail to avoid obscuring aspects of the embodiments.

Reference throughout this specification to "one embodiment" or "an embodiment" means that a particular feature, structure, or characteristic described in connection with the embodiment is included in at least one embodiment. Thus, the appearances of the phrases "in one embodiment" or "in an embodiment" in various places throughout this specification are not necessarily all referring to the same embodiment. Furthermore, the particular features, structures, or characteristics may be combined in any suitable manner in one or more embodiments.

The headings provided herein are for convenience only and do not interpret the scope or meaning of the embodiments .

Antibodies are complex tetramers in which both the heavy and the light chains are composed by multiple Ig domains, each one folding independently. At the very beginning of the antibody era it has been shown that, through enzymatic treatment, an antibody can originate fragments that maintain the original structure and the antigen-binding properties.

Subsequently, by applying protein engineering techniques, a plethora of different engineered antibody fragments have been generated. According to molecular design, each new antibody fragment is characterized by particular features (i.e. increased avidity, multivalency, multispecif icity, ADCC-def icient , chimerized, etc.) .

However, none of the previous studies have addressed the issue of renal clearance to prolong half-life *in vivo* of antibody fragments.

To this end, the present inventors developed a recombinant antibody fragment comprising a first polypeptide comprising a light chain variable domain and two constant domains and a second polypeptide comprising a heavy chain variable domain and two constant domains, wherein two chain constant domains are light chain constant domains and two constant domains are heavy chain CHI constant domains, fused in different combinations to the variable domains.

In one embodiment, the antibody fragment herein disclosed is more stable *in vivo* than the Fab molecule comprising said light and heavy chain variable domains.

In one embodiment, the antibody fragment has prolonged half-life *in vivo* when administered to a human patient than the Fab molecule comprising said light and heavy chain variable domains because of a reduced renal clearance.

These antibody fragments were named 'Dual Constant Domain Fabs' (DCD-Fabs) .

In a preferred embodiment, the present disclosure concerns an antibody fragment comprising a first polypeptide comprising a light chain variable domain and two constant domains and a second polypeptide comprising a heavy chain variable domain and two constant domains, wherein two constant domains are human light chain constant domains and two constant domains are human heavy chain CHI constant domains, fused in different combinations to the variable domains, wherein the antibody fragment is more stable *in vivo* than the Fab molecule comprising said light and heavy chain variable domains, and wherein the antibody fragment specifically binds the hepatocyte growth factor receptor (HGFR/Met) .

In one embodiment, the light chain variable domain is fused at its C-terminus to one light chain constant domain, that is fused at its C-terminus to one light chain constant domain.

In another embodiment, the light chain variable domain is fused at its C-terminus to a light chain constant domain, that is fused at its C-terminus to one heavy chain CHI constant domain.

In one embodiment, the heavy chain variable domain is fused at its C-terminus to one heavy chain CHI constant domain, that is fused at its C-terminus to one heavy chain CHI constant domain.

In another embodiment, the heavy chain variable domain is fused at its C-terminus to a heavy chain CHI constant domain, that is fused at its C-terminus to a light chain constant domain.

In one embodiment, the constant domains contained in the first and second polypeptide - when coupled together in the antibody fragment - are able to generate disulfide bridges.

In a further preferred embodiment, the present disclosure concerns antibody fragments as defined above wherein the antigen specificity is provided by employing as light and heavy chain variable domains the DN30 light and heavy chain variable domains or humanized light and heavy chain variable domains comprising the complementarity determining regions (CDRs) from DN30 monoclonal antibody. DN30 monoclonal antibody was disclosed in the international patent application WO-A-2007/090807 .

An antibody fragment of the invention is generally a therapeutic antibody. For example, an antibody of the invention may be an antagonistic antibody, a blocking antibody or a neutralizing antibody.

In one aspect, the invention provides methods of treating or delaying progression of a disease administering to a subject having the disease an effective amount of an antibody fragment of the invention, effective in treating or delaying progression of the disease.

In one embodiment, the disease is a tumor or tumor metastasis.

In another embodiment, the disease is associated with dysregulation of hepatocyte growth factor-receptor signalling and/or activation.

An antibody fragment of the invention is suitable for treating or preventing pathological conditions associated with abnormalities within the HGF/HGFR signalling pathway.

In one embodiment, an antibody of the invention is a HGFR antagonist.

In one embodiment, the antibody fragment comprises antigen binding sequences from a non-human donor grafted to a heterologous non-human, human or humanized sequence (e.g. framework and/or constant domain sequences) . In one embodiment, the non-human donor is a mouse.

In one embodiment, the antigen binding sequences comprise all the CDRs and/or variable domain sequences of an anti-HGFR murine antibody.

In one preferred embodiment, the murine light chain variable domain is fused at its C-terminus to one human kappa light chain constant domain, that is fused at its C-terminus to one human kappa light chain constant domain. In another embodiment, the murine light chain variable domain is fused at its C-terminus to a human kappa light chain constant domain, that is fused at its C-terminus to one human IgGl heavy chain CHI constant domain. In one embodiment, the murine heavy chain variable domain is fused at its C-terminus to one human IgGl heavy chain CHI constant domain, that is fused at its C-terminus to one human IgGl heavy chain CHI constant domain. In another embodiment, the murine heavy chain variable domain is fused at its C-terminus to a human IgGl heavy chain CHI constant domain, that is fused at its C-terminus to a human kappa light chain constant domain.

In one preferred embodiment, an antibody fragment of the invention comprises a first polypeptide comprising a light chain variable domain comprising the CDR sequences of an anti-HGFR murine antibody, more preferably the CDRs of DN30, and two constant domains, wherein the two constant domains are: two light chain constant domains or one light chain constant domain and one heavy chain CHI constant domain. In one embodiment the two constant domains are human constant domains.

In one embodiment, an antibody fragment of the invention comprises a second polypeptide comprising a heavy chain variable domain comprising the CDR sequences of an anti-HGFR murine antibody, more preferably the CDRs of DN30, and two constant domains, wherein the two constant domains are: two heavy chain CHI constant domains or one heavy chain CHI constant domain and one light chain constant domain. In one embodiment the two constant domains are human constant domains .

The invention provides, in a most preferred embodiment, a humanized antibody fragment that binds human HGFR, wherein the antibody is effective to inhibit HGF/HGFR activity in vivo, the antibody comprising i) in the heavy chain variable domain (VH) the three CDRs sequence of the heavy chain variable domain of the DN30 monoclonal antibody (SEQ ID No.:19, 20, 21) and substantially a human consensus sequence e.g. substantially the human consensus framework (FR) residues of human heavy chain subgroup and ii) in the light chain variable domain (VL) the three CDRs sequence of the light chain variable domain of the DN30 monoclonal antibody (SEQ ID No.:25, 26,27) and substantially the human consensus framework (FR) residues of human light chain K subgroup I (VKI).

In one embodiment, an antibody fragment of the invention comprises a first polypeptide comprising as the light chain variable domain the light chain variable domain sequence set forth in SEQ ID NO: 12 (DN30 light chain variable domain) and a second polypeptide comprising as heavy chain variable domain the heavy chain variable domain sequence set forth in SEQ ID NO: 4 (DN30 heavy chain variable domain).

In one aspect, the invention provides for use of an antibody fragment of the invention (e.g. a HGFR antagonist antibody fragment of the invention) in the preparation of a medicament for the therapeutic and/or prophylactic treatment of a disease, such as a cancer, a tumor, a cell proliferative disorder.

In one aspect, the invention provides a method of treating a pathological condition associated with dysregulation of HGFR activation in a subject, said method comprising administering to the subject an effective amount of a HGFR antagonist antibody fragment of the invention, whereby said condition is treated.

In one aspect, the invention provides a method of inhibiting the growth of a cell that expresses HGFR, said method comprising contacting said cell with a HGFR antagonist antibody fragment of the invention thereby causing an inhibition of growth of said cell.

In one aspect, the invention provides a method of therapeutically treating a mammal having a cancerous tumor comprising a cell that expresses HGFR, said method comprising administering to said mammal an effective amount of a HGFR antagonist antibody fragment of the invention, thereby effectively treating said mammal .

In one aspect, the invention provides a method for treating or preventing a cell proliferative disorder associated with increased expression or activity of HGFR, said method comprising administering to a subject in need of such treatment an effective amount of a HGFR antagonist antibody fragment of the invention, thereby effectively treating or preventing said cell proliferative disorder.

In one aspect, the invention provides a method of therapeutically treating a tumor in a mammal, wherein the growth of said tumor is at least in part dependent upon a growth potentiating effect of HGFR, said method comprising contacting a tumor cell with an effective amount of a HGFR antagonist antibody fragment of the invention, thereby effectively treating said tumor. The tumor cell can be one selected from breast, colorectal, lung, colon, pancreatic, prostate, ovarian, cervical, central nervous system, renal, hepatocellular, bladder, gastric, head and neck tumor cell, papillary carcinoma (e.g. the thyroid gland), melanoma, lymphoma, myeloma, glioma/glioblastoma (e.g. anaplastic astrocytoma, glioblastoma multiforme, anaplastic oligodendroglioma, anaplastic oligodendroastrocytoma), leukemia cell. In one embodiment, a cell that is targeted in a method of the invention is a hyperprolif erative and/or hyperplastic cell. In one embodiment, a cell that is targeted in a method of the invention is a dysplastic cell. In yet another embodiment, a cell that is targeted in a method of the invention is a metastatic cell. In a further embodiment, a cell that is targeted in a method of the invention is a HGFR expressing cell belonging to the microenvironment sustaining the tumor and/or the metastasis.

Methods of the invention can further comprise additional treatment steps. For example, in one embodiment, a method further comprises a step wherein a targeted tumor cell and/or tissue is exposed to radiation treatment or a chemotherapeutic agent. In another embodiment, a targeted tumor cell and/or tissue is treated, in addition to the antagonist antibody fragment of the invention, with HGF inhibitors (i.e. anti-HGF antibodies) or other anti-HGFR compounds (i.e. small molecule kinase inhibitors). In a further embodiment, a targeted tumor cell and/or tissue is treated, in addition to the antagonist antibody fragment of the invention, with molecules specifically hitting other targets relevant in the maintenance of the transformed phenotype (i.e. anti-EGFR molecules).

Activation of HGFR is an important biological process; its deregulation leads to numerous pathological conditions. Accordingly, in one embodiment of methods of the invention, a cell that is targeted (e.g. a cancer cell) is one in which activation of HGFR is enhanced as compared to a normal cell of the same tissue origin. In one embodiment, a method of the invention causes the death or cell growth arrest of a targeted cell. For example, contact with an antagonist antibody fragment of the invention may result in a cell's inability to signal through the HGFR pathway, which results in cell death or cell growth arrest.

The invention also pertains to immunoconjugates, or antibody-drug conjugates (ADC), comprising an antibody fragment conjugated to a cytotoxic agent such as a chemotherapeutic agent, a drug, a growth inhibitory agent, a toxin (e.g. an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (i.e. a radioconjugate).

The use of antibody-drug conjugates for the local delivery of cytotoxic or cytostatic agents, i.e. drugs to kill or inhibit tumor cells growth in the treatment of cancer, allows targeted delivery of the drug moiety to tumors, and intracellular accumulation therein, where systemic administration of these unconjugated drug agents may result in unacceptable levels of toxicity to normal cells as well as the tumor cells sought to be eliminated.

Therapeutic formulations comprising an antibody fragment of the invention are prepared for storage by mixing the antibody fragment having the desired degree of purity with physiologically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of aqueous solutions, lyophilized or other dried formulations. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers; antioxidants; preservatives; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids; monosaccharides, disaccharides, and other carbohydrates; chelating agents; sugars; salt-forming counter-ions; metal complexes and/or non-ionic surfactants.

The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

The active ingredients may also be entrapped in microcapsule prepared by means of techniques disclosed i.a. in Remington's Pharmaceutical Sciences 16th edition, Osol, A . Ed. (1980) .

The formulations to be used for *in vivo* administration must be sterile.

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody fragment of the invention, which matrices are in the form of shaped articles, e.g. films, or microcapsule.

An antibody fragment of the present invention may be used in *in vitro, ex vivo* and *in vivo* therapeutic methods. The invention provides various methods based on using antibody fragments having superior properties compared to conventional monovalent antibodies.

The present invention provides antibody fragments, which can be used for a variety of purposes, for example as therapeutics, prophylactics and diagnostics.

Antibody fragments of the invention can be used either alone or in combination with other compositions in a therapy. For instance, an antibody fragment of the invention may be co-administered with another antibody, chemotherapeutic agent (s) (including cocktails of chemotherapeutic agents), other cytotoxic agent (s), anti-angiogenic agent (s), cytokines, and/or growth inhibitory agent (s). Such combined therapies noted above include combined administration (where the two or more agents are included in the same or separate formulations), and separate administration, in which case, administration of the antibody of the invention can occur prior to, and/or following, administration of the adjunct therapy or therapies.

The antibody fragment of the invention (and adjunct therapeutic agent) is/are administered by any suitable means, including parenteral, subcutaneous, intraperitoneal, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. The antibody fragment is suitably administered by pulse infusion, particularly with declining doses of the antibody. Dosing can be by any suitable route, e.g. by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. The antibody fragment of the invention can be also delivered by gene transfer by mean of viral vectors (i.e. lentiviral vectors), administered locally or systemically .

The antibody fragment of the invention will be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The antibody need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibodies of the invention present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as used hereinbefore or about from 1 to 99% of the heretofore employed dosages.

For the prevention or treatment of disease, the appropriate dosage of an antibody fragment of the invention (when used alone or in combination with other agents such as chemotherapeutic agents) will depend on the type of disease to be treated, the type of antibody, the severity and course of the disease, whether the antibody fragment is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody fragment is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 mg/kg to 15 mg/kg of antibody is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 mg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the antibody fragment would be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, e.g. every week or every three weeks (e.g. such that the patient receives from about two to about twenty, e.g. about six doses of the antibody). An initial higher loading dose, followed by one or more lower doses may be administered. An exemplary dosing regimen comprises administering an initial loading dose of about 4 mg/kg, followed by a weekly maintenance dose of about 2 mg/kg of the antibody. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

RESULTS

Generation of the chimeric DN30 Fab and characterization of its biochemical and biological properties.

Like other monoclonal antibodies with therapeutic potential, DN30 has been raised in mice. Thus, its direct employment in humans for therapeutic purpose is not applicable, as the murine molecule would be recognized by human anti-murine antibodies (HAMA) that leads to immuno-mediated clearance of the antibody activity. Substitution of the murine constant regions of the antibody with sequences derived from human immunoglobulins (antibody chimerization) is sufficient to strongly reduce the HAMA response. Chimerized mAbs and Fabs are currently used in the clinic. Through classical molecular biology techniques, the present inventors have substituted the constant domains of the DN30 Fab heavy and light chains with constant domains derived from human immunoglobulins: the light chain constant domain has been substituted with the human kappa type domain, the one more represented in the natural human antibodies, while the heavy chain CHI constant domain has been substituted with the homologous domain derived from the human IgGl. This combination is effective: the chimerized DN30 Fab (MvDN30) binds Met with high affinity, induce Met shedding and inhibits proliferation of Met-addicted cells, overlapping the properties of the corresponding murine molecule (Fig 1 and Fig. 2).

Molecular design of the Dual Constant Domain Fab.

Using the MvDN30 sequence as a template, the present inventors duplicated the constant domains in each light and heavy chain (Dual Constant Domain-Fab). The new engineered molecule has a predicted molecular weight of 75 kD. The present inventors generated two different DCD-Fabs. In the first molecule the human constant domains were duplicated in tandem, thus generating a VH-CH1-CH1 chimeric heavy chain and a VL-CL-CL chimeric light chain. In the second molecule the terminal domain were swapped reciprocally, thus generating a VH-CH1-CL chimeric heavy chain and a VL-CL-CH1 chimeric light chain (Fig. 3). The corresponding dimeric recombinant molecules were named DCD-MvDN30.1 and DCD-MvDN30.2. cDNAs encoding for these new molecules were cloned into an expression plasmid and then expressed into eukaryotic cells. Protein were purified from cell culture supernatant thank to the StrepTAG that was inserted at the C-terminus of the sequence. Figure 4 shows the SDS-Page separation under reducing condition of the purified recombinant molecules having the correct molecular weight size.

DCD-MvDN30.1 and DCD-MvDN30.2 bind to Met with high affinity.

Purified DCD-MvDN30.1 and DCD-MvDN30.2 were characterized for their ability to bind the Met receptor. To this end, the present inventors performed ELISA assays using Met ectodomain in solid phase and MvDN30, DCD-MvDN30.1 and DCD-MvDN30.2 in liquid phase. Binding was revealed using anti-strepTAG antibodies (Fig. 5). This analysis showed that the three DN30-

derived monovalent molecules bind to Met with a similar affinity (MvDN30, K_d = 0.141 ± 0.03 nM; DCD-MvDN30 .1, K_d = 0.133 ± 0.02 nM; DCD-MvDN3 0.2, K_d = 0.130 ± 0.03 nM) .

DCD-MvDN30.1 and DCD-MvDN30.2 do not induce Met phosphorylation .

The present inventors tested whether the new DN30-derived molecules could display a Met agonistic activity in Met phosphorylation assay. This was analysed using A459 human lung carcinoma cells, which represent a standard system for determining Met activation in response to acute ligand stimulation. In fact, A549 cells express physiological levels of Met, inactive in basal conditions, but prone to be activated by HGF or a ligand-mimetic molecule (4, 10) . Cells were stimulated for 15 minutes with increasing amounts of MvDN30, DCD-MvDN3 0.1 and DCD-MvDN3 0.2 . Cells were also stimulated with HGF and DN-30 mAb as positive controls. Met activation was determined by immunoblotting with anti-phosphoMet antibodies. As shown in Fig. 6, the new molecules did not show any significant agonistic activity. DCD-MvDN30.1 was indistinguishable from MvDN30, being devoid of any agonistic activity. DCD-MvDN30.2 retained a minimal residual agonist activity, which was in any case negligible compared with the DN30 mAb or HGF. The present inventors also checked the activation of molecules acting as downstream effectors of Met. While stimulation with HGF induced the activation of both Extracellular signal-Regulated Kinases 1 and 2 (ERK-1 and ERK-2) and AKT/Protein Kinase B (AKT) , DCD-MvDN30.1 and DCD-MvDN30 .2, as MvDN30, did not affect the phosphorylation status of these signal transducers (Fig. 7) .

DCD-MvDN30.1 and DCD-MvDN30.2 induce Met shedding.

The present inventors also investigated whether the new molecules derived from MvDN30 maintain the ability to promote receptor shedding and downregulation . A549 cells were incubated with DCD-MvDN3 0.1, DCD-MvDN3 0.2 and MvDN3 0 . After 48 hours, the presence of Met ectodomain in the conditioned medium was analyzed by immunoblotting using a monoclonal antibody directed against the extracellular portion of Met. Total cellular levels of Met were also determined on cell lysates using the same antibody. This analysis revealed that both DCD-MvDN30.1 and DCD-MvDN30.2 efficiently induced Met shedding and promoted Met down-regulation, resulting in release of soluble Met ectodomain in the extracellular space and decreased Met levels in the cell (Fig. 8). Therefore, the new MvDN30 derived molecules, like MvDN30, achieved complete disassociation between the antagonistic and agonistic properties of the parental DN-30 mAb .

DCD-MvDN30.1 and DCD-MvDN30.2 inhibit HGF-induced Met phosphorylation and down-stream signaling.

The present inventors investigated if DCD-MvDN30.1 and DCD-MvDN3 0.2 could inhibit HGF-induced Met phosphorylation and down-stream signaling. A549 cells were incubated with DCD-MvDN30 .1, DCD-MvDN30.2 and MvDN30 for 24 hrs and then stimulated for 15 minutes with HGF. Met activation was determined by immunoblotting with anti-phosphoMet antibodies. As shown in Fig. 9, the two engineered molecules, as MvDN30, efficiently down-regulated Met receptor and strongly impaired the level of its phosphorylation. This resulted in an inhibition of AKT and ERK-1,2 activation (Fig. 9).

DCD-MvDN30.1 and DCD-MvDN30.2 inhibit *MET*-addicted anchorage-dependent cell growth.

Anchorage-dependent growth can be impaired by a Met-inhibitor only in the cells that rely on Met-signalling for proliferation/survival, the so called MBT-addicted cells. The present inventors analysed the complete panel of MBT-addicted tumor cells (GTL-16, SNU-5, Hs746T, MKN-45 - human gastric carcinoma cells - and H1993, EBC-1 - human lung carcinoma cells) . Exponentially growing cells were incubated with increasing concentrations of DCD-MvDN30.1 and DCD-MvDN30.2. MvDN30 was included in the assay as positive control. After 72 hours cell growth was determined using a luminescence-based ATP assay. Both the MvDN30-derived molecules inhibited all the MBT-addicted cell growth in a dose-dependent fashion (Fig. 10) . Inhibitory properties of the two DCD molecules were comparable to the ones of MvDN30 in all cells tested.

DCD-MvDN30.1 and DCD-MvDN30.2 inhibit anchorage-independent cell growth.

The present inventors tested the ability of DCD-MvDN30.1 and DCD-MvDN30.2 to inhibit anchorage independent growth of A549 cells. Cells were seeded in semi-solid medium incubated or not with HGF and treated with a single dose of DCD-MvDN30 .1, DCD-MvDN30.2 and MvDN30. After two weeks, cell colonies were stained and quantified. In this assay as well, DCD-MvDN30.1 and DCD-MvDN30.2 reduced HGF-dependent colony formation in a fashion similar to that of MvDN30 (Fig. 11) .

DCD-MvDN30.1 and DCD-MvDN30.2 show improved pharmacokinetic profile *in vivo* compared to MvDN30 .

The present inventors studied the pharmacokinetic properties of DCD-MvDN30.1 and DCD-MvDN30 .2, in comparison with MvDN30. A single dose of the above mentioned molecules were delivered by intraperitoneal injection to immunodef icient mice. Peripheral blood from the treated mice was collected at different time points after the delivery. The circulating concentrations of the studied molecules were determined by ELISA performed on the serum samples. DCD-MvDN30.1 and DCD-MvDN30.2 reached higher circulating levels compared to MvDN30. Moreover both the molecules showed increased half-life and are longer lasting in the circulation, being biological available for a longer time. DCD-MvDN3 0.1 and DCD-MvDN30.2 clearance is strongly improved compared to MvDN30 (clearance reduction compared to MVDN30: 9.6 and 13.7 fold respectively for DCD-MvDN30.1 and DCD-MvDN30 .2)(Fig. 12 and Table 1).

Table 1. Pharmacokinetic parameters of the different DN30-derived molecules

	t1/2	**CL** (ml/h)	**Vss** (ml)	**Cmax** (ng/ml)	**Tmax** (h)	**AUCtot** (ng/ml)h	**kel** (1/h)
MVDN30	8.41	4.36	11.96	7595	0.5	22933	0.082
DCD-MvDN30.1	10.53	0.45	5.77	24130	4	220188	0.066
DCD-MvDN30.2	10.27	0.32	4.36	24952	4	314667	0.068

tl/2: half-life; CL: clereance; Vss: Volume of distribution; Cmax: maximal molecule concentration; Tmax: time to reach Cmax; AUCtot: area under the Curve; Kel: constant of elimination.

MATHERIAL AND METHODS

Cell Culture

EBC-1 human lung carcinoma cell and MKN-45 gastric carcinoma cell line were obtained from the Japanese Collection of Research Bioresources (Osaka, Japan). GTL-16 human gastric carcinoma cells were derived from MKN-45 cells as described (11). All other cell lines were obtained from the ATCC-LGC Standards partnership (Sesto San Giovanni, Italy). All cell lines were maintained in RPMI except Hs746T - in DMEM - and SNU-5 - in IMDM. Cell media were supplemented with 10% (20% for SNU-5) Fetal Bovine Serum and 2 mM glutamine (Media, serum and glutamine were from Sigma Life Science, St. Louis, Missouri).

Protein engineering

DCD-MvDN30.1 and DCD-MvDN30.2 are comprised of a heavy chain and a light chain.

The DCD-MvDN30.1 heavy chain (SEQ ID NO.:1 and 2) corresponds to the VH domain of wild-type DN30 Fab (2; SEQ ID No.: 3 and 4) fused to the CHI domain of human immunoglobulin G1 repeated in tandem (SEQ ID NO.: 5 and 6). At the C-terminus, a STREP tag (ST, SEQ ID NO.:7) and a poly-histidine tag (HT, SEQ ID NO.: 8) have been added for purification and detection purposes. The overall structure corresponds to (from the N- to the C-terminus): VH-CH1-CH1-ST-HT. The nucleotide and amino acid sequences of the heavy chain of DCD-MvDN30.1 are reported in Figure 14A and B, respectively.

The DCD-MvDN30.1 light chain (SEQ ID No.:9 and 10) corresponds to the VL domain of wild-type DN30 Fab (SEQ ID No.: 11 and 12) fused to the CL domain of human immunoglobulin kappa (SEQ ID NO.: 13 and 14) repeated in tandem. The overall structure corresponds to (from the N- to the C-terminus): VL-CL-CL. The nucleotide and amino acid sequences of the light chain of DCD-MvDN30.1 are reported in Figure 13A and B, respectively.

The DCD-MvDN30.2 heavy chain (SEQ ID No.: 15 and 16) corresponds to the VH domain of wild-type DN30 Fab (SEQ ID No.:3 and 4) fused to the CHI domain of human immunoglobulin G1 (SEQ ID NO.: 5 and 6) plus the CL region of human immunoglobulin kappa (SEQ ID NO.: 13 and 14). The overall structure corresponds to (from the N- to the C-terminus): VH-CH1-CL. The nucleotide and amino acid sequences of the heavy chain of DCD-MvDN30.2 are reported in Figure 16A and B, respectively.

The DCD-MvDN30.2 light chain (SEQ ID No.: 17 and 18) corresponds to the VL domain of wild-type DN30 Fab (SEQ ID No.: 11 and 12) fused to the CL domain of human immunoglobulin kappa (SEQ ID NO.: 13 and 14) plus the CHI of human immunoglobulin Gl (SEQ ID NO.: 5 and 6) plus the STREP tag (ST, SEQ ID NO.:7) and the poly-histidine tag (HT, SEQ ID NO.: 8). The overall structure corresponds to (from the N- to the C-terminus): VL-CL-CH1-ST-HT. The nucleotide and amino acid sequences are of the light chain of DCD-MvDN30.2 reported in Figure 15A and B, respectively.

The cDNAs encoding DCD-MvDN30.1 and DCD-MvDN30.2 were synthesized chemically by the GeneArt® service (Life Technologies, Paisley, United Kingdom). The nucleotide and amino acid sequences of DN30 Fab light and heavy chain variable domains are reported in Figures 17 and 18, respectively. The CDR regions are underlined both in the nucleotide and amino acid sequences, wherein the CDRs of the heavy chain variable domain have the amino acid and nucleotide sequences set forth in SEQ ID No.: 19 to 24, and the CDRs of the light chain variable domain have the amino acid and nucleotide sequences set forth in SEQ ID No.: 25 to 30.

All constructs were engineered to contain a BamHI site at the 5' end and a Notl site at the 3' end. The BamHI-NotI fragments were subcloned into the pUPEX expression vector (U-Protein Express, Utrecht, The Netherlands). Medium-scale production of DCD-MvDN30.1 and DCD-MvDN30.2 was outsourced to U-Protein Express that achieved it by transient transfections into HEK (Human Epithelial Kidney) cells. Proteins were purified by affinity chromatography using the STREP tag and the poly-histidine tag. Purified proteins were conserved in

PBS plus 0.02% Tween-80 (Sigma-Aldrich) and stored at 4° C. Purity was determined by SDS-PAGE in both reducing and non-reducing conditions followed by Coomassie staining.

Iiranunoprecipitation and Western Blotting

Immunoprecipitation was performed as described (12) using the DO-24 anti-Met mAb (4). Western blotting was performed using the following antibodies: anti-human Met mAb clone DL-21 that recognizes a domain located in the extracellular portion of Met (4); anti-phosphotyrosine mAb clone 4G10 mAb (Millipore, Temecula, California); anti-phospho-Met (Tyr 1234/1235), anti-phospho-Met (Tyr 1349), anti-phospho-Akt (Ser 473), anti-Akt, anti-phospho-ERK (Thr 202/Tyr 204) and anti-ERK polyclonal Abs (Cell Signaling Technology, Beverly, Massachusetts).

ELISA binding assays

Binding of MvDN3 0, DCD-MvDN3 0.1 and DCD-MvDN3 0.2 was determined by ELISA using a Met-Fc chimera in solid phase (R&D Systems, Minneapolis, Minnesota) and increasing concentrations of FLAG-tagged recombinant antibody in liquid phase. Binding was revealed using an anti-strepTAG II antibody conjugated with horseradish peroxidase (IBA, Olivette, Missouri). Data were analyzed and fit using Prism software (Graph Pad Software, San Diego, California). Met-Fc chimera is a fusion protein wherein the Fc domain derived from a human IgG is fused in frame with the Met extracellular portion.

Met activation analysis

Subconfluent A549 human lung carcinoma cells were incubated in serum-free medium for 48 hours and then stimulated for 10 minutes with the indicated concentrations of recombinant HGF (R&D Systems) or purified DN-30 mAb, MvDN30, DCD-MvDN30.1 and DCD-MvDN30.2 as described (13). Following stimulation, cells were immediately lysed and processed as described (12). Cell extracts were immunoprecipitated with anti-Met antibodies (DO-24), resolved by SDS-PAGE and analyzed by Western blotting using anti-phosphotyrosine antibodies (Millipore). The same blots were re-probed with anti-Met antibodies (DL-21) to normalize the amount of Met immunoprecipitated.

For the inhibition of HGF-induced Met phosphorylation A549 cells were treated for 24 hrs in serum free medium with MvDN30, DCD-MvDN30.1 and DCD-MvDN30.2 and than stimulated with HGF as described above. Cell monolayers were lysated with Laemmli buffer and equal amounts of total proteins, separated into acrylammide gel by SDS-PAGE and analyzed by immunoblotting with anti-phospho-Met (Tyr 1234/1235) antibodies.

Analysis of Met shedding

Subconfluent A549 monolayers were washed twice with PBS and then incubated in serum-free medium with the indicated concentrations of DN-30 FAb or mAb. After 48 hours, conditioned medium was collected and cells were lysed with Laemmli Buffer. Met protein levels were determined in 50 µg of total cell lysates and in 50 µl of cell culture supernatant by Western blotting using the anti-Met DL-21 mAb.

In vitro biological assays

For cell growth analysis, cells were seeded in 96 well-dishes (1,000 cells/well) in medium containing 10% FBS. After 24 hours, the medium was replaced with fresh one containing the DN30-derived molecules plus 5% FCS antibodies at the indicated concentrations. Cell number was evaluated after 72hrs using the CellTiter-Glo luminescent cell viability assay (Promega Corporation, Madison, Wisconsin) according to manufacturer' s instructions. Chemo-luminescence was detected with a Multilabel Reader PerkinElmer 2030 apparatus (PerkinElmer Life and Analytical Sciences, Turku, Finland) .

For anchorage-independent growth assays, cells were seeded in 48 well-dishes (500 cells/well) in medium containing 2% FBS and 0.5% SeaPlaque agarose (BMA, Rockland, Maine) . Antibodies (1.5 uM) and HGF (50 ng/ml) were added in the culture medium every 3 days. After 21 days of culture, colonies were stained by tetrazolium salts (Sigma Life Science) and scored by MetaMorphOf fline Software (Molecular Device LLC, Sunnyvale, California) .

Pharmakokinetic analysis

Adult immunodef icient NOD-SCID mice (body weight between 18 and 22 gr, on average 20 gr) were injected IP with 100 μg of DCD-MvDN30.1 or DCD-MvDN30.2 or MvDN30. Peripheral blood was collected at different time (for MvDN30: 10, 20 and 30 min, 1, 2, 4, 6, 8, 10, 16, 24, 48 hours; for DCD-MvDN3 0.1 and DCD-MvDN30 .2 : 30 min, 1, 2, 4, 6, 8, 10, 16, 24, 48, 72, 96, 144 hrs after the delivery) . Therapeutic molecule concentrations were evaluated by ELISA as described above in binding assay section, interpolating the absorbance values of the samples on the linear part of a standard curve obtained by serial dilutions of the different purified molecules. Each time point was the average value of a least 3 mice.

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CLAIMS

1. Antibody fragment comprising a first polypeptide comprising a light chain variable domain and two constant domains and a second polypeptide comprising a heavy chain variable domain and two constant domains, wherein two constant domains are light chain constant domains and two constant domains are heavy chain CHI constant domains.

2. Antibody fragment according to claim 1, wherein said light chain variable domain is selected between a non-human light chain variable domain or a human or humanized light chain variable domain comprising the complementarity determining regions (CDRs) from a non-human antibody.

3. Antibody fragment according to any one of claims 1 to 2, wherein said heavy chain variable domain is selected between a non-human heavy chain variable domain or a human or humanized heavy chain variable domain comprising the complementarity determining regions (CDRs) from a non-human antibody.

4. Antibody fragment according to any one of claims 1 to 3, wherein the first polypeptide comprises a light chain variable domain and two human light chain constant domains, wherein the light chain variable domain is fused to one human light chain constant domain and the two human light chain constant domains are fused to each other.

5. Antibody fragment according to any one of claims 1 to 4, wherein the second polypeptide comprises a heavy chain variable domain and two human heavy chain constant domains, wherein the heavy chain variable domain is fused to one human heavy chain CHI constant domain and the two human heavy chain CHI constant domains are fused to each other.

6. Antibody fragment according to any one of claim 1 to 3, wherein the first polypeptide comprises a light chain variable domain, one human light chain constant domain and one human heavy CHI constant domain, wherein the light chain variable domain is fused to the human light chain constant domain, and the human light chain constant domain is fused to the human heavy chain CHI constant domain.

7. Antibody fragment according to any one of claims 1 to 3 or 6, wherein the second polypeptide comprises a heavy chain variable domain, one human heavy chain CHI constant domain and one human light chain constant domain, wherein the heavy chain variable domain is fused to the human heavy chain CHI constant domain, and the human heavy chain CHI constant domain is fused to the human light chain constant domain.

8. Antibody fragment according to any one of claims 1 to 7, wherein the light chain constant domain is a human kappa light chain constant domain.

9. Antibody fragment according to any one of claims 1 to 8, wherein the heavy chain CHI constant domain is a human gamma heavy chain CHI constant domain .

10. Antibody fragment according to any one of claims 1 to 9, wherein the heavy chain CHI constant domain is from a human IgGl.

11. Antibody fragment according to any one of claims 1 to 10, wherein the antibody fragment is more stable *in vivo* than the Fab molecule comprising said light and heavy chain variable domains.

12. Antibody fragment according to any one of claims 1 to 11, wherein said antibody fragment specifically binds the hepatocyte growth factor receptor (HGFR) .

13. Pharmaceutical composition comprising the antibody fragment according to one of claims 1 to 12 and a pharmaceutically acceptable carrier.

14. The antibody fragment according to any one of claims 1 to 12 for use in the treatment of a patient suffering from a tumor and/or metastasis.

15. The antibody fragment according to claim 14, wherein said tumor and/or metastasis is associated with dysregulation of HGFR signaling and/or activation.

16. Product containing an antibody fragment according to any one of claims 1 to 12 and at least one of an HGF inhibitor and an HGFR inhibitor, as a combined preparation for simultaneous, separate or sequential use in the treatment of tumors and/or metastases, preferably a tumor and/or metastasis associated with dysregulation of HGFR signaling and/or activation .

17. Product according to claim 16, wherein the at least one inhibitor is selected from anti-HGF antibodies, recombinant molecules competing with HGF, small molecule HGFR inhibitors, anti-HGFR antibodies or inhibitors .

18. Isolated nucleic acid encoding the antibody fragment according to any one of claims 1 to 12.

19. Vector comprising at least a nucleotide sequence encoding the antibody fragment according to any one of claims 1 to 12.

20. Composition comprising two or more nucleic acids encoding the antibody fragment according to any one of claims 1 to 12.

21. Isolated nucleic acid according to claim 18, vector according to claim 19, or composition according to claim 20 for use in the treatment of a patient suffering from a tumor and/or metastasis, preferably a tumor and/or metastasis associated with dysregulation of HGFR signaling and/or activation .

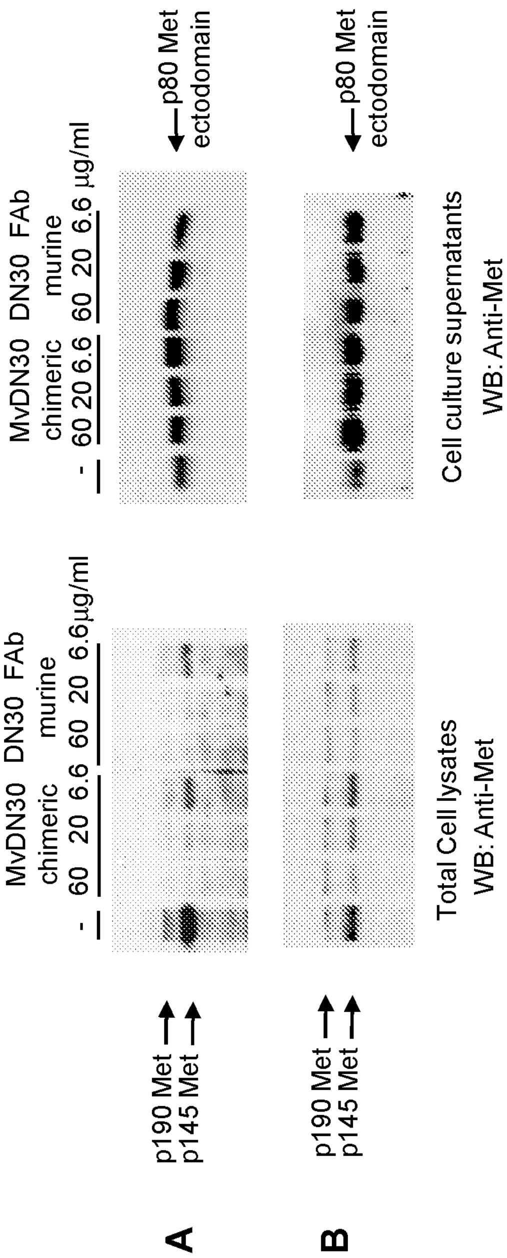
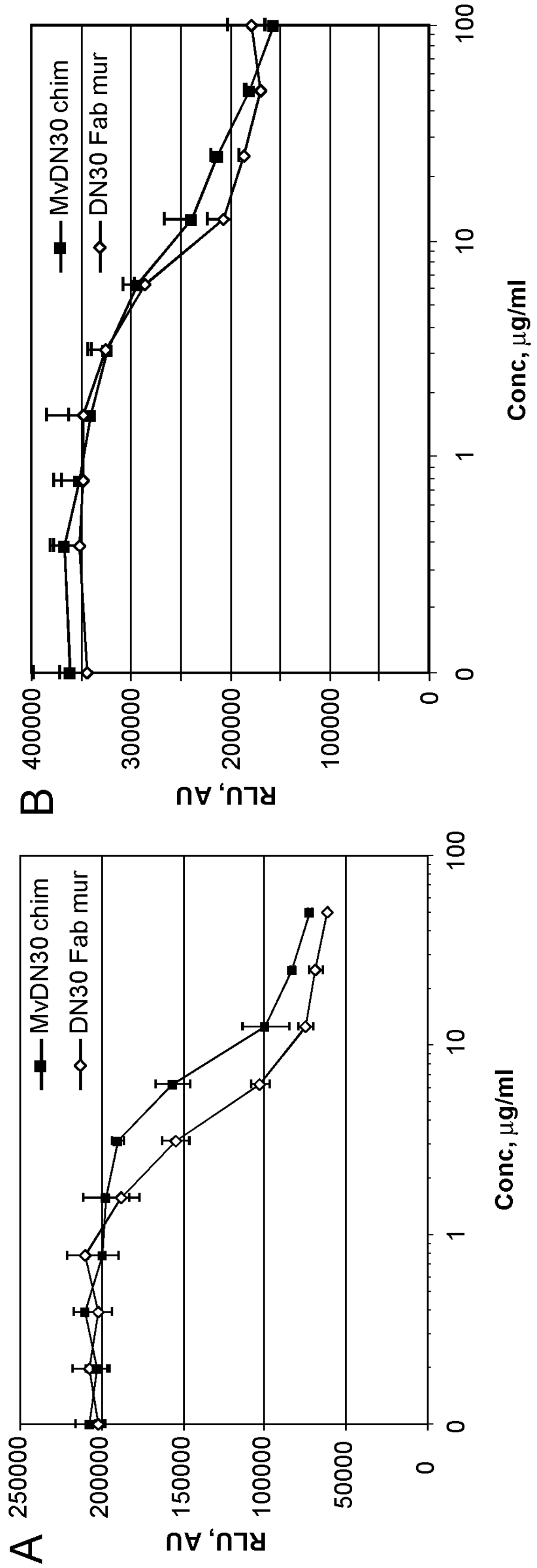

A
B
p190 Met
p145 Met
MvDN30 chimeric
DN30 FAb murine
- 60 20 6.6 60 20 6.6 μg/ml
Total Cell lysates
WB: Anti-Met
p80 Met ectodomain
Cell culture supernatants
WB: Anti-Met

Figure 1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200000
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0
1
10
100
Conc, µg/ml
MvDN30 chim
DN30 Fab mur
B
RLU, AU
400000
300000
200000
100000
0
0
1
10
100
Conc, µg/ml
MvDN30 chim
DN30 Fab mur

Figure 2

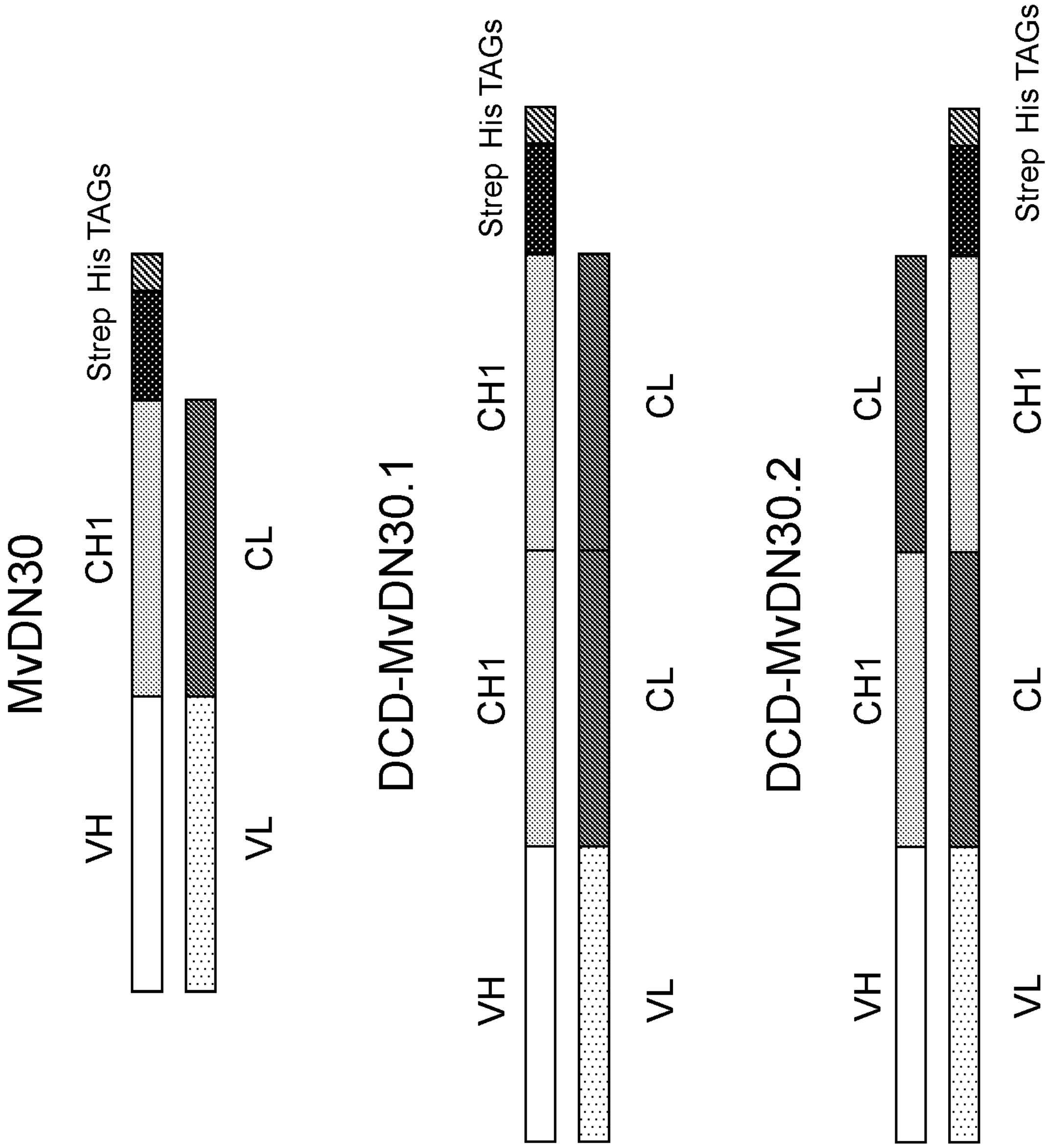

MvDN30
VH
CH1
Strep His TAGs
VL
CL
DCD-MvDN30.1
VH
CH1
CH1
Strep His TAGs
VL
CL
CL
DCD-MvDN30.2
VH
CH1
CL
VL
CL
CH1
Strep His TAGs

Figure 3

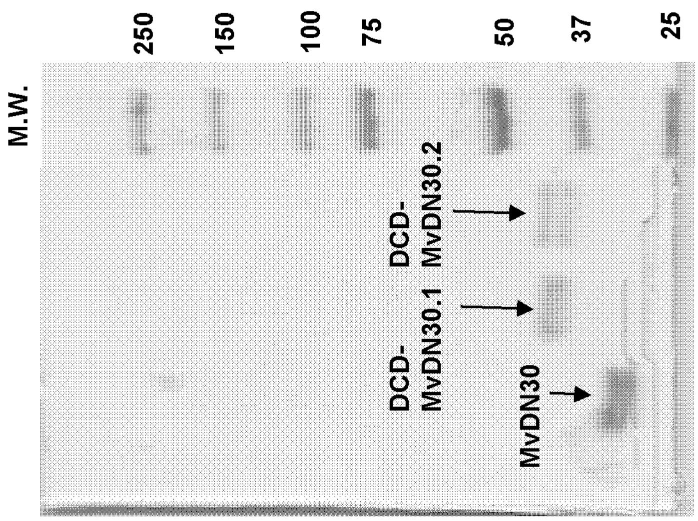
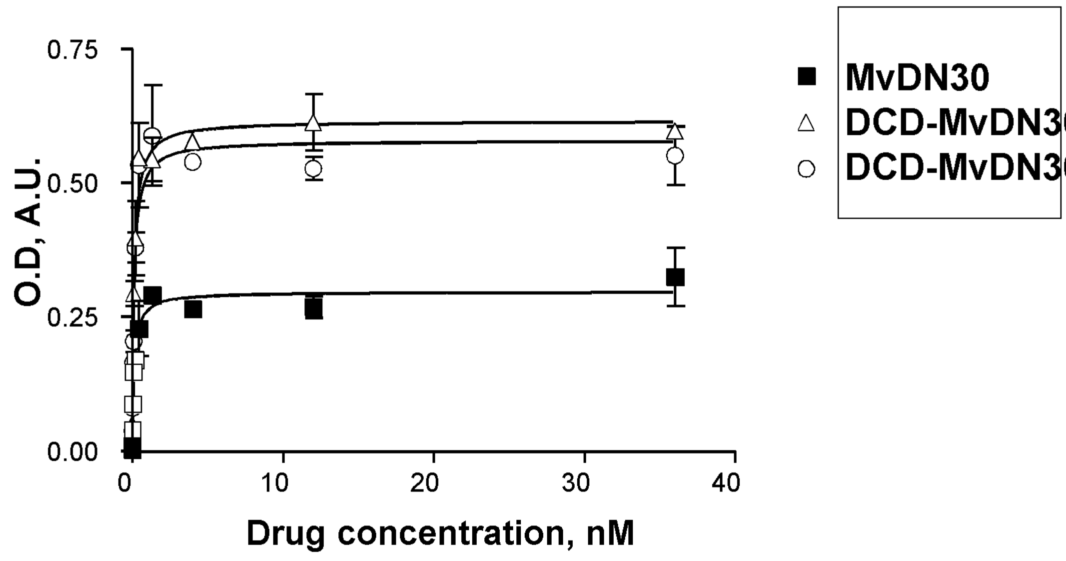


Figure 4



MvDN30	Kd= 0.141 +/- 0.03 nM	Vmax= 0.30 +/- 0.012
DCD-MvDN30.1	Kd= 0.133 +/- 0.02 nM	Vmax= 0.62 +/- 0.018
DCD-MvDN30.2	Kd= 0.130 +/- 0.03 nM	Vmax= 0.58 +/- 0.024

Figure 5

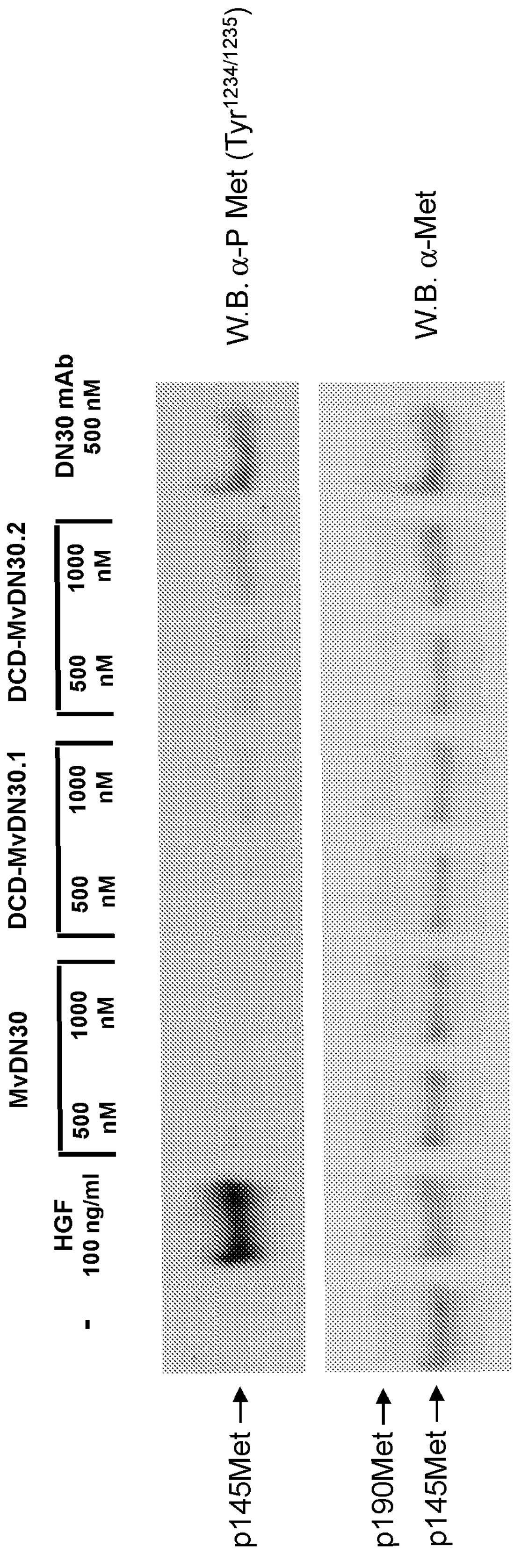

-
HGF
100 ng/ml
MvDN30
500 nM
1000 nM
DCD-MvDN30.1
500 nM
1000 nM
DCD-MvDN30.2
500 nM
1000 nM
DN30 mAb
500 nM
W.B. α-P Met (Tyr1234/1235)
W.B. α-Met
p145Met
p190Met
p145Met

Figure 6

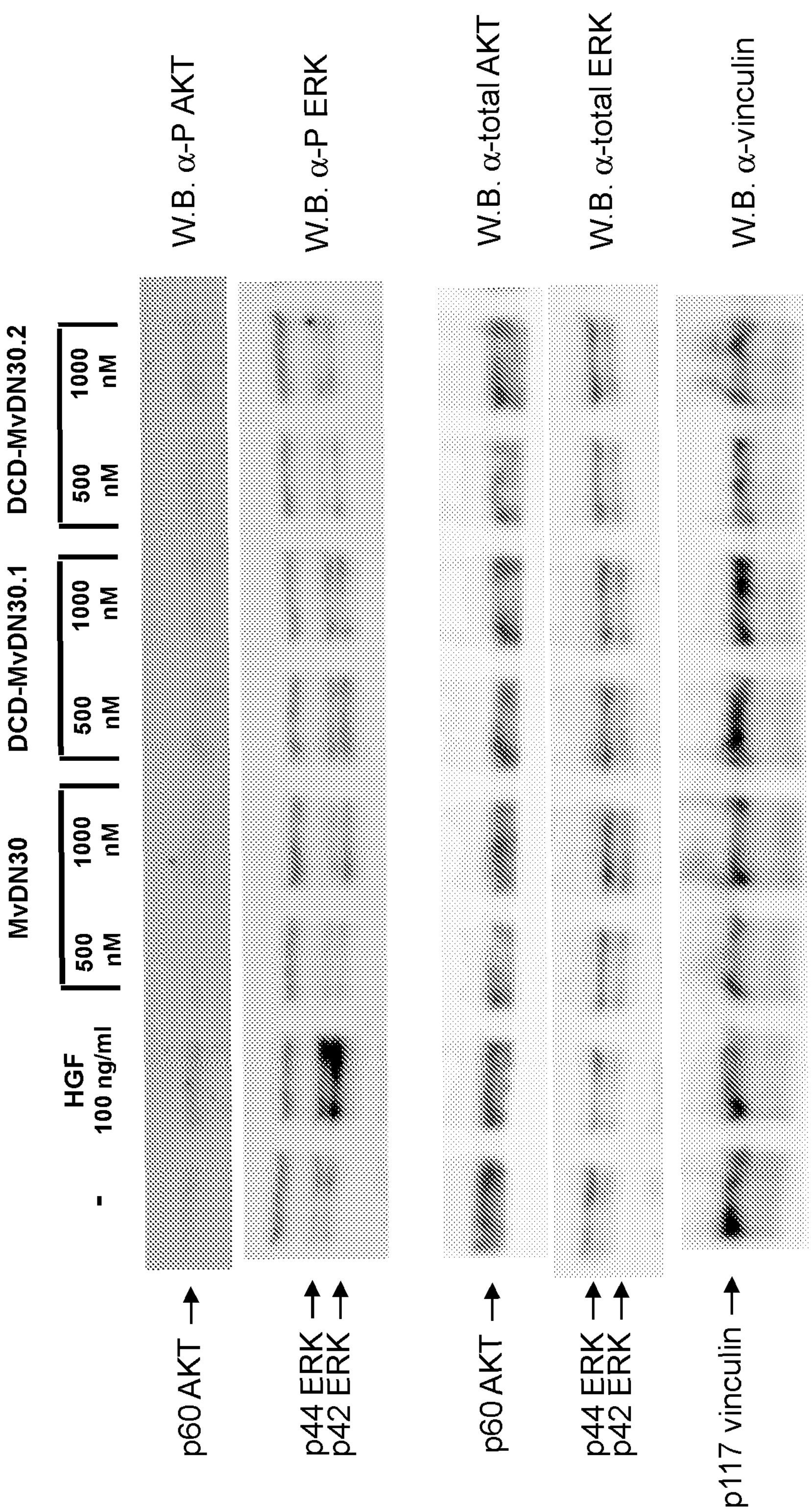

-
HGF
100 ng/ml
MvDN30
500
nM
1000
nM
DCD-MvDN30.1
500
nM
1000
nM
DCD-MvDN30.2
500
nM
1000
nM
W.B. α-P AKT
W.B. α-P ERK
W.B. α-total AKT
W.B. α-total ERK
W.B. α-vinculin
p60 AKT
p44 ERK
p42 ERK
p60 AKT
p44 ERK
p42 ERK
p117 vinculin

Figure 7

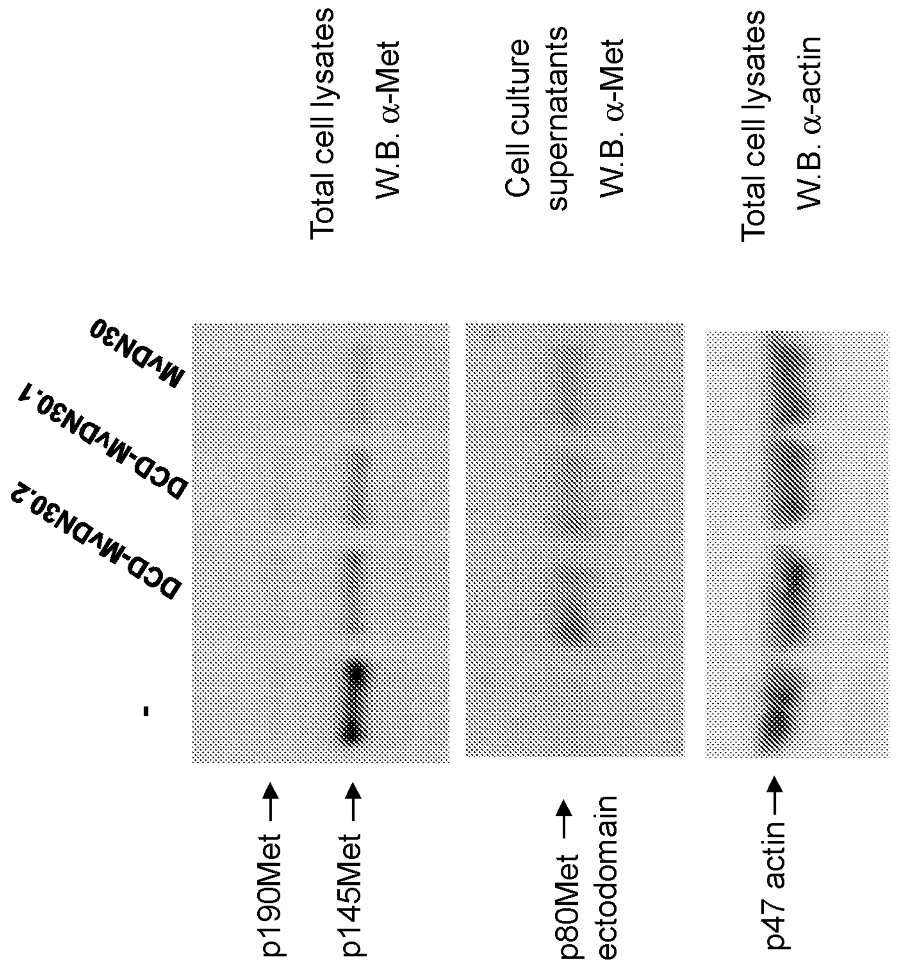

Total cell lysates
W.B. α-Met
Cell culture supernatants
W.B. α-Met
Total cell lysates
W.B. α-actin
MvDN30
DCD-MvDN30.1
DCD-MvDN30.2
-
p190Met
p145Met
p80Met ectodomain
p47 actin

Figure 8

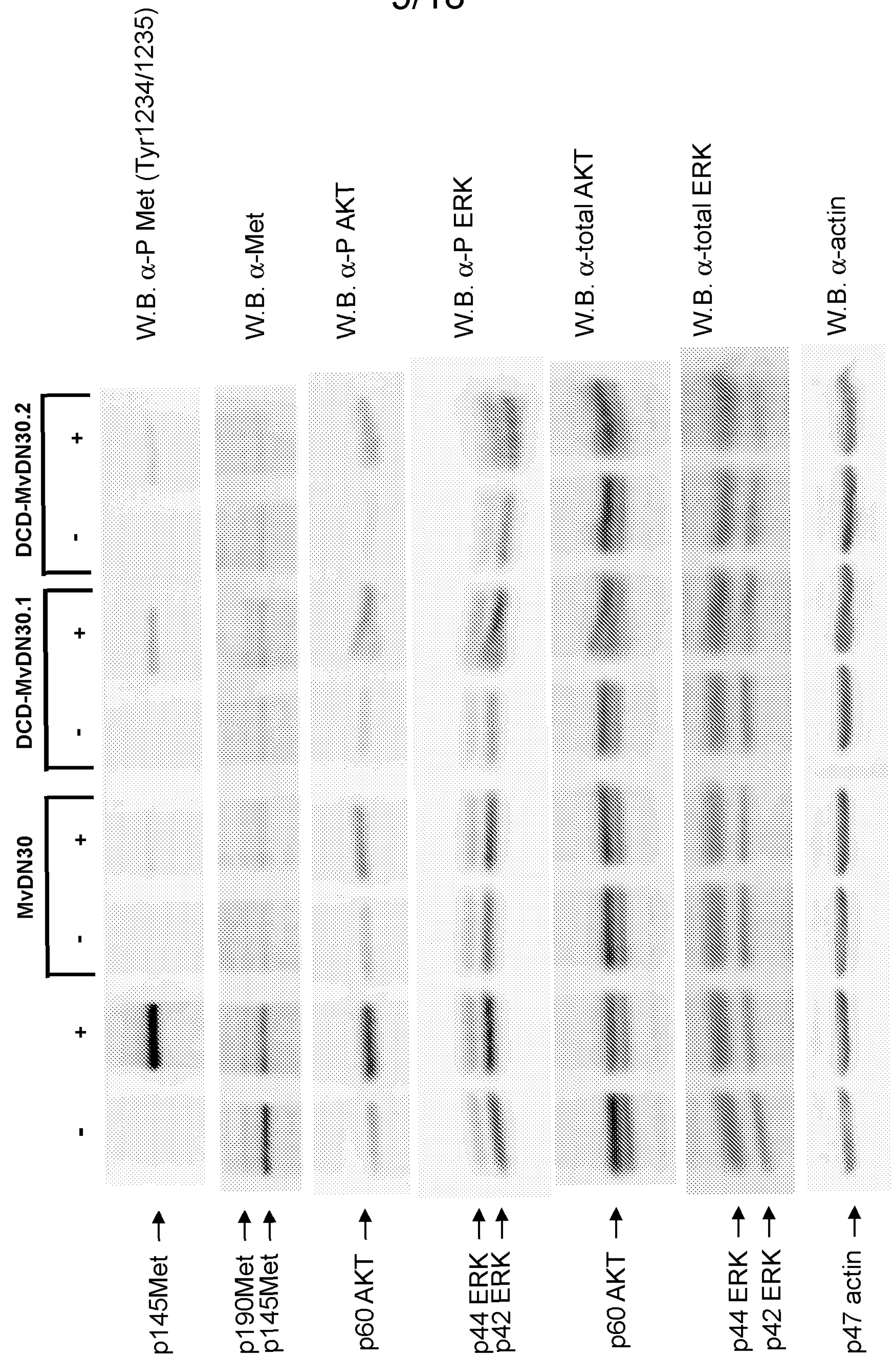

MvDN30
DCD-MvDN30.1
DCD-MvDN30.2
- + - + - + - +
W.B. α-P Met (Tyr1234/1235)
W.B. α-Met
W.B. α-P AKT
W.B. α-P ERK
W.B. α-total AKT
W.B. α-total ERK
W.B. α-actin
p145Met
p190Met
p145Met
p60 AKT
p44 ERK
p42 ERK
p60 AKT
p44 ERK
p42 ERK
p47 actin

Figure 9

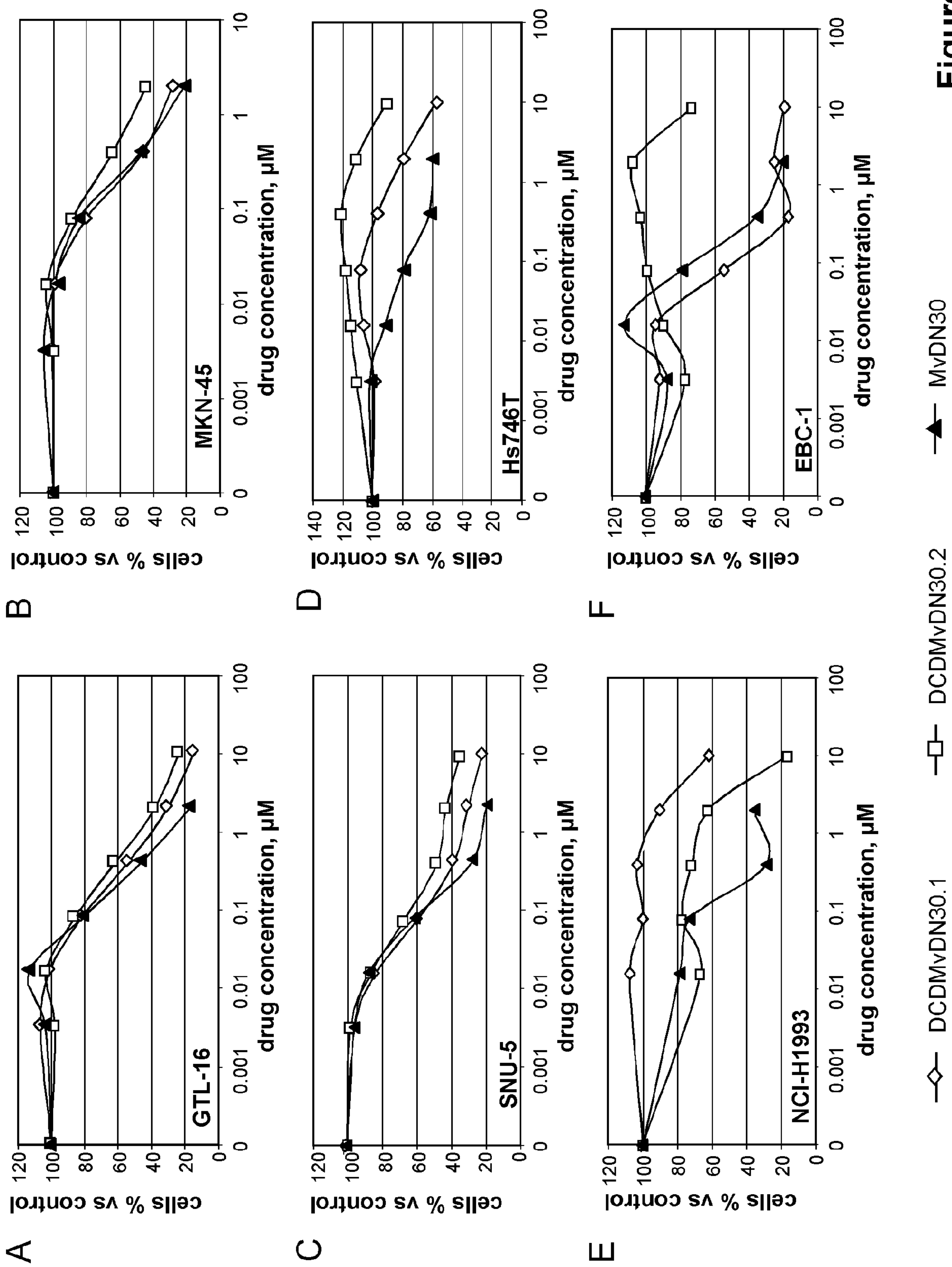

A
GTL-16
B
MKN-45
C
SNU-5
D
Hs746T
E
NCI-H1993
F
EBC-1
cells % vs control
drug concentration, µM
DCDMvDN30.1
DCDMvDN30.2
MvDN30

Figure 10

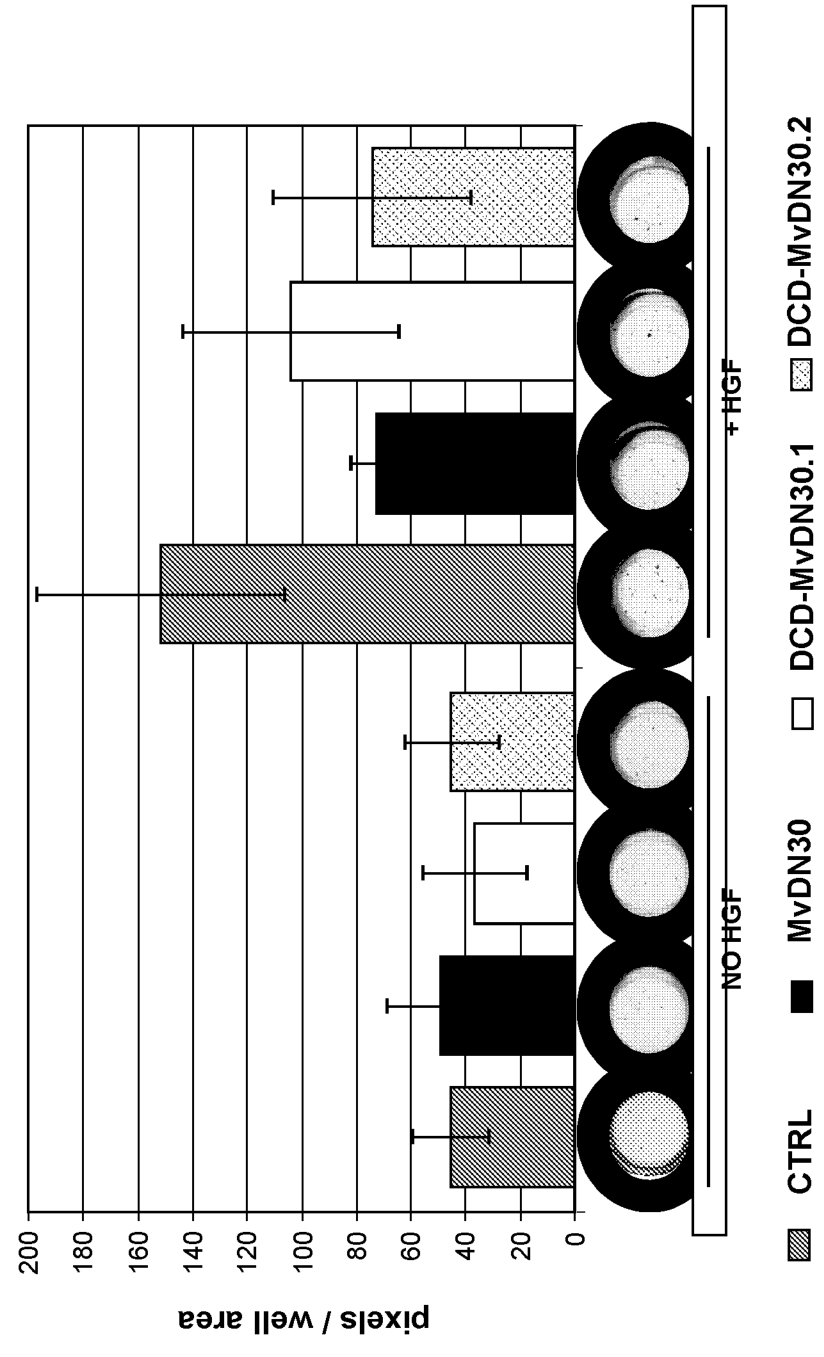

pixels / well area
200
180
160
140
120
100
80
60
40
20
0
NO HGF
+ HGF
CTRL
MvDN30
DCD-MvDN30.1
DCD-MvDN30.2

Figure 11

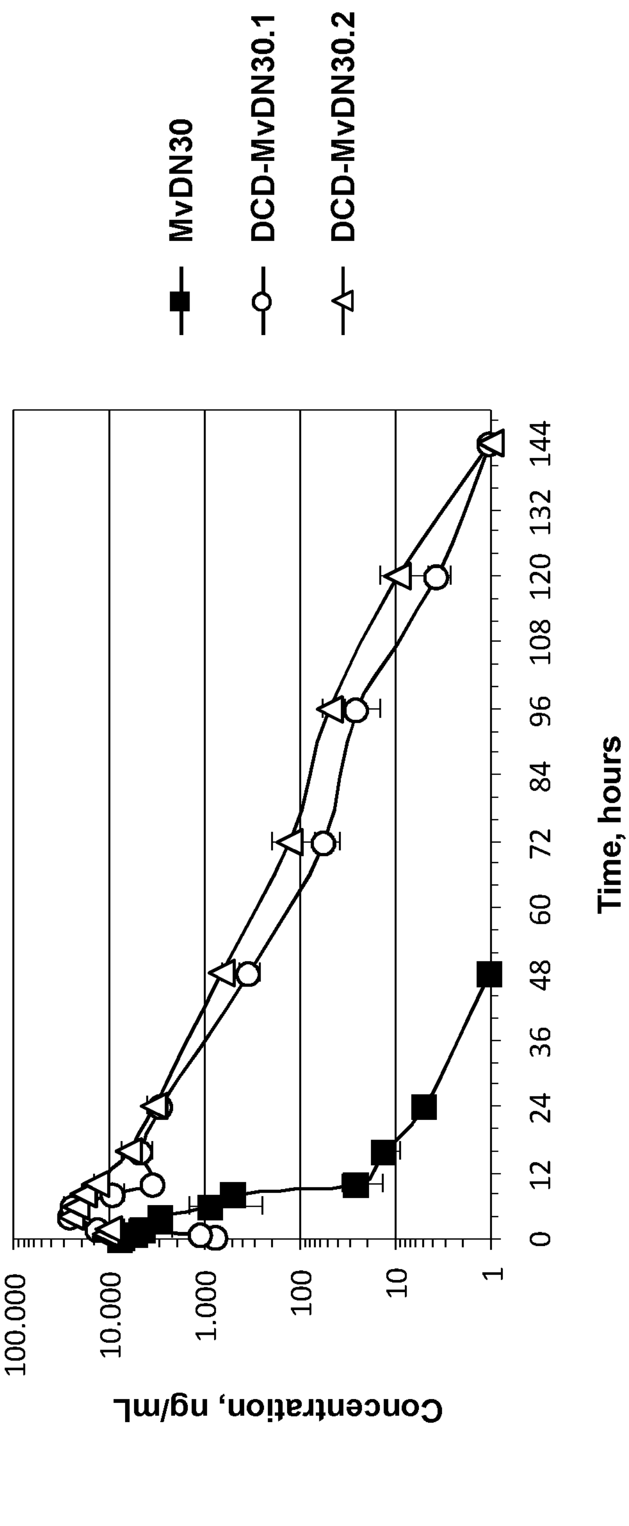

MvDN30
DCD-MvDN30.1
DCD-MvDN30.2
Concentration, ng/mL
100.000
10.000
1.000
100
10
1
Time, hours
0
12
24
36
48
60
72
84
96
108
120
132
144

Figure 12

VL-CL-CL

A- Nucleotide sequence (SEQ ID No.:9)

5'-
atggagacagacacaatcctgctatgggtgctgctgctctgggttccaggctccactggtgacattgtgctgacccaatctccagcttctttgg
ctgtgtctctagggcagagggccaccatctcctgcaaggccagccaaagtgttgattatgatggtggtagttatatgagttggttccaacaga
gaccaggacagccacccaaactcctcatctctgctgcatccaaccttgaatctggcatcccagccaggtttagtggcagtggctctgggac
agacttcaccctcaatatccatcctgtggaggaggaggatgttgcaacctattactgtcagcaaagttatgaagacccgctcacgttcggtg
ctggtaccaaggtggagatcaaacgaactgtggctgcaccatctgtcttcatcttcccgccatctgatgagcagttgaaatctggaactgcct
ctgttgtgtgcctgctgaataacttctatcccagagaggccaaagtacagtggaaggtggataacgccctccaatcgggtaactcccagg
agagtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcaccctgacgctgagcaaagcagactacgagaaac
acaaagtctacgcctgcgaagtcacccatcagggcctgagctcgcccgtcacaaagagcttcaacaggggagagtgtactgtggctgc
accatctgtcttcatcttcccgccatctgatgagcagttgaaatctggaactgcctctgttgtgtgcctgctgaataacttctatcccagagagg
ccaaagtacagtggaaggtggataacgccctccaatcgggtaactcccaggagagtgtcacagagcaggacagcaaggacagcac
ctacagcctcagcagcaccctgacgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggcct
gagctcgcccgtcacaaagagcttcaacaggggagagtgttaa-3'

B- Amino acid sequence (SEQ ID No.:10)

N term-
METDTILLWVLLLWVPGSTGDIVLTQSPASLAVSLGQRATISCKASQSVDYDGGSYMSWFQQRPG
QPPKLLISAASNLESGIPARFSGSGSGTDFTLNIHPVEEEDVATYYCQQSYEDPLTFGAGTKVEIKR
TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST
YSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC- C term

Figure 13

VH-CH1-CH1-*TAGs*

A- Nucleotide sequence (SEQ ID No.:1)

5'-
atgggatggagctatatcatcctctttttggtagcaacagctacagatggccactcccaggtccaactgcaacagcctgggactgaactggt
gaagcctggggcttcagtgaagctgtcctgcaaggcttctggctacaccttcaccagttactggatacactgggtgaagcagaggcctgga
caaggccttgagtggattggagagattaatcctagcagcggtcgtactaactacaacgagaaattcaagaacaaggtcacagtgactgta
gacaaatcttccaccacagcctacatgcaactcagcaacctgacatctgaggactctgcggtctattactgtgcaagtaggggctactgggg
ccaaggcaccactctcacagtctcctcagctagcacgaagggcccatcggtcttccccctggcaccctcctccaagagcacctctggggg
cacagcggccctgggctgcctggtcaaggactacttccccgaaccggtgacggtgtcgtggaactcaggcgccctgaccagcggcgtgc
acaccttcccggctgtcctacagtcctcaggactctactccctcagcagcgtggtgaccgtgccctccagcagcttgggcacccagacctac
atctgcaacgtgaatcacaagcccagcaacaccaaggtggacaagaaagttgagcccaaatcttgtgcaagcacgaagggcccatcg
gtcttccccctggcaccctcctccaagagcacctctgggggcacagcggccctgggctgcctggtcaaggactacttccccgaaccggtga
cggtgtcgtggaactcaggcgccctgaccagcggcgtgcacaccttcccggctgtcctacagtcctcaggactctactccctcagcagcgtg
gtgaccgtgccctccagcagcttgggcacccagacctacatctgcaacgtgaatcacaagcccagcaacaccaaggtggacaagaaa
gttgagcccaaatcttgtgacaaaactcacaca*GGTGCCGCATGGAGCCACCCCCAGTTCGAAAAAGGGGCC*
GCATGGAGCCACCCCCAGTTCGAAAAAGGGGCCGCATGGAGCCACCCCCAGTTCGAAAAAGG
*GGCCGCACACCATCACCATCACCAT*TAG-3'

B- Amino acid sequence (SEQ ID No.:2)

N term-
MGWSYIILFLVATATDGHSQVQLQQPGTELVKPGASVKLSCKASGYTFTSYWIHWVKQRPGQGLE
WIGEINPSSGRTNYNEKFKNKVTVTVDKSSTTAYMQLSNLTSEDSAVYYCASRGYWGQGTTLTVSS
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS
SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCASTKGPSVFPLAPSSKSTSGGTAALGCLV
KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD
KKVEPKSCDKTHT*GAAWSHPQFEKGAAWSHPQFEKGAAWSHPQFEKGAAHHHHHH*-C term

Figure 14

VL-CL-CH1-*TAGs*

A- Nucleotide sequence (SEQ ID No.:17)

5'-
atggagacagacacaatcctgctatgggtgctgctgctctgggttccaggctccactggtgacattgtgctgacccaatctccagcttctttggct
gtgtctctagggcagagggccaccatctcctgcaaggccagccaaagtgttgattatgatggtggtagttatatgagttggttccaacagaga
ccaggacagccacccaaactcctcatctctgctgcatccaaccttgaatctggcatcccagccaggtttagtggcagtggctctgggacaga
cttcaccctcaatatccatcctgtggaggaggaggatgttgcaacctattactgtcagcaaagttatgaagacccgctcacgttcggtgctggt
accaaggtggagatcaaacgaactgtggctgcaccatctgtcttcatcttcccgccatctgatgagcagttgaaatctggaactgcctctgttgt
gtgcctgctgaataacttctatcccagagaggccaaagtacagtggaaggtggataacgccctccaatcgggtaactcccaggagagtgtc
acagagcaggacagcaaggacagcacctacagcctcagcagcaccctgacgctgagcaaagcagactacgagaaacacaaagtct
acgcctgcgaagtcacccatcagggcctgagctcgcccgtcacaaagagcttcaacaggggagagtgtgcaagcacgaagggcccatc
ggtcttccccctggcaccctcctccaagagcacctctgggggcacagcggccctgggctgcctggtcaaggactacttccccgaaccggtg
acggtgtcgtggaactcaggcgccctgaccagcggcgtgcacaccttcccggctgtcctacagtcctcaggactctactccctcagcagcgt
ggtgaccgtgccctccagcagcttgggcacccagacctacatctgcaacgtgaatcacaagcccagcaacaccaaggtggacaagaaa
gttgagcccaaatcttgtgacaaaactcacaca*GGTGCCGCATGGAGCCACCCCCAGTTCGAAAAAGGGGCC*
GCATGGAGCCACCCCCAGTTCGAAAAAGGGGCCGCATGGAGCCACCCCCAGTTCGAAAAAGG
*GGCCGCACACCATCACCATCACCAT*TAG-3'

B- Amino acid sequence (SEQ ID No.:18)

N term-
METDTILLWVLLLWVPGSTGDIVLTQSPASLAVSLGQRATISCKASQSVDYDGGSYMSWFQQRPGQ
PPKLLISAASNLESGIPARFSGSGSGTDFTLNIHPVEEEDVATYYCQQSYEDPLTFGAGTKVEIKRTV
AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL
SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECASTKGPSVFPLAPSSKSTSGGTAALGC
LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV
DKKVEPKSCDKTHT*GAAWSHPQFEKGAAWSHPQFEKGAAWSHPQFEKGAAHHHHHH*- C term

Figure 15

VH-CH1-CL

A- Nucleotide sequence (SEQ ID No.:15)

5'-
atgggatggagctatatcatcctctttttggtagcaacagctacagatggccactcccaggtccaactgcaacagcctgggactgaactgg
tgaagcctggggcttcagtgaagctgtcctgcaaggcttctggctacaccttcaccagttactggatacactgggtgaagcagaggcctgg
acaaggccttgagtggattggagagattaatcctagcagcggtcgtactaactacaacgagaaattcaagaacaaggtcacagtgactg
tagacaaatcttccaccacagcctacatgcaactcagcaacctgacatctgaggactctgcggtctattactgtgcaagtaggggctactg
gggccaaggcaccactctcacagtctcctcagctagcacgaagggcccatcggtcttcccccctggcaccctcctccaagagcacctctg
ggggcacagcggccctgggctgcctggtcaaggactacttccccgaaccggtgacggtgtcgtggaactcaggcgccctgaccagcg
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gacctacatctgcaacgtgaatcacaagcccagcaacaccaaggtggacaagaaagttgagcccaaatcttgtactgtggctgcacca
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gcctcagcagcaccctgacgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggcctgagc
tcgcccgtcacaaagagcttcaacaggggagagtgttaa-3'

B- Amino acid sequence (SEQ ID No.:16)

N term-
MGWSYIILFLVATATDGHSQVQLQQPGTELVKPGASVKLSCKASGYTFTSYWIHWVKQRPGQGLE
WIGEINPSSGRTNYNEKFKNKVTVTVDKSSTTAYMQLSNLTSEDSAVYYCASRGYWGQGTTLTVS
SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS
LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCTVAAPSVFIFPPSDEQLKSGTASVVCL
LNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ
GLSSPVTKSFNRGEC- C term

Figure 16

VL

A- Nucleotide sequence (SEQ ID No.:11)
5'-

CDRL1
gacattgtgctgacccaatctccagcttctttggctgtgtctctagggcagagggccaccatctcctgcaaggccagccaaagtgttgatt
CDRL2
atgatggtggtagttatatgagttggttccaacagagaccaggacagccacccaaactcctcatctctgctgcatccaaccttgaatctgg
catcccagccaggtttagtggcagtggctctgggacagacttcaccctcaatatccatcctgtggaggaggaggatgttgcaacctatta
CDRL3
ctgtcagcaaagttatgaagacccgctcacgttcggtgctggtaccaaggtggagatcaaacga-3'

B- Amino acid sequence (SEQ ID No.:12)
N term-

CDRL1 CDRL2
DIVLTQSPASLAVSLGQRATISCKASQSVDYDGGSYMSWFQQRPGQPPKLLISAASNLESGIPAR
CDRL3
FSGSGSGTDFTLNIHPVEEEDVATYYCQQSYEDPLTFGAGTKVEIKR- C term

Figure 17

VH

A- Nucleotide sequence (SEQ ID No.: 3)

5'-

CDRH1

ggtccaactgcaacagcctgggactgaactggtgaagcctggggcttcagtgaagctgtcctgcaaggcttctggctacaccttcaccagttac

CDRH2

tggatacactgggtgaagcagaggcctggacaaggccttgagtggattggagagattaatcctagcagcggtcgtactaactacaacgaga
aattcaagaacaaggtcacagtgactgtagacaaatcttccaccacagcctacatgcaactcagcaacctgacatctgaggactctgcggtct

CDRH3

attactgtgcaagtaggggctactggggccaaggcaccactctcacagtctcctca-3'

B- Amino acid sequence (SEQ ID No.:4)

N term-

CDRH1 *CDRH2*

QVQLQQPGTELVKPGASVKLSCKASGYTFTSYWIHWVKQRPGQGLEWIGEINPSSGRTNYNEKFKN

CDRH3

KVTVTVDKSSTTAYMQLSNLTSEDSAVYYCASRGYWGQGTTLTVSS-C term

Figure 18

INTERNATIONAL SEARCH REPORT

International application No
PCT/ I B2014/058098

A. CLASSIFICATION OF SUBJECT MATTER
I NV . C97 K16/28 A6 1K39/395 A61 P35/G0
ADD .

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO- I nternal , BI OS I S, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CONSTANTI NOU A ET AL: "Modu l ati ng the pharmaco ki net ics of therapeut i c anti bodi es", BI OTECHNO LOGY LETTERS, SPRI NGER NETH ERLANDS, DORD RECHT, vol . 32, no. 5, 4 February 2010 (2010-02 -04) , pages 609 -622 , XP0 19787094 , I SSN : 1573-6776 the whol e document ----- -/--	1-21

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 April 2014	11/04/2014

Name and mailing address of the ISA/
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Covone -van Hees, M

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2014/058098

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WEIR A N C ET AL: "Formatting antibody fragments to mediate specific therapeutic functions", BIOCHEMICAL SOCIETY TRANSACTIONS, PORTLAND PRESS LTD, GB, vol. 30, no. 4, 1 August 2002 (2002-08-01), pages 512-516, XP009135661, ISSN: 0300-5127 page 513 - page 517, paragraph 1 page 515 figure 3 -----	1-21
A	JAZAYERI J A ET AL: "Fc-based cytokines: Prospects for engineering superior therapeutics", BI0DRUGS: CLINICAL IMMUNOTHERAPEUTICS, BIOPHARMACEUTICALS AND GENE THERAPY, ADIS INTERNATIONAL, FR, vol. 22, no. 1, 1 January 2008 (2008-01-01), pages 11-26, XP009148905, ISSN: 1173-8804, D0I: 10.2165/00063030-200822010-00002 pg.21 point 8.4 - pg.22 point 8.5 figure 5 -----	1-21
A	WARD E S ET AL: "THE EFFECTOR FUNCTIONS OF IMMUNOGLOBULINS: IMPLICATIONS FOR THERAPY", THERAPEUTIC IMMUNOLOGY, BLACKWELL SCIENTIFIC PUBL. LONDON, GB, vol. 2, no. 2, 1 January 1995 (1995-01-01), pages 77-94, XP008057562, ISSN: 0967-0149 "catabolims"; page 84 - page 87 -----	1-21
A	KNAUF M J ET AL: "Relationship of Effective Molecular Size to Systemic Clearance in Rats of recombinant Interleukin-2 Chemically Modified with Water-soluble Polymers", JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY, vol. 263, no. 29, 15 October 1988 (1988-10-15), pages 15064-15070, XP002256084, ISSN: 0021-9258 the whole document -----	1-21

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category"	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to olaim No.
A	GI OVANNI PACCHI ANA ET AL: "Monoval ency Un l eashe s the Ful l Therapeut i c Potenti al of the DN-30 Anti -Met Anti body" , JOU RNAL OF BI OLOG I CAL CH EMI STRY, AMERI CAN SOC I ETY FOR BI OCH EM I STRY AND MOLECU LAR BI OLOGY , vol . 285 , no . 46 , 12 November 2010 (2010- 11- 12) , pages 36149 -36 157 , XPQ0262 1766 , I SSN : 002 1-9258 , D0 I : 10 . 1074/ JBC.MI 10 . 13403 1 [retri eved on 2010-09 - 10] the whol e document -----	1-21
A	PRAT M ET AL: "AGON I STI C MONOCLONAL ANTI BOD I ES AGAI NST THE MET RECEPTOR DI SSECT THE BI OLOG I CAL RESPONSES TO HGF", JOU RNAL OF CELL SCI ENCE , CAMB RI DGE UN I VERS I TY PRESS , LONDON , GB , vol . I l l , no . PART 02 , 1 January 1998 (1998-01 -01) , page s 237 -247 , XP000943567 , I SSN : 002 1-9533 the whol e document -----	1-21
A	W0 2012/083370 AI (CEPHA LON AUSTRALI A PTY LTD [AU] ; WI LSON DAVI D [US] ; TAURA TETSUYA [US]) 28 June 2012 (2012 -06-28) page 2, line 29 - page 12, line 19 -----	1-21

INTERNATIONAL SEARCH REPORT	International application No. PCT/IB201 4/058098

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. (means)
      - [ ] on paper
      - [X] in electronic form

   b. (time)
      - [X] in the international application as filed
      - [ ] together with the international application in electronic form
      - [ ] subsequently to this Authority for the purpose of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent Tamlly members

International application No

PCT/ I B2014/058098

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