

(12) STANDARD PATENT
(19) AUSTRALIAN PATENT OFFICE

(11) Application No. **AU 2013349703 B2**

(54) Title
CD44v6-derived peptides for treating metastasizing cancers

(51) International Patent Classification(s)
A61K 38/17 (2006.01) **A61P 35/00** (2006.01)

(21) Application No: **2013349703** (22) Date of Filing: **2013.11.21**

(87) WIPO No: **WO14/079943**

(30) Priority Data

(31)	Number	(32)	Date	(33)	Country
	1220889.8		2012.11.21		GB

(43) Publication Date: **2014.05.30**

(44) Accepted Journal Date: **2016.12.22**

(71) Applicant(s)
Amcure GmbH

(72) Inventor(s)
Orian-Rousseau, Veronique;Matzke, Alexandra;Ponta, Helmut

(74) Agent / Attorney
AJ PARK, Level 9 Nishi 2 Phillip Law Street, Canberra, ACT, 2601

(56) Related Art
ORIAN-ROUSSEAU ET AL, EUROPEAN OF JOURNAL OF CANCER, VOL 46, NO 7, 1 MAY 2010, PAGES 1271-1277
EP 1647556 A1
EP 2266593 A1
ALEXANDRA MATZKE ET AL, CANCER RESEARCH, 15 JULY 2005, PAGES 6105-6110
TREMEL M. ET AL, BLOOD, VOL 114, NO 25, 22 SEP 2009, PAGES 5236-5244



(43) International Publication Date
30 May 2014 (30.05.2014)

(10) International Publication Number
WO 2014/079943 A1

- (51) **International Patent Classification:**
A61K 38/17 (2006.01) *A61P 35/00* (2006.01)
- (21) **International Application Number:**
PCT/EP2013/074404
- (22) **International Filing Date:**
21 November 2013 (21.11.2013)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
1220889.8 21 November 2012 (21.11.2012) GB
- (71) **Applicants:** KARLSRUHER INSTITUT FÜR TECHNOLOGIE [DE/DE]; Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen (DE). **AMCURE GMBH** [DE/DE]; Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen (DE).
- (72) **Inventors:** **ORIAN-ROUSSEAU, Véronique**; 41, Rue de Betschdorf, F-67690 Rittershoffen (FR). **MATZKE, Alexandra**; Waldstr. 3e, 76706 Dettenheim (DE). **PONTA, Helmut**; Bismarckstr. 62, 76676 Graben-Neudorf (DE).
- (74) **Agent:** **MAIWALD PATENTANWALTS GMBH**; Dirk Bühler, Elisenhof, Elisenstr. 3, 80335 München (DE).
- (81) **Designated States** (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

- (84) **Designated States** (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))
- with sequence listing part of description (Rule 5.2(a))

(54) **Title:** CD44V6-DERIVED PEPTIDES FOR TREATING METASTASIZING CANCERS

(57) **Abstract:** The present invention relates to compounds, pharmaceutical compositions and methods for treating different forms of pancreatic cancer.



WO 2014/079943 A1

5

10

CD44v6-derived peptides for treating metastasizing cancers

15

Technical Field of the Invention

The present invention relates to methods for treating metastasizing forms of cancer.

20 Background of the invention

Different types of cancer have been shown to involve at least in part over activation of receptor-tyrosine-kinases such as cMET, and VEGFR. Cancers include e.g. colorectal cancer, breast cancer, and pancreatic cancer.

25

CD44 has been discussed as having a role in e.g. HGF dependent activation of receptor-tyrosine-kinases such cMET, ERK and VEGFR (see inter alia, Ponta et al., Nature Reviews (2003), 4, 33-45 and Tremmel et al., Blood (2009), 25, 5236-5244).

30

Further, expression of an alternatively spliced form of CD44, namely CD44v6 has been shown to occur in some of the cancers being characterized by over-activation of receptor-tyrosine-kinases. Peptides, which are able to block CD44v6 mediated activation of receptor-tyrosine-kinases have been discussed as being potentially

useful in treatment of such cancers.

However, there is a continuing interest for pharmaceutically active agents that allow treatment of such cancers, such as e.g. pancreatic cancer.

5

In the United States, pancreatic cancer is the second most common malignant tumor of the gastrointestinal tract and the fourth leading cause of cancer-related death in adults (Cancer Staging Manual, 7th Edition, 2010, American Joint Committee on Cancer, Springer). It is a malignant neoplasm originating from transformed cells arising in tissues forming the pancreas. The most common type of pancreatic cancer is adenocarcinoma or exocrine pancreatic cancer, which are tumors exhibiting glandular architecture on light microscopy arising with the exocrine component of the pancreas. A minor type arises from pancreatic duct cells and are classified as neuroendocrine tumors.

15

Treatment of pancreatic cancer typically depends on the stage of the cancer.

Although only localized cancer is considered suitable for surgery with curative intent at present, only about 20% of cases are diagnosed with localized disease. Surgery can also be performed for palliation, if the malignancy is invading or compressing the duodenum or colon. Further treatment options include radiation and palliative chemotherapy. At present chemotherapy includes treatment with gemcitabin or combination therapies with gemcitabin such as gemcitabin/oxaliplatin or gemcitabin/cisplatin. Despite intensive research efforts, no treatment is currently available which would be considered to provide a long-term progression-free survival. Pancreatic cancer is therefore to date one of the malignancies with the worst prognosis of all neoplasias. Particularly if metastases have spread across the body such as to the liver, the peritoneal cavity and the lungs, no efficient treatment is available, which would allow to effectively regression of existing metastases.

30 Similar problems exist for other cancers, which have already formed metastases. For

many of these cancers, palliative chemotherapy may be the only therapeutic option.

Thus, there is a need for new methods which can be used to treat cancers such as pancreatic cancers, when metastatic spreading has occurred.

5

In this specification where reference has been made to patent specifications, other external documents, or other sources of information, this is generally for the purpose of providing a context for discussing the features of the invention. Unless specifically stated otherwise, reference to such external documents is not to be

10 construed as an admission that such documents, or such sources of information, in any jurisdiction, are prior art, or form part of the common general knowledge in the art.

15 In the description in this specification reference may be made to subject matter that is not within the scope of the claims of the current application. That subject matter should be readily identifiable by a person skilled in the art and may assist in putting into practice the invention as defined in the claims of this application.

Objective and Summary of the Invention

20

It is an objective of the present invention to provide

methods for treating cancer in a human being when metastatic spreading has occurred; and/or to at least provide the public with a useful choice.

25

These and other objectives as they will become apparent from the ensuing description are attained by the subject-matter of the independent claims. Some of the preferred embodiments of the present invention are mentioned in the dependent claims.

30

The present invention, to some extent, is based on the experimental data described

hereinafter that in part aim to elucidate the molecular function of the co-receptor molecule CD44v6. The CD44 transmembrane glycoproteins form part of a large family of cell adhesion molecules (CAMs). It has been recognized that CD44 comprises alternatively spliced variants, some of which are involved in the process of metastasis through its activation of the receptor tyrosine kinase Met and VEGFR (see 5 Matzke et al., Cancer Res (2005), 65(14) 6105-6109).

The experiment described hereinafter shows that an alternatively spliced version of CD44 comprising exon v6 (CD44v6) is responsible for inducing metastasis, e.g. in 10 animal models of human pancreatic cancer. CD44v6 has also been implicated for other cancers such as Hodgkin lymphoma, colorectal cancer, cervical cancer, head and neck cancer, gastric cancer and breast cancer, probably due to its role in HGF dependent activation of receptor-tyrosine-kinases such cMET, ERK and VEGFR.

15 The experiments described hereinafter further show that a peptide having as a minimal requirement the tri-peptide sequence R-W-H being embedded in a 5-amino acid peptide backbone such as N-R-W-H-E is capable of blocking the formation of metastases in an animal model of human pancreatic cancer. Furthermore, the data described herein show that in an orthotopic model of a human pancreatic cancer in 20 mice, these peptides also allow efficient regression of metastases that have already spread and formed across the body. Before this background it seems reasonable to conclude that these peptides will allow regression of metastases, which have formed and spreaded across the body, not only for pancreatic cancer, but also for other cancers such as Hodgkin lymphoma, colorectal cancer, cervical cancer, head and 25 neck cancer, gastric cancer and breast cancer, for which CD44v6 expression has been shown.

As already mentioned above it has been found that the peptides described herein are efficient not only for inhibiting metastasis but also for actually removing already formed metastases if the tri-peptide sequence R-W-H is embedded in a 5-amino acid 30 peptide backbone. This was found by changing the amino acids N and E of the

pentapeptide N-R-W-H-E to A respectively. Even though these were non-conservative amino acid substitution, the peptide is still active in inhibiting CD44v6 mediated activation of Met. Thus, it seems justified to conclude that the N in the first position of the pentapeptide N-R-W-H-E can not only be conservatively substituted by amino acids, such as K, R, or Q but also by any other amino acids or amino acids with non-polar side chains that are comparable to A such as V, L or I. The same considerations of course apply to the fifth position in the pentapeptide such that the amino acid E may not only be replaced by a conservative substitution, such as by K, but also by any other amino acid or particularly amino acids resembling the properties of alanine such as V, L or I.

Further, the pentapeptide N-R-W-H-E has also been shown to be effective for inhibiting CD44v6-mediated activation of Met signaling when being embedded in a larger peptide, the upper limit of which is reasonably to assume being a 14 mer. Given the findings of the possibility to substitute the first and last position in the pentapeptide not only by conservative amino acid substitutions, but also by non-conservative amino acid substitutions, it seems reasonable to conclude that any amino acid outside the essential motive of R-W-H could be replaced according to the same reasoning.

Thus, the present invention in one embodiment relates to a method of treating metastasizing cancer in a human being, comprising administering to said human being a compound,

wherein said compound comprises:

- a peptide comprising at least the amino acid sequence X_1 -R-W-H- X_5 with X_1 being selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y and X_5 being selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y (SEQ ID No.: 1), or a peptidomimetic thereof, or
- a peptide comprising at least 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 amino acids of

amino acid sequence X_1 - X_2 - X_3 - X_4 - X_5 - X_6 - X_7 -R-W-H- X_{11} - X_{12} - X_{13} - X_{14} ,
wherein X_1 , X_2 , X_3 , X_4 , X_5 , X_6 , X_7 , X_{11} , X_{12} , X_{13} , or X_{14} is selected from the
group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or
Y (SEQ ID No.: 7), and wherein the peptide comprises at least X_7 -R-W-H-
5 X_{11} of SEQ ID No.: 7, wherein X_7 , and X_{11} is selected from the group
comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y, or a
peptidomimetic thereof.

Also described is a compound for use in treating a cancer in a human being when the
10 cancer has already formed metastases,
wherein said compound comprises:

- a peptide comprising at least the amino acid sequence X_1 -R-W-H- X_5
with X_1 being selected from the group comprising A, C, D, E, F, G, H, I, K,
L, M, N, P, Q, R, S, T, V, W, or Y and X_5 being selected from the group
15 comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y
(SEQ ID No.: 1), or a peptidomimetic thereof, or
- a peptide comprising at least 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 amino
acids of amino acid sequence X_1 - X_2 - X_3 - X_4 - X_5 - X_6 - X_7 -R-W-H- X_{11} - X_{12} - X_{13} -
 X_{14} , wherein X_1 , X_2 , X_3 , X_4 , X_5 , X_6 , X_7 , X_{11} , X_{12} , X_{13} , or X_{14} is selected from
20 the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W,
or Y (SEQ ID No.: 7), and wherein the peptide comprises at least X_7 -R-W-H-
 X_{11} of SEQ ID No.: 7, wherein X_7 , and X_{11} is selected from the group
comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y, or a
peptidomimetic thereof.

25 Such cancers may metastasizing forms of Hodgkin lymphoma, colorectal cancer,
cervical cancer, head and neck cancer, gastric cancer, lung cancer, pancreatic cancer
and breast cancer, as for these cancer an expression of CD44v6 has been shown.

30 Given the observations described above, namely that the amino acid N and amino

acid E in the pentapeptide N-R-W-H-E can be replaced by alanine, it seems reasonable to assume that peptides that comprise in these positions either conservative amino acid substitutions or amino acid substitutions, which in terms of their physical chemical properties are comparable to alanine, such as V, L, or I will also provide the same activity as N-R-W-H-E. The same considerations apply to peptides derived from the 14 mer for positions that flank the essential tripeptide motive R-W-H.

Thus, in a preferred embodiment the present invention relates to a method wherein said compound comprises:

- a peptide comprising at least the amino acid sequence X_1 -R-W-H- X_5 , wherein X_1 is selected from the group consisting of amino acids with an NH_2 amino acid side group and amino acids with a non-polar side chain, and wherein X_5 is selected from the group consisting of amino acids with negatively charged side chains, and amino acids with non-polar side chains (SEQ ID No. 4) or a peptidomimetic thereof, or
- a peptide comprising at least 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 amino acids of amino acid sequence X_1 - X_2 - X_3 - X_4 - X_5 - X_6 - X_7 -R-W-H- X_{11} - X_{12} - X_{13} - X_{14} , wherein X_1 is selected from the group consisting of amino acids with an NH_2 amino acid side group and amino acids with non-polar side chains, wherein X_2 is selected from the group consisting of amino acids with negatively charged side chains and amino acids with non-polar side chains, wherein X_3 is selected from the group consisting of amino acids with an NH_2 amino acid side group and amino acids with non-polar side chains, wherein X_4 is selected from the group consisting of amino acids with non-polar side chains, non-charged side chains, aromatic ring structures and amino acids with non-polar side chains, wherein X_5 is selected from the group consisting of amino acids with non-polar or non-charged side chains and aromatic rings structures and amino acids with non-polar side chains, wherein X_6 is selected from the group consisting of G and amino acids with non-polar side chains, wherein

5 X₇ is selected from the group consisting of amino acids with an NH₂ amino acid side group and amino acids with non-polar side chains, wherein X₁₁ is selected from the group consisting of amino acids with negatively charged side chains and amino acids with non-polar side chains, wherein X₁₂ is selected from the group consisting of G and amino acids with non-polar side chains, wherein X₁₃ is selected from the group consisting of amino acids with non-polar or non-charged side chains and aromatic rings structures and amino acids with non-polar side chains, and wherein X₁₄ is selected from the group consisting of amino acids with an NH₂ amino acid side group and amino acids with non-polar side chains (SEQ ID No. 8), and wherein the peptide comprises at least X₇-R-W-H-X₁₁ of SEQ ID No.: 8, wherein X₇ is selected from the group comprising amino acids with an NH₂ amino acid side group and amino acids with non-polar side chains, and wherein X₁₁ is selected from the group consisting of amino acids with negatively charged side chains and amino acids with non-polar side chains, or a peptidomimetic thereof.

Also described is a compound for use in treating a cancer in a human being, when the cancer has already formed metastases, wherein said compound comprises:

- 20 • a peptide comprising at least the amino acid sequence X₁-R-W-H-X₅, wherein X₁ is selected from the group comprising amino acids with an NH₂ group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I and wherein X₅ is selected from the group comprising amino acids with negatively charged side chains such as E or D, or amino acids with non-polar side chains such as A, V, L or I (SEQ ID No. 4) or a peptidomimetic thereof, or
- 25 • a peptide comprising at least 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 amino acids of amino acid sequence X₁-X₂-X₃-X₄-X₅-X₆-X₇-R-W-H-X₁₁-X₁₂-X₁₃-X₁₄, wherein X₁ is selected from the group comprising amino acids with an NH₂ group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I, wherein X₂ is selected from the group comprising amino
- 30

acids with negatively charged side chains such as E or D, or amino acids with non-polar side chains such as A, V, L or I, wherein X_3 is selected from the group comprising amino acids with an NH_2 group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I, wherein X_4 is selected from the group comprising amino acids with non-polar or non-charged side changes and aromatic ring structures such as F, W, or Y, or amino acids with non-polar side chains such as A, V, L or I, wherein X_5 is selected from the group comprising amino acids with non-polar or non-charged side changes and aromatic rings structures such as F, W, or Y, or amino acids with non-polar side chains such as A, V, L or I, wherein X_6 is selected from the group comprising G or amino acids with non-polar side chains such as A, V, L or I, wherein X_7 is selected from the group comprising amino acids with an NH_2 group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I, wherein X_{11} is selected from the group comprising amino acids with negatively charged side chains such as E or D, or amino acids with non-polar side chains such as A, V, L or I, wherein X_{12} is selected from the group comprising G or amino acids with non-polar side chains such as A, V, L or I, wherein X_{13} is selected from the group comprising amino acids with non-polar or non-charged side chains and aromatic rings structures such F, W, or Y, or amino acids with non-polar side chains such as A, V, L or I, and wherein X_{14} is selected from the group comprising amino acids with an NH_2 group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I (SEQ ID No. 8), and wherein the peptide comprises at least X_7 -R-W-H- X_{11} of SEQ ID No.: 8, , wherein X_7 is selected from the group comprising amino acids with an NH_2 group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I and wherein X_{11} is selected from the group comprising amino acids with negatively charged side chains such as E or D, or amino acids with non-polar side chains such as A, V, L or I, or a peptidomimetic thereof.

Such cancers may metastasizing forms of Hodgkin lymphoma, colorectal cancer, cervical cancer, head and neck cancer, gastric cancer, pancreatic cancer and breast cancer, as for these cancer an expression of CD44v6 has been shown.

- 5 Even though the pentapeptides or any longer peptide derived from the 14 mer as described hereinafter should be effective not only in preventing metastasis but actually removing already formed metastases in cancers such as Hodgkin lymphoma, colorectal cancer, cervical cancer, head and neck cancer, gastric cancer, pancreatic cancer and breast cancer, a preferred embodiment of the methods and uses of the
- 10 present invention refers to the pentapeptide sequences described hereinafter, with a particular preferred embodiment focusing on the amino acid sequence N-R-W-H-E (SEQ ID NO:2).

- 15 A person skilled in the art will understand that any compound that provides for the same amino acids or at least the same overall configuration of the peptide as peptides described herein such as the pentapeptide of SEQ ID NO:1 or SEQ ID NO:2 will also be efficient in not only preventing formation of metastasis, but also removing already formed metastases in cancers such as Hodgkin lymphoma, colorectal cancer, cervical cancer, head and neck cancer, gastric cancer, pancreatic cancer and breast cancer.

- 20 The invention therefore in some embodiments contemplates the use of peptidomimetics of any of the peptides described hereinafter particularly peptidomimetics of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO: 4 or 5. These peptidomimetics will preferably have the same amino acids but an
- 25 altered backbone which provides for the same overall configuration of the peptidomimetic as does the peptide itself, but which is e.g. more resistant to protease cleavage. Preferred peptidomimetics are e.g. isosteric peptoids, which comprise poly-N-substituted glycines in the peptide bonds of the backbone.

- 30 The present invention also considers further modified forms of the peptides and

peptidomimetics described herein. Such modified peptides or peptidomimetics may comprise e.g. chemically or enzymatically attached modifications that render the peptides more stable, e.g. against protease degradation, that allow to provide the peptides or peptidomimetics as pharmaceutically acceptable salts, or which e.g.
5 improve the biological properties of the peptides or peptidomimetics such as half-life. Such preferred modified forms of such peptides or peptidomimetics refer to pegylated, hesylated, pasylated, myristoylated, and glycosylated, and/or cyclic forms of these peptides and peptidomimetics, and in particular peptides of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4 and SEQ ID NO:5 or peptidomimetics
10 of these sequences.

Such modified peptides or peptidomimetics are generally referred to, in the context of the present invention, as compounds or peptide compounds. These compounds or peptide compounds may be formulated for oral administration, e.g. by inhalation, for
15 nasal administration, or administration by injection such as subcutaneous administration.

In one embodiment also described are pharmaceutical compositions for use in treating cancers when metastases have already formed such as metastasizing forms of
20 Hodgkin lymphoma, colorectal cancer, cervical cancer, head and neck cancer, gastric cancer, pancreatic cancer and breast cancer in a human being wherein these pharmaceutical compositions comprise the compounds/peptide compounds as described above. These pharmaceutical compositions may comprise pharmaceutically acceptable excipients and may be formulated for oral
25 administration such as by inhalation, nasal administration or administration by injection.

The invention also relates to a use of a compound as defined above, in the manufacture of a medicament for use in treating metastasizing cancer in a human
30 being.

The invention also provides a compound as defined above, when used in treating metastasizing cancer in a human being.

- 5 The present invention also refers to the use of such peptides, peptidomimetics thereof, or modified peptides and peptidomimetics in the manufacture of a medicament for use in treating cancers when metastases have already formed such as metastasizing forms of Hodgkin lymphoma, colorectal cancer, cervical cancer, head and neck cancer, gastric cancer, pancreatic cancer and breast cancer in a human
10 being.

- Further, the invention relates to methods of treating cancers when metastases have already formed such as metastasizing forms of Hodgkin lymphoma, colorectal cancer, cervical cancer, head and neck cancer, gastric cancer, pancreatic cancer and
15 breast cancer in a human being in need thereof by administering the peptides, peptidomimetics thereof or modified forms thereof, i.e. the compounds in accordance with the present invention, or pharmaceutical compositions comprising compounds in accordance with the present invention to a human being in need thereof.

- 20 The compounds described, i.e. the peptides, peptidomimetics thereof or modified forms thereof, the pharmaceutical compositions described, and the methods and uses in accordance with the present invention are considered for treating cancers where metastases have already formed and may have even spread across the body. These
25 cancers are also designated as metastasizing cancers.

Metastasizing cancers in accordance with the invention include metastasizing forms of cancers for which expression of CD44v6 has been observed on cancer tissues or can be observed upon corresponding testing e.g. with CD44v6 antibodies.

- 30 Metastasizing cancers in accordance with the invention include metastasizing forms

of Hodgkin lymphoma, colorectal cancer, cervical cancer, head and neck cancer, gastric cancer, pancreatic cancer, head and neck squamous cell cancer and breast cancer.

- 5 Such metastasizing cancer forms of the various cancers such as Hodgkin lymphoma, colorectal cancer, cervical cancer, head and neck cancer, gastric cancer, pancreatic cancer and breast cancer can be identified according to the TNM Anatomic Stage/Prognostic Group System of the Cancer Staging Manual of the American Joint Committee on Cancer (7th edition, 2010, Springer).Metastasizing cancers in
10 accordance with the invention will typically be classified as a Stage IV cancer according to the TNM Anatomic Stage/Prognostic Group System, particularly if M is set as 1. The Cancer Staging Manual of the American Joint Committee on Cancer (7th edition, 2010, Springer) describes the TNM Anatomic Stage/Prognostic Group System and under which conditions a cancer is considered to be of e.g. Stage I, II, III
15 and IV for the various cancers mentioned herein and is thus included by reference as far.

- For example, in a particularly preferred embodiment the described compounds, i.e. the peptides, peptidomimetics thereof or modified forms thereof, the pharmaceutical
20 compositions described hereinafter and methods and uses of the invention are considered for treating breast cancer or colorectal cancer in a human subject, for which the breast or colorectal cancer is classifiable as Stage IV according to the TNM Anatomic Stage/Prognostic Group System of the Cancer Staging Manual of the American Joint Committee on Cancer (7th edition, 2010, Springer, see pages 145 to
25 166 for colorectal cancer and pages 347 to 378 for breast cancer).

- It is to be understood that for all aspects and embodiments of the present invention, i.e. the methods and uses hereinafter and for the compounds and pharmaceutical compositions described, it is always preferred to use the pentapeptides as described
30 herein, such as those of SEQ ID NO:1, SEQ ID NO:2; SEQ ID NO:3, SEQ ID NO:4

and SEQ ID NO:5 and in particular of SEQ ID NO:2 for treating metastasizing cancers in a human being, and in particular for treating metastasizing cancers which are classifiable as Stage IV according to the TNM Anatomic Stage /Prognostic Group System of the Cancer Staging Manual of the American Joint Committee on Cancer (7th edition, 2010, Springer).

Figure Legends

Figure 1: The co-receptor function of CD44v6 for Met is required for tumor metastasis. A: BSp73AS and its transfectants were induced with HGF where indicated and the activation of Met and ERK was determined as described in Materials and Methods. The numbers indicate the fold induction as calculated by the computer program ImageJ. All experiments were performed at least 3 times and gave similar results. B: The cells used in A were subcutaneously injected into the right posterior flank of syngeneic rats (Materials and Methods). Four weeks later the lymph nodes and lungs were prepared for immunohistochemical analysis. The represented lymph nodes are the auxiliary lymph nodes. Two pictures of the lungs are shown, the arrows indicate metastases. C: Immunohistochemical analysis of paraffin sections of ASs6 tumors infected with lentivirus expressing ctrl-shRNA or Met-shRNA. Slices were stained with anti-GFP antibody to monitor shRNA-transduced areas or with a phospho-Met antibody. Magnification 20x.

Figure 2: A CD44v6 specific peptide blocks metastatic spreading of tumor cells. A: BSp73ASs6 cells were induced with HGF in the presence of the CD44v6-specific rat peptide, a CD44v6 specific antibody or a control peptide (mouse) as indicated. The activation of Met and Erk was determined using phospho-specific antibodies. The numbers refer to

the fold induction. B: BSp73ASs6 cells were injected subcutaneously into the right posterior flank of BD10 rats. After one week of tumor growth the animals were treated with the CD44v6 peptide (i.t. or i.v.), the control peptide, the CD44v6 antibody or PBS (Materials and Methods). Axillary lymph nodes (left side) and lungs (right side) were analyzed for metastases as described in Figure 1. C: Sections of lungs of animals treated either with the CD44v6 peptide or with the control peptide were stained with H&E and PAS. H&E- hematoxylin and eosin; PAS - Periodic acid-Schiff reaction. Magnification 1.5x.

D: Growth curve of BSp73ASs6 tumors in animals upon treatment for 28 days as indicated. The mean tumor volume of rats treated with either PBS, CD44v6 antibody, CD44v6 peptide or control peptide (mouse) was measured weekly after the start of the treatment and continued for 28 days using a caliper.

Figure 3: Specific binding of the CD44v6 peptide to primary tumors and metastases in vivo. A: Left side: BSp73ASs6 cells were fixed and stained either with the DY681 labeled CD44v6 rat or the mouse peptide (control) for one hour. Images were taken using a laser scanning confocal microscope (Leica TCS2 SP2) with a 20x objective. Right side: BSp73ASs6 cells were induced with HGF in the presence of the DY681 rat v6 peptide respectively mouse peptide and the activation of Erk was determined. The numbers indicate fold induction. B: Rats bearing a subcutaneous tumor of BSp73ASs6 cells (grown for three weeks) were injected i.v. with 200 µg of DY681 rat v6 peptide or DY681 mouse v6 peptide (control) and analyzed by NIRF imaging using Optix MX2 (ART, Montreal, Canada). Fluorescence intensities are displayed in NC (Normalized Correlation). Series of fluorescent data sets obtained at various time points after injection of the indicated peptide show fluorescent signals

after 24 and 48 hours indicating binding of the rat v6 peptide but not the mouse v6 peptide in the range of two days to the tumor. C: Ex vivo scans of tumors and lungs from rats injected with DY681 rat v6 peptide showed specific fluorescent signals not only over the tumor area but also in specific areas of the lung indicating binding of the rat peptide to metastases.

Figure 4: A CD44v6 peptide prevents metastasis of human tumor cells in an orthotopic model of pancreatic cancer. A: L3.6pl cells were treated with the human v6 peptide or the rat v6 peptide as control prior to induction with 10 ng/ml HGF. Activation of Erk was determined in western blot. The numbers refer to the fold induction. B: L3.6pl cells were injected orthotopically into the head of the pancreas of male nude mice (Materials and Methods). 7 days later the animals were injected with the human CD44v6 peptide or control (rat) peptide (20 µg each). The peptide injection was repeated 3 times per week. Animals were killed 23 days after the first peptide treatment. Tumors were isolated and stained for CD44v6 expression (BIWA) or secondary antibody as control. Nuclei were stained with hematoxylin. C: Immunofluorescence staining of L3.6pl tumors from the v6 peptide or control peptide treated animals using the phospho-Met and Met antibodies. Nuclei are stained with DAPI. D top: Tumor volume of animals treated with either v6 or control peptide was determined at the end of the experiment using the formula $\text{volume} = (\text{width})^2 \times \text{length} / 2$. Bars represent average tumor volume at the end of the experiment. D bottom: Each group of animals treated with one of the peptides consisted of 15 animals. Bars show the percentage of animals bearing metastases. E: Staining of L3.6pl tumors treated with control or v6 peptide with a CD31-specific antibody. The magnification is 50x. The graphs show the average vessel numbers respectively average vessel

size calculated from five independent tumors. F: Human VEGF levels produced by L3.6pl cells in presence and absence of the v6 peptide (200ng/ml in the culture medium). Bars reflect average VEGF levels from triplicates obtained in 3 independent experiments. G: Left side: Livers of v6 peptide or control peptide treated animals were examined for macroscopic metastases. Right side: Bars show the average number of metastases. Each group of animals treated with one of the peptides consisted of 15 animals. In all graphs the significance was calculated using Student's t test: ***p < 0,001.

Figure 5: Specific accumulation of the CD44v6 peptide in primary tumors and metastases of the human pancreatic cancer model. A: Left side: L3.6pl cells were stained with either the DY681 labeled CD44v6 human or rat peptide. Images were taken with the laser scanning confocal microscope (Leica TCS2 SP2). Right side: L3.6pl cells were induced with HGF in the presence of the DY681 human v6 peptide respectively rat v6 peptide and the activation of Erk was determined. The numbers refer to the fold induction. B: L3.6pl tumors were orthotopically induced for 3 weeks as described in Figure 4 followed by one intravenous injection of DY681 human v6 peptide or DY681 rat v6 peptide, (each 20 µg). Binding of the peptide was analyzed 24h after injection in anesthetized mice using the Pearl® Impulse Small Animal Imaging System (Li-Cor). Tumor free and tumor bearing animals that received no peptide treatment were used as control. C: Primary tumors, livers and spleens were excised from the animals shown in B and fluorescence of the labeled peptides was monitored ex-vivo. The scales at the side indicate the synchronized signal intensity.

Figure 6: Reversion of pre-existing metastases by the CD44v6 peptides.

A: Schematic representation of the experimental procedure.

BSp73ASs6 cells or L3.6pl cells were injected in rats respectively nude mice. During the following 3 weeks, the tumors developed and metastases were detected after 3 weeks of time. In the groups treated with the v6 peptide or the control peptide (200 µg in rats, 20 µg in nude mice), the treatment started after three weeks. After 21 additional days the animals were sacrificed and analyzed for lung respectively liver metastases. B: Lungs of rats bearing a BSp73ASs6 subcutaneous tumor and treated with the v6 peptide (rat) or the control peptide (mouse) are shown. Bottom left: The quantification represents the average number of metastases. The number of animals used in each group is given in Table 3. Bottom right: A graphic evaluation of the number of animals bearing metastases is presented. C: Livers from mice with L3.6pl pancreatic tumors and treated with the v6 peptide (human) or the control peptide (rat) are shown. Bottom left: A graph evaluating the number of liver metastases in control peptide treated animals and CD44v6 peptide treated animals is shown. Bottom right: A graphic evaluation of the number of animals bearing metastases is presented.

Figure 7: The CD44v6 peptide induces apoptosis in already established metastases. Animals bearing lung metastases three weeks after tumor cell injection received an injection of CD44v6 peptide or control peptide every second day. At the indicated days one animal of each group was sacrificed. Apoptosis in lung metastasis was monitored on paraffin sections using an antibody against cleaved Caspase-3 and cleaved Caspase-8 (Materials and Methods). The area of the metastasis is marked (M). The magnification is 4.5x. The experiment was performed 2 times with similar outcome.

- Figure 8: depicts Tables 1 to 3.
- Figure 9: depicts effects of pegylated rat CD44v6 peptides on activation of ERK in rat pancreatic cells.
- 5 Figure 10: depicts effects of pegylated rat CD44v6 peptides on activation of ERK and Met in rat pancreatic cells.
- 10 Figure 11: depicts effects of pegylated rat CD44v6 peptides on HGF induced clustering in colon cancer cells.
- 15 Figure 12: CD44v6 is required for activation of Met and downstream signaling in several ECs. Different ECs (HUVEC, HAOEC and HCMEC) were serum starved and incubated with several reagents (α CD44v6, v6 14mer and the controls IgG and ctrl pep). The growth factor HGF was added and the cells were lysed. The proteins in the cell lysates were separated by SDS-PAGE and subjected to Western blot analysis. Activation of Met and of its downstream target Erk was detected by means of phospho-specific antibodies that detect phosphorylated forms of Met and Erk. In order to check equal loading of the different samples the membrane was incubated with stripping solution and reprobed with antibodies recognising all Met and Erk proteins. The numbers indicate the fold induction as calculated with the computer programme ImageJ.
- 20
- 25
- Figure 13: Activation of VEGFR-2 and signal transduction induced by VEGF-A165 depends on CD44v6. Different ECs (HUVEC, HAOEC and HCMEC) were serum starved and incubated with several reagents (α CD44v6, v6 14mer and the controls IgG and ctrl pep). The cells were then induced with VEGF-A165 and lysed. The cell lysates were
- 30

loaded on SDS-PAGE and subjected to Western blot analysis. Activation of the receptor tyrosine kinase VEGFR-2 and of its downstream target Erk was detected by means of antibodies that detect phosphorylated forms of VEGFR-2 and Erk. The loading control was performed with antibodies recognising total VEGFR-2 and Erk levels. The numbers indicate the fold induction as calculated with the computer programme ImageJ.

Figure 14: A: Activation of Met and signal transduction induced by HGF depends on CD44v6 in L3.6pl cells. L3.6pl cells were treated with the human v6 peptide (human v6 14mer) or the rat v6 peptide as control prior to induction with 10 ng/ml HGF. Activation of Met and ERK was determined in western blot. The numbers refer to the fold induction. The experiment was repeated at least 5 times. B: Activation of c-Met and signal transduction induced by HGF depends on CD44v6 HeLa cells. Starved HeLa cells respectively HT29 cells were incubated with the v6 peptide (human v6 14mer) or a control peptide for 10 minutes at 37°C and then induced with 25 ng/ml of HGF for the indicated time points. Cells were then either lysed and the lysates were subjected to Western Blot analysis for phospho-Met and Met. C: Activation of c-Met and signal transduction induced by HGF depends on CD44v6 in HT29 cells. HGF-induced c-Met and Erk phosphorylation in HT29 was determined as described above using phospho-specific antibodies. Where indicated the cells were pretreated with the CD44v6 peptide (human v6 14mer) or the control peptide (see Materials and Methods in Example 3). The loading controls were developed with c-Met respectively tubulin antibodies.

Figure 15: In contrast to EGF and ER, induction of the ErbB-receptors via TGF- α , BC, Her, HB-EGF or AR is completely independent of CD44v6.

Serum-starved HT29 cells were pre-incubated for 5 min with 100ng/ml of a CD44v6-specific peptide (human v6 14mer) or a control peptide. Afterwards the cells were induced with 20 ng/ml of various ErbB ligands as indicated. The cell lysates were resolved by SDS-PAGE. Western-blotting was used to detect ErbB ligands-induced Erk-kinase phosphorylation.

Figure 16: EGF-dependent induction of the ErbB receptors can be blocked by a CD44v6 specific peptide in HT29 cells. Serum-starved HT29 cells were pre-incubated for 5 min with 100 ng/ml of CD44v6-specific peptide (pep1=14mer, pep2=5mer) or a control peptide. Afterwards the cells were induced with 20 ng/ml of EGF or TGF α and lysed. The cell lysates were resolved by SDS-PAGE. Western-blotting was used to detect EGF and TGF α induced Erk-kinase phosphorylation.

Figure 17: The human CD44 v6 specific peptides block HGF induced cell scattering and invasion into matrigel. Scattering of HT29 cells was determined in 24-well plates. First, 4.5×10^4 cells were seeded at 37°C; after 24 h the cells were serum starved for additional 24 h. the cells were either left untreated (control) or later treated with HGF (50 ng/ml) for 24 h. Where indicated the cells were treated with the v6 specific peptide (human v6 5mer, 10mer, 14mer) or control peptide prior to induction with HGF for 30 min. Pictures were made using phase contrast microscopy. Magnification 40x.

Figure 18: CD44v6 is required for EC migration. HUVECs and HAOECs were grown to confluence and a wound was made into the cell monolayer with a sterile pipette tip. After washing the cells were incubated with the antibody against CD44v6, the CD44v6 peptide (human v614mer) or the control peptide as indicated. The ligands HGF, VEGF-A165 or

VEGF-A121 were added and migration of the ECs was analysed after 24 hours. The numbers indicate the percentage of free area in the scratch area quantified by the computer program ImageJ.

5 Figure 19: Spheroids of HUVECs (750 cells) were generated in hanging drops. They were suspended in Endothelial Cell Growth Medium containing 1 mg/ml rat tail collagen I and 0.6% (wt/vol) methylcellulose and distributed in 48-well plates (30 spheroids/well). After solidification at 37°C the mixture was overlaid with Endothelial Cell Growth Medium containing 100 ng/ml v6 peptide (human v6 14mer) or control peptide and VEGFs (40 ng/ml). Pictures were taken 48h later and sprouting was quantified with the computer program ImageJ.

15 Figure 20: The response of HUVECs to a mixture of angiogenic factors produced by angiogenic Langerhans islets can be reduced by CD44v6 specific inhibitors. HUVEC cells were mixed with CD44v6 antibody, CD44v6 peptide or control peptide and then suspended in a collagen gel. Hyperplastic angiogenic Langerhans islets isolated from the pancreas of 8-9 week old RIP1Tag2 mice were placed on top of the HUVEC/collagen mixture and overlaid with medium. Every third day new antibody or peptide was added and pictures were taken after 5 to 7 days. The number of islets that induced an angiogenic response of the ECs is given as percentage of all analysed islets. For quantification 60 islets per condition were analysed. Both the CD44v6 antibody and the CD44v6 peptide (human v6 14mer) reduced the angiogenic response, and the reduction obtained with the antibody is significant ($p < 0.05$).

30 Figure 21: Inhibition of metastasis and primary tumor growth using the human v6 peptide and PEGylated derivatives thereof (PEG840 and PEG2000) at

different concentrations (2 µg, 20 µg and 200 µg per injection). A: L3.6pl tumors were orthotopically implanted in nude mice for one week, followed by i.p. injection of the indicated peptides three times per week for three weeks. At the end of the experiment animals were sacrificed and examined. Red arrows indicate metastases. Significance was calculated using Student's t test: ***p < 0,001. Each group consisted of 3 animals. B: Quantification of the primary tumor volume. C: Overview of the average number of macrometastases per animal.

Figure 22: Inhibition of metastasis and primary tumor growth using the CD44v6 peptide (linear 14mer) and a human cyclic 8mer at different concentrations (0, 2 µg, 2 µg, 10 µg and 20 µg per injection). A: L3.6pl tumors were orthotopically implanted in nude mice for one week, followed by i.p. injection of the indicated peptides three times per week for three weeks. At the end of the experiment, animals were sacrificed and examined. Red arrows indicate metastases. Significance was calculated using Student's t test: * p<0,01, ***p < 0,001. Each group consisted of 5 animals. B: Overview of average number of macroscopic metastases in each animal. C showed a decrease in metastatic spreading starting from a dosage of 2 µg and full inhibition at 20 µg. C: The v6 14mer and the cyclic 8mer reduces growth of the primary tumor.

Figure 23: Side-by-side comparison of the v6 14mer with derivatives thereof (14merPEG840, 14mer PEG2000, myristoylated 14mer, D-amino acid derivatives DOTA CD44v6-14[daa7] and DOTA CD44v6-14[r14], cyclic 8mer, cyclic 5mer and myristoylated cyclic 8mer) for primary tumor growth. L3.6pl tumors were orthotopically implanted in nude mice for one week, followed by i.p. injection of the indicated peptides

three times per week for three weeks. At the end of the experiment animals were sacrificed and examined. A: Average tumor size of animals treated with the indicated compounds (n=5) is shown. Significance was calculated using Student's t test: ***p < 0,001. Each group consisted of 5 animals. B: Individual tumor size of animals in each group.

5

10

15

Figure 24: Side-by-side comparison of the v6 14mer with derivatives thereof (14merPEG840, 14mer PEG2000, myristoylated 14mer, D-amino acid derivatives DOTA CD44v6-14[daa7] and DOTA CD44v6-14[r14], cyclic 8mer, cyclic 5mer and myristoylated cyclic 8mer) for inhibition of metastasis. A: Representative examples of primary tumor and liver metastasis for the indicated compounds. Red arrows point towards a metastase. Red circle: Note the very strong vascularization of the primary tumor in the control group. B: Summary of the number of animals with metastasis and the average number of metastases per animal.

20

25

Figure 25: The CD44v6 peptide reduces the number of tumors in the duodenum. 18 week old APC/Min/+ mice were treated with the v6 14mer for 6 weeks (i. p. injection) and sacrificed to estimate the total tumor number in the duodenum. A: Macroscopic analysis of tumor number. B: Statistical evaluation of the total tumor number according to the size of the tumor.

30

Figure 26: Treatment with the v6 14mer reduces growth of the primary tumor and macroscopic metastases formation in the lung. 1×10^6 murine 4T1 cells per mouse were diluted in 50 μ l RPMI plus 50 μ l Matrigel and injected into the 6th mammary fat pad of 10 female BALB/c mice. One week after the injection, the mice were either injected

intraperitoneally with 20 µg of the murine v6 14mer dissolved in 100 µl PBS or with 20 µg of control peptide (rat v6 14mer) dissolved in 100 µl PBS. The injection was performed 3 times per week for two weeks. After 2 weeks the mice were sacrificed and the primary tumors and the lungs were extracted. A: Overall reduction of the tumor volume in the v6 peptide treated group. B: Immunofluorescent staining for the endothelial marker CD34 to monitor vascularization of the primary tumor. Graphic representation of the reduction of the total vessel area. C: The v6 peptides led to a reduction in macroscopic lung metastasis. The black arrows mark examples for metastases. The scale bar corresponds to a size of 1 cm. Statistical evaluation of the average number of lung metastases. Significance was calculated using Student's t test: $p < 0.01$; $**p < 0.005$. Each group consisted of 5 animals.

Figure 27: A: MALDI-TOF spectrum of v6-PEG3000-palmitic amide and V6-PEG2000. B: SEM images of PEG-QD, 10% v6-PEG-QD, and 30% v6-PEG-QD.

Figure 28: CD44v6 peptide coupled Quantum Dots bind specifically to CD44v6 expressing tumor cells A: Panel 1-3: AS10 or ASs6 cells incubated with 100 µg/ml PEGylated Quantum Dots (control), 10% CD44v6-peptide-PEG-QDs or 30% CD44v6-peptide-PEG-QDs were co-stained with DAPI and visualized by fluorescence microscopy. Panel 4: AS10 cells transfected with mCherry (red) and ASs6 cells transfected with CFP (cyan) were co-cultured and stained with 30% v6-pep-QDs. Magnification, 40x. B: Percentages of Quantum Dot stained cells was quantified. C: AS10 or ASs6 cells were incubated with PBS or 100 µg/ml PEG-QDs, 10% CD44v6-peptide-PEG-QDs or 30% CD44v6-peptide-PEG-QDs for 10 min and analysed for QD

binding via FACS measurement.

Detailed Description of the Invention

5

Before the invention is described in detail with respect to some of its preferred embodiments, the following general definitions are provided.

10

The present invention as illustratively described in the following may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein.

15

The present invention will be described with respect to particular embodiments and with reference to certain figures but the invention is not limited thereto but only by the claims.

20

Where the term “comprising” is used in the present description and claims, it does not exclude other elements. For the purposes of the present invention, the term “consisting of” is considered to be a preferred embodiment of the term “comprising of”. If hereinafter a group is defined to comprise at least a certain number of embodiments, this is also to be understood to disclose a group, which preferably consists only of these embodiments.

25

For the purposes of the present invention, the term “obtained” is considered to be a preferred embodiment of the term “obtainable”. If hereinafter e.g. an antibody is defined to be obtainable from a specific source, this is also to be understood to disclose an antibody, which is obtained from this source.

30

Where an indefinite or definite article is used when referring to a singular noun, e.g. “a”, “an” or “the”, this includes a plural of that noun unless something else is

specifically stated. The terms “about” or “approximately” in the context of the present invention denote an interval of accuracy that the person skilled in the art will understand to still ensure the technical effect of the feature in question. The term typically indicates deviation from the indicated numerical value of $\pm 20\%$, preferably $\pm 15\%$, more preferably $\pm 10\%$, and even more preferably $\pm 5\%$.

Furthermore, the terms "first", "second", "third" or "(a)", "(b)", "(c)", "(d)" or "(i)", "(ii)", "(iii)", "(iv)" etc. and the like in the description and in the claims, are used for distinguishing between similar elements and not necessarily for describing a sequential or chronological order. It is to be understood that the terms so used are interchangeable under appropriate circumstances and that the embodiments of the invention described herein are capable of operation in other sequences than described or illustrated herein.

In case the terms "first", "second", "third" or "(a)", "(b)", "(c)", "(d)" or "(i)", "(ii)", "(iii)", "(iv)" etc. relate to steps of a method or use or assay there is no time or time interval coherence between the steps unless indicated otherwise, i.e. the steps may be carried out simultaneously or there may be time intervals of seconds, minutes, hours, days, weeks, months or even years between such steps, unless otherwise indicated in the application as set forth herein above or below.

Technical terms are used by their common sense. If a specific meaning is conveyed to certain terms, definitions of terms will be given in the following in the context of which the terms are used.

As mentioned above, described are peptides or peptide compounds for use in treating metastasizing cancer in a human being.

The present invention is based to some extent on the experimental findings described hereinafter that a peptide of amino acid sequence N-R-W-H-E (SEQ ID No. 2) or of

amino acid sequence K-E-Q-W-F-G-N-R-W-H-E-G-Y-R (SEQ ID No.: 6) is not only capable of inhibiting metastasis in human pancreatic cancer cells but can also eliminate already established metastases in an orthotropic animal model of human pancreatic cancer. It thus seems reasonable to assume that the same efficacy can be
5 observed in different metastasizing cancers, particularly where these cancers show expression of CD44v6. It was moreover shown that upon mutating N to A and E to A, a peptide of amino acid sequence A-R-W-H-A (SEQ ID No. 3) is capable of abrogating Met activation (see Matzke et al., Cancer Res. (2005), 65 (14), 6105-6110). Given that the effects on Met activation by N-R-W-H-E (SEQ ID No. 2) are
10 maintained despite the non-conservative amino acids substitutions of K to A and F to A (Matzke et al., vide supra), it seems reasonable to assume that a peptide of amino acid sequence X₁-R-W-H-X₅ (SEQ ID No. 1), wherein X₁ is selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y and wherein X₅ is selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R,
15 S, T, V, W, or Y can be used not only for treatment of pancreatic cancer, but also for metastasizing cancers in general.

Thus described is a compound for use in treating metastasizing cancer in a human being, wherein said compound comprises a peptide comprising at least the amino
20 acid sequence X₁-R-W-H-X₅, wherein X₁ is selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y and wherein X₅ is selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y (SEQ ID No. 1) or a peptidomimetic thereof.

25 Also described is a compound for use in treating metastasizing cancer in a human being, wherein said compound comprises a peptide comprising at least the amino acid sequence X₁-R-W-H-X₅, wherein X₁ is selected from the group comprising amino acids with an NH₂ group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I and wherein X₅ is selected from the group
30 comprising amino acids with negatively charged side chains such as E or D, or amino

acids with non-polar side chains such as A, V, L or I (SEQ ID No. 4) or a peptidomimetic thereof. An example is a peptide of amino acid sequence A-R-W-H-A (SEQ ID No. 3) or a peptidomimetic thereof.

- 5 Also described is a compound for use in treating metastasizing cancer in a human being, wherein said compound comprises a peptide comprising at least the amino acid sequence X_1 -R-W-H- X_5 , wherein X_1 is selected from the group comprising K, R, N, or Q and wherein X_5 is selected from the group comprising E or D (SEQ ID No. 5). As an example of one of the most preferred embodiments the peptide may
10 comprise and preferably consist of amino acid sequence N-R-W-H-E (SEQ ID No. 2).

The term "peptide" as used herein refers to any compound comprising at least the above mentioned amino acids five and a maximum of fourteen amino acids.

15

- If peptides useful in accordance with the invention have more than the above-mentioned five amino acids, these amino acids may e.g. be those found in a peptide of amino acid sequence K-E-Q-W-F-G-N-R-W-H-E-G-Y-R (SEQ ID No.: 6) or variations thereof. It is noted that amino acids 7 to 11 of SEQ ID No.: 6 correspond
20 to SEQ ID No.: 2. As for a peptide of SEQ ID No. 2, it has been found in a linker screen analysis with alanine substitutions that amino acids 1, 2, 3, 4, 5, 6, 12, 13, or 14 can be substituted by alanine without having detrimental effects on Met activation. Such peptides may thus be any peptide comprising amino acids 6 to 12, 5 to 13, 4 to 14, etc. of X_1 - X_2 - X_3 - X_4 - X_5 - X_6 - X_7 -R-W-H- X_{11} - X_{12} - X_{13} - X_{14} , wherein X_1
25 is selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y, wherein X_2 is selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y, X_3 is selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y, X_4 is selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y, X_5 is
30 selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T,

- V, W, or Y, X₆ is selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y, X₇ is selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y, X₁₁ is selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y, X₁₂ is
- 5 selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y, X₁₃ is selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y and wherein X₁₄ is selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y (SEQ ID No. 7). Preferably the selection is made according to the principles laid out above.
- 10 Thus, X₁ may either be an amino acid similar to K or it may be an amino acid with a non-polar side chain such as A, V, L, or I. Similar considerations apply to X₂, X₃, X₄, X₅, X₆, X₇, X₁₁, X₁₂, X₁₃ or X₁₄. In one preferred embodiment such longer peptide comprise amino acids 6 to 12, 5 to 13, 4 to 14, etc. of X₁-X₂-X₃-X₄-X₅-X₆-X₇-R-W-H-X₁₁-X₁₂-X₁₃-X₁₄, wherein X₁ is selected from the group comprising amino acids
- 15 with an NH₂ group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I, wherein X₂ is selected from the group comprising amino acids with negatively charged side chains such as E or D, or amino acids with non-polar side chains such as A, V, L or I, wherein X₃ is selected from the group comprising amino acids with an NH₂ group such as K, R, N, or Q, or amino acids with non-polar
- 20 side chains such as A, V, L or I, wherein X₄ is selected from the group comprising amino acids with non-polar or non-charged side changes and aromatic rings structures such as F, W, or Y, or amino acids with non-polar side chains such as A, V, L or I, wherein X₅ is selected from the group comprising amino acids with non-polar or non-charged side changes and aromatic rings structures such as F, W, or Y,
- 25 or amino acids with non-polar side chains such as A, V, L or I, wherein X₆ is selected from the group comprising G or amino acids with non-polar side chains such as A, V, L or I, wherein X₇ is selected from the group comprising amino acids with an NH₂ group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I, wherein X₁₁ is selected from the group comprising amino acids with
- 30 negatively charged side chains such as E or D, or amino acids with non-polar side

chains such as A, V, L or I, wherein X_{12} is selected from the group comprising G or amino acids with non-polar side chains such as A, V, L or I, wherein X_{13} is selected from the group comprising amino acids with non-polar or non-charged side chains and aromatic rings structures such F, W, or Y, or amino acids with non-polar side chains such as A, V, L or I, and wherein X_{14} is selected from the group comprising amino acids with an NH_2 group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I (SEQ ID No. 8). In an even more preferred embodiment such longer peptide comprise amino acids 6 to 12, 5 to 13, 4 to 14, etc. of X_1 - X_2 - X_3 - X_4 - X_5 - X_6 - X_7 -R-W-H- X_{11} - X_{12} - X_{13} - X_{14} , , wherein X_1 is selected from the group comprising amino acids with an NH_2 group such as K, R, N, or Q, wherein X_2 is selected from the group comprising amino acids with negatively charged side chains such as E or D, wherein X_3 is selected from the group comprising amino acids with an NH_2 group such as K, R, N, or Q, wherein X_4 is selected from the group comprising amino acids with non-polar or non-charged side changes and aromatic rings structures such as F, W, or Y, wherein X_5 is selected from the group comprising amino acids with non-polar or non-charged side changes and aromatic rings structures such as F, W, or Y, wherein X_6 is selected from the group comprising G or amino acids with non-polar side chains such as A, V, L or I, wherein X_7 is selected from the group comprising amino acids with an NH_2 group such as K, R, N, or Q, X_{11} is selected from the group comprising amino acids with negatively charged side chains such as E or D, wherein X_{12} is selected from the group comprising G or amino acids with non-polar side chains such as A, V, L or I, wherein X_{13} is selected from the group comprising amino acids with non-polar or non-charged side chains and aromatic rings structures such F, W, or Y, and wherein X_{14} is selected from the group comprising amino acids with an NH_2 group such as K, R, N, or Q (SEQ ID No. 9).

Peptides may be linear, branched and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic peptides may result from post-translation natural processes or may be made by synthetic methods.

In some of the most preferred embodiments, peptides useful in accordance with the invention comprise and more preferably consist of five or 14 amino acids as mentioned above and include peptides of SEQ ID No.: 1, 2, 3, 4, 5 or 6 to 10. The
5 most preferred embodiment of the present invention relates to a peptide of SEQ ID No.: 2 or SEQ ID NO: 6.

The term "compound comprising a peptide" refers to compounds which comprise a peptide e.g. in the form of a pharmaceutically acceptable salt. The term equally refer
10 to peptides which have been e.g. chemically or enzymatically modified such that e.g. a peptide of SEQ ID No. 1, 2, 3, 4 or 5 comprises additional modifications as they are described hereinafter. Modified forms of a peptide of SEQ ID No. 2 are particularly preferred.

15 The term "compound comprising a peptide" and its grammatical variation such as "peptide compound" thus includes salts, preferably pharmaceutically acceptable salts of the peptides described herein. Salts encompassed within the term "pharmaceutically acceptable salts" refer to non-toxic salts of the peptide compounds described herein. Representative salts and esters include the following: acetate,
20 ascorbate, benzenesulfonate, benzoate, bicarbonate, bisulfate, bitartrate, borate, caamsylate, carbonate, citrate, dihydrochloride, methanesulfonate, ethanesulfonate, p-toluenesulfonate, cyclohexylsulfamate, quinate, edetate, edisylate, estolate, esylate, fumarate, gluconate, glutamate, glycerophosphates, hydrobromide, hydrochloride, hydroxynaphthoate, lactate, lactobionate, laurate, malate, maleate, mandelate,
25 mesylate, mucate, napsylate, nitrate, n-methylglucamine, oleate, oxalate, palmoates, pamoate (embonate), palmitate, pantothenate, perchlorates, phosphate/diphosphate, polygalacturonate, salicylates, stearate, succinates, sulfate, sulfamate, subacetate, succinate, tannate, tartrate, tosylate, trifluoroacetate, and valerate. Other salts include
30 guanidine, diethanolamine or choline; ammonium, substituted ammonium salts or

aluminum salts. The salts are prepared by conventional methods.

The peptide component of "compound comprising a peptide" may, however, in addition to the peptide sequence of any of SEQ ID Nos.: 1 to 9 comprise amino acid sequences derived from other proteins. Therefore, the peptide compound described includes heterologous fusion peptides consisting essentially of SEQ ID Nos.: 1 to 9 fused to a heterologous amino acid sequence. The heterologous amino acid sequence may comprise or consist of 1, 2, 3, 4 or more amino acids. The heterologous amino acid sequence may for example comprise or consist of at least 5 or at least 10 or at least 20 heterologous amino acids. The heterologous amino acids may be fused to the N- and/or C-terminus of the CD44-derived sequences SEQ ID Nos.: 1 to 9 to provide other functionalities such as improved translocation across cellular membranes.

It is preferred that the peptide component useful in the invention is an isolated peptide. The term "isolated" means altered "by the hand of man" from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

It is also preferred that the peptide useful in the invention is in a pure state. Preferably, the peptide is $\geq 80\%$ pure, preferably $\geq 90\%$ pure, more preferably $\geq 95\%$ pure, even more preferably $\geq 99\%$ pure and particularly preferred is a pharmaceutically pure state that is greater than 99.9% pure with respect to contaminating macromolecules, particularly other peptides. It is preferred that the peptide is free of infectious and pyrogenic agents.

Preferably, a purified peptide is substantially free of other peptides. When used in this context, the term "pure" does not exclude the presence of the same peptide in

alternative physical forms, such as dimers.

The peptides useful in the invention may be prepared by chemical synthesis or by recombinant expression in host cells. The preparation by chemical synthesis is preferred. As protein products, compounds of e.g. SEQ ID NO: 2 or any of the other peptides useful in the present invention are amenable to production by the technique of solution- or solid-phase peptide synthesis. The synthetic peptide synthesis approach generally entails the use of automated synthesizers and appropriate resin as solid phase, to which is attached the C-terminal amino acid of the desired peptide. Extension of the peptide in the N-terminal direction is then achieved by successively coupling a suitably protected form of the next desired amino acid, using either Fmoc- or BOC-based chemical protocols typically, until synthesis is complete. Protecting groups are then cleaved from the peptide, usually simultaneously with cleavage of peptide from the resin, and the peptide is then isolated and purified using conventional techniques, such as by reversed phase HPLC using acetonitrile as solvent and tri-fluoroacetic acid as ion-pairing agent. Such procedures are generally described in numerous publications and reference may be made, for example, to Stewart and Young, "Solid Phase Peptide Synthesis," 2nd Edition, Pierce Chemical Company, Rockford, Ill. (1984).

The term "peptidomimetic" refers to a small protein-like chain designed to mimic a corresponding peptide. Peptidomimetics can typically arise either from modification of an existing peptide, or by designing similar systems that mimic peptides, such as peptoids and β -peptides. Irrespective of the approach, the altered chemical structure is designed to advantageously adjust the molecular properties such as metabolic stability and bioavailability without negatively affecting biological activity.

Typically a peptidomimetic will have an altered backbone such as a methylated amide group instead of the amide group of a peptide bond to increase the stability of the peptidomimetic against degradation by proteases. Alternatively or in addition, the

peptidomimetic may comprise non-natural amino acids or D-enantiomers. A common theme of peptidomimetics is that the molecular changes in the backbone structure and/or in the amino acids should not have a substantial effect on the overall conformation of the peptidomimetic in comparison to the corresponding peptide in order to not negatively affect the biological activity of the peptidomimetic. Thus a peptidomimetic is an isostere of the corresponding peptide. Preferred peptidomimetics are e.g. isosteric peptoids, which comprise poly-N-substituted glycines in the peptide bonds of the backbone. In accordance with the invention, peptidomimetic shall therefore have the same activity in the experiments described hereinafter as the peptides as described hereinafter, such as e.g. a peptide of SEQ ID NO. 1, 2, 3, 4, or 5. The most preferred peptidomimetics are those having five amino acids such as a peptidomimetic of a peptide of SEQ ID No. 2, 8 amino acids (such as a peptidomimetic of SEQ ID NO: 16, 17 or 18) or 14 amino acids (cf. SEQ ID NO: 6, 7, 8, 9, or 10). Such peptidomimetics are preferably isosteric peptoids, which comprise poly-N-substituted glycines in the peptide bonds of the backbone.

The present invention also contemplates the use of modified forms of peptides or peptidomimetics, e. g. as pharmaceutical compositions for treating metastasizing cancer in a human being. Such modified forms relate e.g. to peptides or peptidomimetics which have been chemically modified at their respective N- and/or C-terminus by blocking groups such as Fmoc or BOC or alkylation such as methylation to reduce degradation of the peptides or peptidomimetics e.g. by proteases and to increase stability thereof. Other modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, i.e. a cyclic peptide, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, including a

myristoylated cyclic peptide, such as a myristoylated cyclic 5mer (based on SEQ ID No: 2), 6mer, 8mer or 14mer, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, ubiquitination and sumoylation.

- 5 Examples of such peptidomimetics are given in Table 4. In a preferred embodiment the peptide is a 14mer pegylated with PEG having a molecular weight of 2000 Da or a PEG having a molecular weight in the range of 200 to 20000 Da, a 14mer, a cyclic 8mer or a cyclic 5mer. A typical peptidomimetic can have one or more modification, i.e. can be cyclic and additionally be myristoylated, pegylated, and/or modified in
10 any other way described herein, such as by use of D-amino acids. Typical examples of peptidomimetics include those which have SEQ ID Nos: 1 to 18 with L- and/or D-amino acids.

- Cyclization of peptides is performed by methods generally known by a person skilled
15 in the art, such as described in Zitzmann et al. (2005, Journal of Nuclear Medicine, 46(5):782).

- The compounds described can also be administered in combination with cytotoxic compounds and/or chemotherapeutic agents. It is possible to administer the
20 compounds along with one or more cytotoxic compounds and/or chemotherapeutic agents or it is possible to covalently conjugate the cytotoxic compound to the peptide or compound described. The preferred cytotoxic compounds are maytansinoids and preferred chemotherapeutic compounds are taxanes.

- 25 In addition or alternatively preferred modified forms of peptides or peptidomimetics include e.g. chemically or enzymatically modified forms thereof which have improved biological properties such as improved solubility, absorption, biological half-life, etc. The modifications may alternatively decrease the toxicity of the molecule, eliminate or attenuate any undesirable side effect of the molecule, etc.
30 Modifications which increase e.g. the biological half-life include pegylation,

hesylation, pasylation, glycosylation with glycosyl structure having sialic acid residues at their end, etc.

5 The terms “pegylated” and its grammatical variations such as “pegylation” all describe that the peptide or peptidomimetics thereof in their different forms (e.g. in isolated form, as pharmaceutically acceptable salts etc) comprise a PEG moiety, i.e. a polyethyleneglycol chain, which is covalently attached to the peptides or peptidomimetics as described herein.

10 As is described hereinafter, a rat CD44v6 pentapeptide of sequence NEWQG (SEQ ID No.: 11), which is the counterpart to the human CD44v6 pentapeptide of SEQ ID No.2 and which has been modified with PEG750 or PEG3000 is capable of inhibiting HGF stimulated and CD44 mediated activation of Met and Erk even though at least the PEG3000 moiety has a molecular weight, i.e. 3000 Da, which is
15 significantly higher than the calculated molecular weight of the sequence of SEQ ID No.: 11, being approximately 620 Da. Moreover, PEGs of such a molecular weight are known to have an extended zigzag structure. It therefore is surprising that modification of comparatively small peptide such as the pentapeptide of SEQ ID No.: 11 does not lead to loss of activity. It seems justified to conclude that the same
20 should apply to the human counterparts such as a pentapeptide of SEQ ID No.: 2, peptidomimetics thereof or even longer peptides such as those of SEQ ID No.: 6. It is furthermore surprising that inhibition of ERK signaling is even more efficient with pegylated peptides than non-pegylated peptides.

25 The term “PEG moiety” or “Polyethyleneglycol moiety” as used hereinafter refers to PEGs of an average molecular weight of about 200 Da to about 35,000,000 Da. It is preferred to use PEGs which have an average molecular weight of about 400 Da to about 20,000 Da, preferably of about 600 to about 10,000 Da, even more preferably of about 700 Da to about 10,000 Da. The most preferred PEGs have an average
30 molecular weight of about 800 Da to about 8,000 Da, of about 900 Da to about

7,000 Da, of about 1,000 Da to about 6,000 Da such as of about 2,000 Da, of about 3,000 Da, of about 4,000 Da or of about 5,000 Da. In a preferred embodiment, the PEG has an average molecular weight in the range of 200 to 20000 Da.

- 5 PEGs are typically named by their average molecular weight. Thus, a PEG with nine repeating units has an average molecular weight of 400 Da and would be labeled as PEG 400. The PEGs contemplated for use in the present invention may thus be PEGs such as PEG 400, PEG 600, PEG 50, PEG 840, PEG 1000, PEG 1500, PEG 2000, PEG 3000, PEG 3350, PEG 4000, PEG 4600, PEG 8000, PEG 10000, PEG 12000 as
10 they are commercially available e.g. from Sigma-Aldrich.

- PEGs may come as straight, branched, star or comb PEGs. Branched PEGs have three to ten PEG chains emanating from a central core group. Star PEGs have 10 to 100 PEG chains emanating from a central core group. Comb PEGs have multiple
15 PEG chains normally grafted onto a polymer backbone.

- For the purposes of the invention it is generally preferred to use straight PEGs and particularly preferred to use straight PEGs with an average molecular weight in the range of about 1,000 to 6,000 Da, such as a PEG 2000, PEG 3000, PEG 4000 or
20 PEG 5000 or a PEG of higher molecular weight.

- The PEG moieties may be further modified. For example, the PEG moieties may be covalently modified with fatty alcohol and fatty acids. Such additional modifications may allow the modified pegylated peptides and peptidomimetics to build or be at
25 least incorporated in micelle- or liposome-like structures.

- Also described are such micelle- or liposome-like structures. The pegylated peptides or pegylated peptidomimetics thereof and pharmaceutical compositions comprising such pegylated peptides or pegylated peptidomimetics thereof may provide e.g. for
30 improved drug solubility, reduced dosage frequency, extended circulating half-life,

increased drug stability, enhanced protection from proteolytic degradation, etc. The micelle- or liposome-like structures may additionally allow delivering the pegylated peptides or pegylated peptidomimetics more efficiently as the additional modification such as fatty alcohol- or fatty acid-chains may allow improved
5 interaction with cellular membranes. Moreover, the micelle- or liposome-like structures may comprise additional pharmaceutically active agents such as chemotherapeutic agents. In case of pancreatic cancer, these agents may include e.g. gemcitabin. In case of breast cancer, these agents may include Herceptin.

10 Also described are such micelle- or liposome-like structures comprising additional pharmaceutically active agents. Such micelle- or liposome-like structures will allow targeting of such chemotherapeutic agents through the pegylated peptides or pegylated peptidomimetics thereof to e.g. the tumors expressing CD44v6 and thus allowing targeted and improved therapy.

15

The person skilled in the art knows how to modify peptides or peptidomimetics with PEG moieties. The covalent attachment of a PEG moiety may be done chemically or enzymatically.

20 The first step in chemical modification is typically functionalizing either one or both ends of the PEG polymer. Depending on the fictionalization, one can differentiate between monofunctional, bihomofunctional or biheterofunctional PEGs.

The chemical Pegylation process can be generally categorized into two types, namely
25 a solution phase batch process or an on-column fed-batch process. The commonly adopted batch process involves the mixing of reagents in a suitable buffer solution, preferably at a temperature of 4 to 6°C, followed by separation and purification of the desired product using techniques such as size exclusion chromatography, ion exchange chromatography or hydrophobic interaction chromatography.

30

The choice of the suitable functional group for the PEG derivative is based on the type of available reactive group on the molecule that will be coupled to the PEG. For proteins and peptides, typical reactive amino acids include lysine, cysteine, histidine, arginine, aspartic acid, glutamic acid, serine, threonine, tyrosine. The N-terminal amino group and the C-terminal carboxylic acid can also be used as a site specific site by conjugation with aldehydes functional polymers. If it is preferred to not react an amino acid with a PEG, but only e.g. the N-terminus of a peptide, one can block the functional groups in amino acids, e.g. with Fmoc, pegylate the peptide and then de-block the amino acids.

10

The techniques used to form PEG derivatives such as pegylated peptides are generally reacting the PEG polymer with a group that is reactive with hydroxyl groups, typically anhydrides, acid chlorides, chloroformates and carbonates. In the second generation pegylation chemistry more efficient functional groups such as aldehyde, esters, amides etc. made available for conjugation.

15

Heterobifunctional PEGs are very useful in linking two entities, where a hydrophilic, flexible and biocompatible spacer is needed. Preferred end groups for heterobifunctional PEGs are maleimide, vinyl sulfones, pyridyl disulfide, amine, carboxylic acids and NHS esters.

20

The pegylated peptides and pegylated peptidomimetics thereof described herein may be provided in the form of pharmaceutical compositions comprising optionally pharmaceutically acceptable excipients.

25

In the following it is set out how the compounds described, i.e. the peptides, peptidomimetics thereof and modified forms thereof, the pharmaceutical compositions comprising these compounds and methods making use of these compounds may be used for the treatment of metastasizing cancer in a human being.

30

It is to be understood that, whenever reference is made in the following to the

treatment of metastasizing cancer, this reference, as a preferred embodiment, always contemplates to use the pentapeptides as described hereinafter, namely those of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4 and SEQ ID NO:5, and in particular those of SEQ ID NO: 2, peptidomimetics thereof, or modified forms thereof such as the pegylated forms thereof.

As can be taken from the experiments described hereinafter, the peptides used in the experiments were capable of inhibiting metastasis formation in pancreatic cancer models as well as inducing regression of already formed metastases. As mentioned, the invention relates to the use of compounds, pharmaceutical compositions and the application of methods as described herein for the treatment of metastasizing cancers.

Metastasizing cancers in accordance with the invention include metastasizing forms of cancers for which expression of CD44v6 has been observed on cancer tissues or can be observed upon corresponding testing e.g. with CD44v6 antibodies. Thus, in order to see whether a patient is eligible for treatment with compounds and pharmaceutical compositions as described herein, one may take a biopsy of tumor tissue and test for expression of CD44v6. If the tumor can be shown to express CD44v6 and if metastases have started to form or have already formed and maybe even spreaded through the body, this tumor is considered as a metastasizing cancer in accordance with the invention. Metastasizing cancers in accordance with the invention include metastasizing forms of Hodgkin lymphoma, colorectal cancer, cervical cancer, lung cancer, skin cancer such as squamous cell cancer or basal cell carcinoma, head and neck cancer, gastric cancer, pancreatic cancer, head and neck squamous cell cancer, breast cancer, and others.

Such metastasizing cancer forms of the various cancers such as Hodgkin lymphoma, colorectal cancer, cervical cancer, lung cancer, skin cancer such as squamous cell cancer or basal cell carcinoma, head and neck cancer, gastric cancer, pancreatic

cancer and breast cancer can be identified according to the TNM Anatomic Stage/Prognostic Group System of the Cancer Staging Manual of the American Joint Committee on Cancer (7th edition, 2010, Springer).

- 5 Metastasizing cancers in accordance with the invention may be classified as Stage III or Stage IV cancers according to the TNM Anatomic Stage/Prognostic Group System of the Cancer Staging Manual of the American Joint Committee on Cancer (7th edition, 2010, Springer). The TNM (tumor node metastasis) staging system of the American Joint Committee on Cancer allows staging, i.e. classification of cancers by
- 10 the size and extent of the primary tumor (T), the question if regional lymph nodes (N) are affected and if distant metastases can already be detected (M). This indication is then typically taken as an indication for different routes to take in treating the patient and also allows a reliable prognosis of the diseases. This is why the TNM system has become an indispensable tool for oncologists. The parameter M
- 15 receives a value of 0, i.e. M0 if no distant metastases can be detected clinically although they may have started to develop. If M is set at M0, a patient depending on the values of T and N may be classified as Stage III. Thus for e.g. any T, N3 and M0, a patient may be classified as Stage III, or Stage IIIc in case of e.g. breast cancer (see page 362, Cancer Staging Manual of the American Joint Committee on Cancer (7th
- 20 edition, 2010, Springer). If such a Stage III patient or a patient having the highest subclass of Stage III (such as Stage IIIC for breast cancer) additionally displays circulating tumor cells and micrometastases in the bone marrow, this will worsen the prognosis. Thus, for the purposes of the present invention a metastasizing cancer may be classifiable as Stage III according to the TNM Anatomic Stage/Prognostic
- 25 Group System of the Cancer Staging Manual of the American Joint Committee on Cancer (7th edition, 2010, Springer).

Preferably, for a metastasizing cancer in accordance with the invention M is M1, i.e. that distant metastasis can be clinically detected so that such a metastasizing tumor

30 can be classified as Stage IV according to the TNM Anatomic Stage/Prognostic

Group System of the Cancer Staging Manual of the American Joint Committee on Cancer (7th edition, 2010, Springer).

5 It is to be understood that wherever the present invention makes reference to a metastasizing cancer, this most preferably relates to a cancer such as Hodgkin lymphoma, colorectal cancer, cervical cancer, lung cancer, skin cancer such as squamous cell cancer or basal cell carcinoma, head and neck cancer, gastric cancer, pancreatic cancer, or breast cancer, which is classified as Stage IV according to the TNM Anatomic Stage/Prognostic Group System of the Cancer Staging Manual of the
10 American Joint Committee on Cancer (7th edition, 2010, Springer). The specific requirements for classifying each cancer according to this system, which can be found in the respective chapters of the Cancer Staging Manual of the American Joint Committee on Cancer (7th edition, 2010, Springer), see e.g. page 143 to 146 for colorectal cancer, page 241 to 250 for pancreatic cancer, page 301 to 314 for
15 squamous cell cancer, page 347 to 376, etc.), are hereby incorporated by reference.

A particularly preferred embodiment thus refers to the use of the pentapeptides described hereinafter, e.g. of SEQ ID NO:1, 2, 3, 4, or 5 and most preferably of SEQ ID NO:2, peptidomimetics thereof or modified forms thereof, and pharmaceutical
20 compositions comprising these compounds for the treatment of metastasizing cancers such as Hodgkin lymphoma, colorectal cancer, cervical cancer, lung cancer, skin cancer such as squamous cell cancer or basal cell carcinoma, head and neck cancer, gastric cancer, pancreatic cancer, or breast cancer, which are classifiable as Stage IV according to the Cancer Staging Manual of the American Joint Committee on
25 Cancer.

The compounds and salts thereof can be formulated as mentioned above as pharmaceutical compositions (e.g. liquids, suspensions, emulsions, lozenges, sachets, ampoules, aerosols, powders, granules, tablets, pills, capsules, injections, solutions
30 etc.) comprising at least one such compound alone or optionally in a mixture with

pharmaceutically acceptable carriers, excipients and/or diluents.

The compounds/salts thereof and pharmaceutical compositions may be formulated for oral administration, e.g. by inhalation, for nasal administration or for
5 administration by injection such as subcutaneous injection.

The invention is now described with respect to experiments which, however, are not to be construed in a limiting sense.

10 **Examples**

Example 1

1. Material and Methods

15

1.1 Cell lines

The rat pancreatic carcinoma cell line BSp73AS (also designated AS) and its transfectants have been described (Orian-Rousseau et al., Genes & Development (2002), 16:3074–3086) and were grown in RPMI (Invitrogen, Karlsruhe, Germany) plus 10% FCS (PAA, Cölbe, Germany). The human pancreatic cancer cells L3.6pl (Bruns et al., Neoplasia (1999), 1, 50-62) were maintained in DMEM (low glucose; Invitrogen, Karlsruhe, Germany) supplemented with 10% FCS (PAA, Cölbe, Germany), sodium pyruvate, nonessential amino acids, L-glutamine, and MEM vitamin solution (Pan Biotech, Aidenbach Germany).

10

1.2 Antibodies and other reagents

The human monoclonal antibody against CD44v6 (VFF18) was a gift from Bender (eBioscience, Campus Vienna Biocenter 2, A-1030, Vienna, Austria), the anti-ERK 1 (K-23), c-Met (C-28) and GFP antibody (sc-101525) were from Santa Cruz Biotechnology (Heidelberg, Germany), the cleaved Caspase-8 antibody (IMG-5703) from Imgenex (San Diego, CA, USA), the CD31 antibody (MEC13.3) from BD Biosciences, Heidelberg, Germany, and the cleaved Caspase-3 (Asp175), Phospho-Met (Tyr1234/1235) (D26), Met (25H2) and the phospho-ERK phospho-p44/42 antibodies from Cell Signaling Technology (Beverly, England). The rat exon v6-specific antibody 1.1ASML has been described (Gunthert et al., Cell (1991), 65, 13-24). Secondary antibodies labeled with horseradish peroxidase were from Dako (Glostrup, Germany). The Alexa Fluor R 546 goat anti-rabbit secondary antibody was purchased from Life Technologies (Darmstadt, Germany). HGF was purchased from Peprotech (Hamburg, Germany). The CD44v6 rat and human peptides (14mer and 5mer) have been described (Matzke et al. Cancer Res. (2005), 65(14), 6105-6110). The sequence of the rat 14mer is KEKWFENEWQGKNP (SEQ ID No. 10), the rat 5mer corresponds to NEWQG (SEQ ID No. 11). The human 14mer corresponds to KEQWFGNRWHEGYR (SEQ ID No.: 6) and the human 5mer has

25

the following sequence: NRWHE (SEQ ID No.: 2). For in vivo imaging experiments the rat 11mer WFENEWQGKNP (SEQ ID No.:12), the mouse 11mer WFQNGWQGKNP (SEQ ID No.: 13) and the human 11mer WFGNRWHEGYR (SEQ ID No.: 14) were labeled with the fluorescent dye DY681. As a control peptide

5 in the case of rat cells or rat syngeneic models, the human v6 peptide of an identical length as the specific rat v6 peptide was used. In the case of human tumor cells or human orthotopic tumor model, the rat v6 peptide was used as a control. All peptides were produced by Bachem (Bubendorf, Switzerland) or Intavis (Köln, Germany). Lyophilized peptides were resuspended in PBS containing 1% BSA to a stock

10 concentration of 1 mg/ml. Final dilutions were obtained by dilution in PBS.

1.3 Lentiviral transfection of shRNA

The lentivirus system used for silencing Met has already been described (Corso et al., Oncogene (2008), 27(5):684-93). Lentiviruses were produced as described elsewhere

15 (Vigna et al., J. Gene Med. (2000), 2(5):308-16). Briefly, 4×10^6 293 T cells (p12-15) were seeded in a 10 cm plate. 24 h later the packaging vectors VSV-G, PMDL and Rev, the TetR and the lentivirus construct (either Met-shRNA or control-shRNA construct) were mixed and brought to a final volume of 450 μ l by addition of TE. 450 μ l of a 2.5 M CaCl_2 solution was added to this mixture. After vortexing and

20 5 minutes incubation 500 μ l of 2x HBS solution was added drop wise to the DNA-TE- CaCl_2 mixture while vortexing at full speed. The precipitate was added to the 293T cells. After 16 h the medium was replaced and fresh medium containing 5 mM sodium butyrate was added. Medium was collected after 24 and 48 hours. Virus containing medium was then added to the target cells BSp73ASs6 (70% confluency)

25 in presence of 8 μ g/mL polybrene. 24 hours after infection the medium was replaced and production of shRNA was induced by addition of Doxycycline to a final concentration of 1 μ g/ml. BSp73ASs6 cells transduced with TetR and control-shRNA or Met-shRNA were treated with doxycycline for 72 hours before starting an assay or injection in the animal.

1.4 Western Blot analysis

Serum-starved cells (24 hours) were induced with the growth factor HGF (10 ng/mL) at 37°C for 5 minutes. Where indicated, the cells were treated with peptides (100 ng/ml) prior to induction at 37°C for 10 minutes (100 ng/mL CD44v6 peptide or control peptide). Following the induction with HGF, cells were washed with ice-cold phosphate-buffered saline (PBS). To detect activated Met and Erk, cells were lysed in sodium dodecyl sulfate (SDS)–sample buffer containing 100 mM dithiothreitol (DTT), boiled and subjected to western blot analysis using antibodies against phospho- Met and ERK. The loading controls were performed on the same blot after stripping (62.5 mM Tris, pH 6.8, 2% SDS, 0.8% DTT) by probing with the Met respectively ERK antibody. Blots were stained using the enhanced chemiluminescence system (Thermo Fisher Scientific, Schwerte, Germany). Bands in western blot analysis were quantified with the program Image J (National Institutes of Health).

15

1.5 Quantitative determination of HGF and VEGF in cell culture supernatant

Determination of human HGF and VEGF levels in cell culture medium of L3.6pl cells were performed using the Quantikine Human HGF Immunoassay and Quantikine Human VEGF Immunoassay from Roche (Mannheim, GER) and R&D Systems (Wiesbaden, Germany). For this purpose 3×10^6 cells were cultured for 5 days in a 15 cm plate in the presence of the respective peptides as indicated. The supernatant (20 ml) was centrifuged (1200 rpm) and the assay was performed according to manufacturer's instructions.

25 1.6 Animal experiments

Male athymic nude mice (NCI-nu) were purchased from Harlan (Roßdorf, Germany). BD10 and BDX rats were bred in house. The animals were housed and maintained under specific pathogen-free conditions in facilities approved by the Regierungspräsidium Karlsruhe. All animals were handled according to German regulations for animal experimentation. The animal experiments were approved by

30

the Regierungspräsidium Karlsruhe (35-9185.817G-192/10). Imaging experiments (Figure 3) were performed in Göttingen were authorized by the Regierungspräsidium (35-9185.817G-106/09).

- 5 In the case of the rat syngeneic model, 1×10^6 pancreatic cells (BSp73AS and its transfectants) were subcutaneously injected into the right posterior flank of the animals. Tumors developed for four weeks. During this time period animals injected with BSp73ASs6 cells expressing Met-shRNA or control-shRNA received doxycycline in the drinking water. At the end of the experiment, primary tumors
10 were isolated. Lungs and axillary lymph nodes were analyzed. The tissues were incubated for 24 h in a zinc-fixative (0.5 g calcium acetate, 5.0 g zinc acetate, 5.0 g zinc chloride, in 1 L 0.1 M Tris pH 7.4) and embedded in paraffin for further analysis. In case of peptide or antibody treatment tumors developed for one week before the first treatment. Where indicated, animals received 200 μ g of peptide or
15 antibody per injection three times per week for four weeks. Tumor growth was monitored weekly using a caliper. Animals were killed at day 28 or 30 after start of the treatment.

- In the case of the human orthotopic model, L3.6pl pancreatic carcinoma cells
20 (passage 24-26) were suspended in Hank's balanced salt solution (Invitrogen, Karlsruhe, Germany) after trypsinization. The cells were injected orthotopically in the pancreas of male nude mice as described (Bruns CJ et al. (1999) Neoplasia 1(1):50-62). Two groups of 15 mice each were injected i.p. 7 days later with either the human v6 peptide respectively rat control peptide (20 μ g). For all experiments
25 described and depicted herein either the rat 14mer control peptide or the human 14mer peptide were used. All experiments were then repeated with the rat 5mer control peptide or the human 5mer peptide (N-R-W-H-E). The same results as for the 14mer peptides were observed. The injection was repeated three times per week for 21 days. Two days after the last treatment the animals were killed.

In order to examine the regression of metastases (either BSp73ASs6 or L3.6pl) were implanted as described above. Tumor growth was allowed for three weeks. At that time all animals had developed metastases in the control group. Animals were injected i.p. with 20 µg (L3.6pl orthotopic mouse model: human v6 peptide or rat control peptide) or i.v. with 200 µg (rat syngeneic model: rat v6 peptide or mouse control peptide) of peptides three times per week. Animals were killed 23 days after the start of the peptide treatment.

1.7 In vivo imaging using Optix MX2

- 10 In vivo imaging of the subcutaneously grown BSp73ASs6 tumors in BD10 or BDX rats was performed using the near infrared fluorescence (NIRF)-imaging system Optix MX2 (ART, Montreal, Canada) as described earlier (Napp et al., Int J Cancer (2010), 127:1958-1974). To avoid autofluorescence of fur, rats were shaved around the tumor prior to imaging. Subsequently, animals were anesthetized using 2% isoflurane and gently fixed on the devices' heated plate for the entire time of data acquisition. To reduce fluorescence background, rats were fed with chlorophyll-reduced food (Provimi Kliba AG, Kaiseraugst, Switzerland) for one week prior NIRF imaging. All in vivo analyses were preceded by native scans of animals without any injection of the fluorescent probe. For in vivo analysis, rats were injected with 200 µg DY681-labeled rat v6 11mer peptide or the human v6 11mer peptide via the tail vein. Controls were injected with the equal amount of DY681-labeled mouse v6 11mer peptide. The rat 11mer had the sequence WFENEWQGKNP (SEQ ID No.: 12), the mouse 11mer had the sequence WFQNGWQGKNP (SEQ ID No.: 13) and the human 11mer had the sequence WFGNRWHEGYR (SEQ ID No.: 14). All peptides were labeled with the fluorescent dye DY681. Data were acquired at the indicated time after injection of the peptides. For ex vivo monitoring animals were sacrificed after 24 hrs after peptide injection and tumor and organs of interest were scanned ex vivo using Optix MX2.
- 30 DY681 fluorescence was measured using excitation at 670 nm in combination with a

700 nm long-pass emission filter. Scans were performed with 1.5 mm raster, photon collection time of 0.5-1 s per scan point and varying laser power. Data sets were analyzed with OptiView (ART). Fluorescence intensity data are displayed in normalized counts (NC) where the measured fluorescence intensity (counts) was
5 normalized for varying laser power and integration times, allowing comparison of measurements with different settings.

1.8 In vivo imaging using Pearl imager

In vivo imaging of the mice with the L3.6pl tumors was performed using the Pearl™
10 Imager (LI-COR Biosciences, Bad Homburg, Germany). The system uses two lasers (685 and 785 nm) for excitation and a charge-coupled device detector for signal detection. The laser excitation enables a deep tissue penetration. With the near-infrared detection a high sensitivity due to the reduced tissue auto-fluorescence is achieved. In order to standardize the images we made use of the Pearl Cam Software.
15 The animals were fed with chlorophyll-reduced food to reduce the fluorescence background one week before imaging (Regime 210, safe-diets, Augy, France). Prior to imaging the mice were anesthetized with 2.0% isoflurane. Animals were placed on the heated plate of the imager and continuous delivery of isoflurane was achieved through a nose cone in the imaging drawer. Images were captured at white light, 700
20 and 800 nm. Animals were imaged prior to i.v. peptide injection and 24h after injection with either DY681-labeled human v6 peptide or DY681-labeled rat v6 peptide as control. Immediately after each imaging session animals were killed, tumor, liver and spleen isolated and scanned ex vivo at white light, 700 and 800 nm.

25 1.9 Immunofluorescence

ASs6 or L3.6pl cells were seeded at 5,000 cells/well of a Lab-TekR Chamber Slide™ (Nunc, Napierville, IL, USA). On the following day the cells were washed with cold PBS and fixed with 4% Formalin for 30 min at RT. Unspecific binding was blocked with 1% BSA in PBS for 1 hour at RT. The cells were incubated for 1 hour
30 with the DY681-labeled peptides. After three washing steps with PBS the cover slips

were mounted with Fluorescence Mounting Medium (Dako, Glostrup, Denmark) and the immunofluorescence was measured by a laser scanning confocal microscope Leica TCS2 SP2 (Exton, PA, USA) and processed using Leica confocal software. A 20X objective was used for imaging.

5

1.10 Histology

For histo-morphological analysis paraffin-embedded sections of lungs were stained with hematoxylin and eosin and periodic acid-Schiff (H&E or PAS). Serial sections of the whole tissue blocks were examined by analyzing slices every 20 μm ; in each slice, the presence and the extension of the metastatic deposit was assessed, according to the procedure routinely used by pathologists to evaluate the presence of micro-metastases.

1.11 Immunohistological and immunofluorescence analysis

15 7 μm thick paraffin sections were deparaffinized and rehydrated. For P-Met staining antigen unmasking was achieved by boiling the slides in 1 mM EDTA pH 8,0 followed by incubation for 15 minutes at a sub-boiling temperature, for CD31 staining the sections were treated with Proteinase K (8 $\mu\text{g/ml}$) for 10 min at 37°C. For immunofluorescence staining of P-Met, unspecific binding was blocked with 5% goat serum (DAKO, Glostrup, Denmark) (diluted in 1x PBS / 0,3% Triton X-100) for 60 minutes. In the case of immunohistochemistry, endogenous peroxidases were at first blocked with 3% H_2O_2 in PBS followed by incubation with biotin blocking system (Dako, Glostrup, Denmark) and then unspecific binding was inhibited by incubation with 5% FCS in PBS. The sections were incubated with the P-Met antibody (D26, dilution 1:50 in 1 x PBS / 1% BSA / 0.3% Triton X-100), Met antibody (C-28, dilution 1:50), GFP antibody (sc-101525, dilution 1:50) or CD31 antibody (5 $\mu\text{g/ml}$) over night or VFF18 (5 $\mu\text{g/ml}$) o/n at 4°C. After washing in PBS the sections were incubated with Alexa Fluor R 546 goat anti-rabbit (in case of immunofluorescence P-Met and Met staining, dilution 1:500) or a biotinylated secondary antibody (for immunohistochemical stainings rabbit anti-rat antibody for

20

25

30

VFF18 and CD31, goat anti-rabbit for P-Met, cleaved Caspase-3 and cleaved Caspase-8 and rabbit anti-mouse for GFP, dilution 1:500) for 45 minutes. For DAB (3,3'-diaminobenzidine) staining the sections were treated with a streptavidin-peroxidase conjugate (Dako, Glostrup, Denmark) and developed with DAB substrate system (3,3'-diaminobenzidine; Biozol, Eching, Germany). For immunofluorescence DAPI was used for nuclear staining.

2. Results

2.1 The co-receptor function of CD44v6 for Met is the decisive step for metastatic spreading of rat pancreatic tumor cells

To investigate whether the co-receptor function of CD44v6 for the Met receptor is the decisive step for the metastatic spreading of tumor cells it was first examined whether a CD44 isoform containing exclusively the exon v6 or all the variant exons v1-10 included in the CD44v1-10 isoform would confer metastatic potential to the rat BSp73AS cells. In these cells, Met cannot be induced by HGF unless a CD44v6 containing isoform is transfected (Fig.1A). Removal of exon v6 e.g. in CD44v1-10Δv6 impaired the activation of Met by HGF (Fig.1A).

The parental BSp73AS cells and cells stably transfected with the constructs mentioned above (CD44v6, CD44v1-10 and CD44v1-10Δv6) were subcutaneously injected into isogenic rats. In all animals primary tumors were palpable already after one week. In the case of cells transfected with CD44v6 or CD44v1-10 the axillary lymph nodes of the corresponding animals were enlarged already two weeks after injection and the animals were killed after six weeks. Histomorphological examination of the lungs and lymph nodes revealed that all rats bearing CD44v6 or CD44v1-10 expressing tumors developed nodal and intrapulmonary metastases. In contrast, the parental cells or the cells transfected with CD44v1-10Δv6 gave rise to primary tumors but not to metastasis, neither in lymph nodes nor in the lungs (Fig.1B and Table 1). No micrometastasis was detected in the lungs of these animals

(Table 1). Thus, CD44 isoforms such as CD44v6, that allow Met activation, also confer metastatic propensity to BSp73AS cells.

5 Next, BSp73ASs6 cells (BSp73AS cells expressing the CD44v6 isoform in addition to CD44s) were stably transfected with lentivirus expressing Met shRNA sequences. It was confirmed that the cells do not express Met (Fig. 1A, last two lanes). Then, these cells (or cells infected with lentivirus expressing control sh-RNA sequences) were injected into syngeneic animals. The animals were examined six weeks after injection. All animals developed primary tumors (Table 1). However, Met activation
10 was only observed in the primary tumors of cells infected with control sh-RNA expressing lentivirus, but not in primary tumors expressing Met sh-RNA (Fig.1C). Furthermore, when Met expression was abolished no metastasis nor micro-metastasis was detected neither in lung nor in the axillary lymph nodes (Fig.1B, Table 1) demonstrating the importance of Met for the metastatic process. From these data, it
15 can be concluded that both CD44v6 and Met are required for metastatic spreading of BSp73AS cells.

Next it was examined whether the rat counterparts to a peptide of SEQ ID No.: 2 (human CD44v6 5mer peptide) or SEQ ID No.: 6 (human CD44v6 14mer peptide)
20 that interferes with the co-receptor function, also inhibit metastasis formation. Rat peptides had the sequences KEKWFENEWQGKNP (SEQ ID No.: 10) and NEWQG (SEQ ID No.: 11). BSp73ASs6 cells were subcutaneously injected into syngeneic rats and rat CD44v6 peptides of SEQ ID No.: 10 or SEQ ID No. 11 (or mouse control peptides) or the CD44v6 antibody (or IgGs as a control) were injected intra-
25 tumoral or intravenously three times a week. Both, treatment with the v6 specific antibody or with the v6 specific peptide completely inhibited metastasis (Fig. 2B, Table 2). In lung sections from animals treated with the CD44v6 peptide, neither metastasis nor micro-metastasis could be detected upon histological analysis using PAS and H&E staining (Fig. 2C and Table 2). This is in clear contrast with the
30 results obtained from animals treated with the control peptide (or control IgG) where

numerous metastases were detected in the lungs (Fig. 2B, C). Interestingly, the outgrowth of primary tumors was not influenced by either treatment (Fig. 2D). This is compatible with the fact that also BSp73AS cells or BSp73ASv1-10Δv6 cells can induce the formation of primary tumors similarly to the v6 transfectants. Taken
5 together the experiments suggest that the co-receptor function of CD44v6 for Met is the decisive step for metastatic spreading in the rat pancreatic tumor cell system and that it does not account for the outgrowth of the primary tumor.

2.2 Specific binding of a CD44v6 peptide to tumor cells in vivo

- 10 In order to test the specificity of the binding of CD44v6 peptides to the tumor site and to the metastases and to estimate the binding kinetics in vivo, near infrared fluorescence (NIRF) imaging with the Optix MX2 was. For this purpose two CD44v6 peptides were labeled with the fluorophore DY681: a rat specific one, rv6 pep Dy681 and a mouse specific one, mv6 pep Dy681, as a control. In vitro testing
15 showed that indeed, only the labeled rat peptide but not the mouse peptide inhibited Met activation as was demonstrated in Fig. 3A right side. Furthermore, the DY681-labeled rat v6 peptide, but not the DY681-labeled mouse v6 peptide bound to BSp73ASs6 cells in tissue culture (Fig. 3A left side).
- 20 For in vivo experiments, BSp73ASs6 cells were injected subcutaneously into syngeneic rats. Three weeks later, they received either of the labeled v6 peptides by injection into the tail vein. The accumulation of the fluorescence was measured in the tumor region by Optix MX2 at the indicated time points. Prior to intravenous (i.v.) injection of Dy681-labeled peptides mice were natively scanned in order to measure
25 levels of autofluorescence background. Then, in vivo binding kinetics of the Dy681-labeled v6 peptides to the subcutaneous tumors were determined. As shown in a series of representative NIRF images taken 1, 2, 3 and 7 days after peptide injection, high fluorescence intensity was detected over the tumor area for at least 2 days in vivo, with a maximum at 1 day. Injection of the control peptide m6 pep Dy681 did
30 not result in any fluorescent signals over the tumor area (Fig. 3D).

To specifically examine the distribution of the v6 peptides in the tumor and also in the lungs, both tissues were excised 24h after peptide injection and examined ex vivo by Optix MX2 (Fig. 3C). Fluorescence intensities could only be detected in tumors and in specific areas of the lungs scanned ex vivo and excised from mice that received rv6 pep Dy681, but not in tumors and lungs from all control rats previously injected with mv6 pep Dy681 (Fig. 3C). These results confirmed the in vivo observation that the rat peptide binds specifically to the subcutaneous rat pancreatic tumor and furthermore even suggest in vivo binding to metastases. Indeed, histological analysis revealed the presence of metastases in the lungs of all animals.

2.3 Metastasis formation of human pancreatic tumor cells is inhibited by a human CD44v6 peptide of SEQ ID No.: 2 or SEQ ID No.:6

To expand the studies to human pancreatic tumors, the highly metastatic human pancreatic carcinoma cells L3.6pl (Bruns, et al. Neoplasia (1999), 1(1):50-62) were investigated. These cells originate from the COLO 375 cells that have been passaged several times in the liver and the pancreas, a step that rendered them more and more metastatic. It was confirmed that Met is dependent on CD44v6 for its activation and signaling in these cells (Fig. 4A). The L3.6pl cells were treated with HGF in the presence or absence of a human v6 peptide of SEQ ID No.: 2 or SEQ ID No.:6 and Met activation and downstream signaling to ERK was measured. Phosphorylation of both Met and ERK was inhibited by the human v6 peptide. Treatment with a corresponding rat v6 peptide (ctrl peptide, SEQ ID No.: 10 or 11) had no influence on receptor activation and signal transduction (Fig. 4A).

To test the effect of the peptide on tumor growth and metastasis formation the L3.6pl cells were orthotopically injected into the pancreas of immunosuppressed mice (NCI-nu). These mice developed primary tumors and showed numerous metastases in the liver after four weeks. The primary tumor expresses a high amount of CD44v6 (Fig. 4B). Intraperitoneal injection (i.p.) of the human-specific peptide three times per

week for three weeks completely repressed Met activation as shown by the immunofluorescence staining for phospho-Met and Met in the tumor (Fig. 4C).

These data demonstrate that the peptide interferes with the co-receptor function of CD44v6 for Met and abrogates Met phosphorylation *in vivo*. Since the activation of Met is species-specific and the human Met receptor can only be activated by human HGF this result suggests that the L3.6pl cells produce their own HGF. Interestingly, in contrast to the rat system the outgrowth of the primary tumor in the pancreas was strongly repressed by the human v6 peptide (Fig. 4D top). The outgrowth of the primary tumor in the pancreas was also strongly repressed (Fig. 4D bottom). The rat-specific peptide (ctrl peptide) had neither any influence on metastasis formation nor on growth of the primary tumor (Fig. 4D). This result suggests that the L3.6pl cells produce their own HGF. Indeed, human HGF in a concentration of 3375 pg/ml was detected in the supernatant (see Material and Methods) after 5 days of culture (data not shown).

It was observed that the outgrowth of the primary tumor was retarded upon inhibition of tumor angiogenesis with a murine-specific v6 peptide. Here it is shown that the treatment with the human peptide also decreased angiogenesis as exemplified by a reduced vessel number and vessel size in the v6 peptide-treated tumors. The decreased angiogenesis explains the reduced tumor volume (Figure 4D, E). Since the v6 peptides act in a species-specific manner, the inhibition of angiogenesis cannot be due to an effect on the murine endothelial cells but rather to an inhibition of VEGF production by human pancreatic carcinoma cells. Indeed, the human L3.6pl cells produce hVEGF, a secretion that can be blocked by treatment with the human v6 peptide (Figure 4F). The most striking observation was that the human-specific peptide completely inhibited metastasis formation within the liver (Figure 4G). The rat-specific peptide (ctrl peptide) had no influence on metastasis formation or on growth of the primary tumor (Figure 4C-G).

Then, the specificity of binding of the human CD44v6 peptide to the tumor *in vivo*

was examined using the small animal imaging system Pearl Impulse. The human labeled peptide but not the rat peptide inhibited Met activation in L3.6pl cells (Fig. 5A right side) and bound to these cells in cell culture (Fig. 5A left side). In vivo the fluorescently labeled (DY 681) human peptide accumulated specifically in the tumor induced by orthotopically injected L3.6pl cells (Fig. 5B). No fluorescence was detected in animals that were treated with a control peptide. Furthermore, no signal was observed in animals that received no peptide whether or not they had developed a tumor (Fig. 5B). Ex vivo analysis of organs isolated from animals treated with the labeled human v6 peptide allowed detection of binding not only to primary tumors but also to areas of the liver most probably corresponding to metastases (Fig. 5C). Thus these experiments reveal that the CD44v6 peptides not only target the primary tumors but also distant metastases.

2.4 Already established metastases are eliminated by CD44v6 peptides

The specific binding of the CD44v6 peptides to specific areas of the lungs in the rat system and of the liver in the human model raises the question whether these peptides might have an effect on already established metastases. In the experiments described in Figs. 2 and 4 the peptides were applied early after injection of the tumor cells and repressed completely the establishment of metastases. In order to measure the effect of the CD44v6 peptide on already established metastases, other experimental settings were used. BSp73ASs6 cells were injected subcutaneously into syngeneic rats and L3.6pl cells orthotopically into male nude mice and allowed to grow for 3 weeks. At that time all animals from the control group had developed metastases (Table 3) suggesting that this was also the case in the groups used for peptide treatment. The animals were then treated either with the species-specific CD44v6 peptide or with the control peptide (mouse for the rat system and rat for the human system) twice per week for another three weeks (Scheme in Fig. 6A) and then tested for the presence of metastases. At the end of the experiments metastases could be detected in all animals treated with the control peptide (Table 3, Fig. 6B for the BSp73ASs6 cells, Table 3, Fig. 6C for L3.6pl cells) whereas animals treated with the

species-specific CD44v6 peptides were free of metastasis. Thus, already established metastases are eliminated by treatment with the CD44v6 peptides.

One hypothesis for the disappearance of metastases is that the peptides induce
5 apoptosis of CD44v6-expressing metastatic cells in the secondary organs. In order to
test this hypothesis, the experiment described above using the BSp73ASs6 cells was
repeated. The cells were injected subcutaneously in syngeneic rats and the tumor was
allowed to develop for three weeks. After that time the animals were separated in two
groups of 10 animals that were injected either with the specific rat CD44v6 peptide
10 or with the control peptide (mouse) every second day. One day following each
injection of the peptide, the lungs of one animal were removed and apoptosis was
measured using an antibody that detects the cleaved form of Caspase 3. Apoptotic
cells were detected as early as three days after the first injection of the peptides in the
tumor areas of animals treated with the CD44v6 peptide. No apoptosis at all was
15 detected in the animals injected with the control peptide (Fig. 7). A maximum of
apoptosis was measured eight days after the first injection of the CD44v6 peptides.
Twelve days after injection of the CD44v6 peptide, less apoptosis was observed. At
day twenty-two, no metastasis could be detected anymore in animals treated with the
CD44v6 peptide whereas metastatic spreading was evident in the lungs of control
20 animals (Fig. 7). From these experiments, it is concluded that the CD44v6 peptide
induces apoptosis of metastatic cells.

To discriminate whether the intrinsic pathway characterized by the release of
mitochondrial proteins or the extrinsic pathway activated by ligand-bound death
25 receptors for apoptosis was induced by the v6 peptide the expression of cleaved
caspase-8 in the metastases treated with the v6 peptide was investigated. A kinetic of
activation of caspase-8 similar to the one obtained for caspase-3 in the lungs of the
v6 peptide treated animals was observed. From these experiments we conclude that
the CD44v6 peptide induces apoptosis of metastatic cells via the extrinsic apoptotic
30 pathway.

Example 2 – pegylated CD44v6 peptides inhibit HGF dependent CD44v6 mediated signaling

5 1. Material and Methods

1.1 Synthesis of pegylated peptides

Peptide synthesis was performed on an Applied Biosystems automated peptide synthesizer (model 433A) and the peptides were purified by preparative HPLC.

10 Peptides of sequences NEWQG (SEQ ID No.: 11) and a control peptide NAAAG (SEQ ID No.: 15) were synthesized. Crude and purified products were characterized by LC coupled to a mass spectrometer (μ TOF LCMS from Bruker Daltonics-Bremen, Germany).

15 Peptide synthesis was performed using standard Fmoc solid phase peptide synthesis protocols (see e.g. Fields et al., *Int J Pept Protein Res.* (1990), 35, 161-214, Maisch et al., *J Am Chem Soc.* (2009), 131, 15596-15597, Strandberg et al., *Biophys J.* (2006), 90, 1676-1686, and Wadhwani et al., *J Org Chem.* (2006), 71, 55-61). Fmoc deprotection was done with 20-22% piperidine in NMP. Coupling was performed
20 using a mixture of Fmoc-amino acid:HOBt: HBTU:DIEA (4:4:3.9:8) in DMF. Peptides were cleaved from the solid support using a mixture of TFA:H₂O:TIS (93.5:2.5:4). The cleavage reagents were removed under N₂-flow, and the labeled peptides were precipitated using diethyl ether.

25 Preparative HPLC was performed on a reversed phase C18 column (4.6 mm x 240 mm) at 35 °C using a Jasco-HPLC system (Tokyo, Japan) fitted with a diode array detector. Epimeric peptides were separated using acetonitrile/water gradients 0.1% TFA.

30 All amino acids were purchased from Novabiochem (Schwalbach, Germany). The

MeO-PEG-COOH (molecular weight 750 Da and 2000 Da) and the coupling reagents (HBTU, HCTU) and DIEA were from Iris Biotech (Marktredwitz, Germany), and solvents and other reagents were from VWR-Merck (Bruchsal, Germany).

5

The following compounds were synthesized:

PEG-control peptide: PEG-NAAAG,

PEG-rat CD44v6 peptide: PEG-NEWQG

Palmitic-NH-PEG-CONH-control peptide

10 Palmitic-NH-PEG-CONH-rat CD44V6 peptide

Palmitic-NH-PEG-CONH-control peptide and Palmitic-NH-PEG-CONH-rat-CD44V6 peptide were synthesized by first coupling Fmoc-NH-PEG-COOH molecular weight 3000 Da to either SEQ ID No.: 11 or SEQ ID No.: 15. Then the

15

pegylated peptides were reacted with palmitic acid.

1.2 Activation Assays

The PEG-control peptide and PEG-rat CD44v6 peptide were compared for their ability to block HGF induced activation of the receptor tyrosine kinase Met and

20

ERK.

Rat pancreatic cancer cells BSp73ASs6 (also designated Ass6) were incubated for 10 min at 37°C with either ctrl-peptides (SEQ ID No.: 15), non-pegylated v6-peptides (SEQ ID No.: 11) and increasing concentrations of the pegylated peptides, namely

25

PEG-rat control peptide and PEG-rat CD44v6 peptide, before induction with HGF (10ng/ml; 5min at 37°C).

Activation of ERK and Met was determined by standard SDS and Western Blotting techniques using a phospho-Met and phospho-Erk specific antibody (phospho-Met clone D16 cell signaling, phospho-Erk p42/44 cell signaling)

30

1.3 Cellular Assays

A scatter assay was performed additionally. HT29 colon cancer cells were grown in medium containing 10% FCS. One day after seeding, the cells were starved. On the third day, the cells were induced with HGF (10 ng/ml) and pre-incubated with either no peptide, ctrl-peptides (SEQ ID No.: 15), non-pegylated v6-peptides (SEQ ID No.: 11) or the pegylated peptides, namely PEG-rat control peptide and PEG-rat CD44v6 peptide.

2. Results

Fig. 9 and 10 show that pegylated peptides are efficient in inhibiting HGF dependent activation of Met and ERK. Inhibition of ERK is even more efficient with pegylated vs. non-pegylated peptides.

Fig. 11 shows that pegylated as well non-pegylated peptides, but not control peptides lead to dissociation and migration of cells away from these cell cluster

Example 3 – in vitro results

Linear CD44v6 peptides described herein

rat: 14mer KEKWFENEWQGKNP, 5mer NEWQG, DY681labeled 11mer WFENEWQGKNP

human: 14mer KEQWFGNRWHEGYR, 5mer NRWHE, DY681labeled 11mer WFGNRWHEGYR

mouse: DY681labeled 11mer WFQNGWQGKNP

1. In vitro inhibition of RTKs using the human v6 peptides

Linear peptide (human, 14mer KEQWFGNRWHEGYR, 5mer NRWHE)

Cell lines used

- HT29** (colorectal cancer)
- 5 **HeLa** (cervical cancer)
- HUVEC** (human umbilical vein endothelial cells)
- HCMEC** (human cardiac microvascular EC)
- HAOEC** (human aortic EC)
- L3.6pl** (human pancreatic carcinoma cell)
- 10 **MCF7** (human breast cancer)

1.1 Endothelial cells

In order to analyse whether CD44v6 acts as a co-receptor for Met in angiogenesis, several primary ECs isolated from different kinds of human blood vessels were used.

15

ECs can differ in shape, function and in the type of cell-cell junctions depending on the blood vessel where they are localised. ECs in arteries (HAOECs (**human aortic endothelial cells**)) are subjected to high pressure compared to ECs in veins (HUVECs (**human umbilical vein endothelial cells**)). In capillaries major exchange of gases and nutrients takes place. There the ECs (e.g. HCMECs (**human cardiac microvascular endothelial cells**)) are the only barrier to control this exchange.

20

When these ECs were treated with HGF, the receptor tyrosine kinase Met was phosphorylated and signaling to Erk – a downstream target of Met – was promoted (Figure 12). The contribution of CD44v6 to Met signaling in these cells was analysed using different tools. Both, an antibody recognising the exon v6 encoded sequence (referred to as α CD44v6) and a peptide composed of 14 amino acids originating from the v6 region (referred to as v6 peptide) (Matzke et al. 2005), were able to prevent activation of Met and of its downstream target Erk when added to the cells before induction with HGF (see Figure 12). As incubation with a control antibody

30

and a control peptide did not interfere with Met activation and signaling, these results show that CD44v6 is required for Met activation in all types of ECs that were used.

- 5 In order investigate if an isoform of CD44 is necessary for VEGFR-2 activation, the three tools inhibiting CD44v6 activity were used in an experiment where VEGFR-2 was activated by VEGF-A165. As expected all ECs responded to VEGF-A165 treatment with VEGFR-2 phosphorylation and signal transduction to Erk. The antibody recognising CD44v6 as well as the CD44v6 14mer blocked VEGFR-2 activation and downstream signaling to Erk induced by VEGF-A165 (Figure 13).
10 Thus CD44v6 is also necessary for a functional VEGF-A165/VEGFR-2 system in these cells.

1.2 Epithelial cells

- The contribution of CD44v6 to Met signaling in the human epithelial pancreatic cancer cells L3.6pl (derived from Colo 357), the cervix carcinoma cells HeLa and the colon cancer cells HT29 were analysed using the CD44v6 peptide (v6 14mer; v6 5mer tested as well, data not shown). It was able to prevent activation of Met and of its downstream target Erk when added to the cells before induction with HGF (Figures 14A, B and C). As incubation with a control peptide did not interfere with
20 Met activation and signaling, these results show that CD44v6 is required for Met activation in all this cell lines.

- Besides VEGFR and Met the dependency of ErbB1 on CD44v6 was investigated. EGF as well as five other ligands, namely AR, BC, ER, HB-EGF, and TGF- α , can activate the ErbB1 receptor. EGF, AR and TGF- α bind to either an ErbB1-homo- or
25 an ErbB1/2-heterodimer. In addition to these dimers, BC, ER and HB-EGF can also bind to an Erb4/4 homo- or an ErbB2/4 heterodimer. Here it was investigated whether the activation of the ErbB receptors by the indicated ligands is also dependent on CD44v6.

For this purpose HT29 cells were serum-starved for 24 hours and pre-treated with the v6-specific or a control peptide. They were then induced with the indicated ErbB ligands. Erk-kinase phosphorylation was used as a read-out for receptor activation. All ligands could activate the Erk-kinase. EGF- as well as ER-dependent Erk phosphorylation was inhibited by pre-incubation of HT29 cells with the CD44v6 peptide (human v6 14mer) whereas a control peptide showed no effect (Figure 15). This shows that, besides EGF, ER is another ErbB1 ligand that is dependent on CD44v6 as a co-receptor. In contrast, AR, BC, HB-EGF and TGF- α -induced activation of the ErbB receptors could not be inhibited by blocking CD44v6 (Figure 15). These ligands are independent of CD44v6 for their induction of the ErbB receptors. This is especially striking, since EGF and TGF- α address the same receptor pairs (ErbB1/1 or ErbB1/2). These data suggest that the specific CD44 isoform used as a co-receptor for ErbB-activation is determined by the ligand that activates the ErbB receptors and not by the receptor proteins themselves.

15 In MCF7 the CD44v6 specific peptides led to the blocking of Erk phosphorylation upon EGF treatment but not upon TGF α induction. Both peptides, the 5mer and 14mer, showed similar effects. The control peptides had no effect on Erk phosphorylation (Figure 16).

2. Cell assays

2.1 Scatter assay

The receptor tyrosine kinases Met and Ron induce cell migration and scattering upon treatment with their ligands. The effect of v6- blocking peptides on scattering and migration was investigated. The CD44v6 peptides (a human v6 5mer, 10mer and 14mer) prevented completely scattering or invasion through the extracellular matrix (Figure 17).

2.2 Scratch assay

In this assay the requirement for CD44v6 for cell migration was tested. In a scratch closure assay migration of either vein or artery ECs was tested. These cells were grown to confluence and a scratch was made into the confluent monolayer using a sterile pipette-tip. The cells lining the scratch border started to migrate into this empty space in order to close the gap. In presence of either HGF, VEGF-A165, or VEGF-A121 the cells migrated faster than in absence of the ligands and the scratch was closed within one day. The EC migration was reduced to basal levels when the CD44v6 antibody or the CD44v6 peptide (human v6 14mer) interfered with receptor activation (Figure 18).

2.3 Angiogenesis assays

2.3.1 Sprouting assay

Clusters of HUVEC cells, so-called spheroids, were generated in hanging drops and embedded in a three-dimensional collagen matrix. They were overlaid with medium containing the blocking substances and the growth factors. Activated by the growth factors VEGF-A165 and VEGF-A121 the ECs started to grow out of the spheroids (Figure 19). This ability to sprout in response to VEGF-A treatment was blocked completely when the cells were treated with the v6 peptide (human v6 14mer). The control peptide did not influence VEGF-A-induced sprouting. Sprouting of the ECs was quantified by analysing the total length of all sprouts originating from one spheroid.

2.3.2 Matrigel/fibrin plugs

Langerhans islets are able to stimulate an angiogenic response when co-cultured with HUVEC cells in a collagen matrix. Such angiogenic hyperplastic islets of Langerhans were isolated by collagenase perfusion. They produce a mixture of angiogenic factors and thereby attract the ECs to get organised in capillary like structures. The angiogenic response of the ECs was reduced when the human CD44v6 of the ECs was blocked by means of the CD44v6 antibody or the v6 specific blocking peptide (human v6 14mer), whereas the control peptide did not interfere

with the angiogenic response. In agreement with published data around 60 % of islets stimulated an angiogenic response in the control experiment (Figure 20).

Example 4

5

1. Dose reduction and increase by linear peptides and PEGylated v6 5mers
PEGylation, the process of covalent attachment of **P**oly**E**thylene**G**lycol polymer chains, is known to reduce immunogenicity and antigenicity. PEGylation increases the hydrodynamic size of the compound and prolongs the circulation time in animals
10 by reducing renal clearance.

The three CD44v6 specific compounds (v6 5mer, PEG840- v6 5mer und PEG2000- v6 5mer of human origin) and a control peptide were applied in three concentrations (2 µg, 20 µg, 200 µg per injection) three times per week i.p. for 4 weeks. One week
15 before start of the treatment the L3.6pl tumor cells were orthotopically implanted into nude mice. The results showed that PEGylation has no impact on the effect of the peptides. All v6 specific compounds prevented metastatic spreading of tumor cells and lead to a reduction of primary tumor growth. A complete block of metastasis was observed for 20 µg per injection, however inhibitory effects were
20 already observed using a tenfold lower concentration (Figure 21). Furthermore an increase of the treatment dose to 200 µg per injection did not lead to side effects in the animals.

2. Results for reducing the dose of the linear v6 14mer and a cyclic v6 8mer
25 In this experiment a reduction of the dose of peptides was investigated. The treatment cycle of three injections per week was maintained. Furthermore a cyclic v6 8mer based on the linear sequence containing the three important amino acids that are required for the co-receptor function was tested in vivo for its inhibitory effect. In vitro data showed already blocking of Met and Erk activation in phosphorylation-
30 and cellular assays by all peptides.

Modification of linear peptides by cyclization is achieved by linking the amino and carboxyl termini with a peptide bond that forms a ring. Cyclization is an efficient way to increase the stability of peptides and to improve permeability.

5

Animals were implanted orthotopically with L3.6pl cells. Tumor growth was allowed for one week before the treatment with the linear v6 14mer, the cyclic v6 8mer as well as the control peptide (rat v6 14mer) was started. All peptides were injected three times per week at a concentration of 0.2, 2 and 10 µg per injection for 3 weeks.

10 At the end of the treatment animals were sacrificed.

All animals of the control group showed macroscopic metastases. Animals that were injected with 0.2 and 2 µg of v6 14mer showed liver metastases but the average number of metastases per animal was reduced. A dose of 10 µg per injection led to a complete inhibition of metastatic spreading (Figure 22A, B). Furthermore the treatment with the v6 14mer led to a significant decrease of the size of the primary tumor as compared to the control groups (Figure 22C).

15

The cyclic v6 8mer blocked metastatic spreading at 2 and 20 µg per injection very efficiently, indicating that the structural change by cyclization did not lead to loss of function of the peptide (Figure 22A, B). The cyclic v6 8mer showed also a drastic effect on growth of the primary tumor (Figure 22C).

20

3. Side-by-side comparison of different CD44v6 peptide derivatives in the L3.6pl orthotopic Xenograft model

25

A comparison of different CD44v6 peptide derivatives was performed in animals bearing a orthotopic L3.6pl tumor. 10 groups of 5 animals each received a dose of 20 µg peptide per injection three times per week for 3 weeks with the compounds listed in Table 4. Three weeks after starting the treatment the animals were sacrificed and analyzed for macroscopic metastases and tumor volume.

30

Histological analysis for micrometastasis (H&E staining) and immunohistochemical analysis for tumor vascularization (anti-CD31 staining) and activation of different RTKs in the tumor is still ongoing.

5

peptide	sequence
control (14mer rat)	H-KEKWFENEWQGANP-OH
PEG840 14-mer	PEG840-KEQWFGNRWHEGYR-OH
PEG2000 14-mer	PEG2000-KEQWFGNRWHEGYR-OH
Myristoyl-14-mer	Myr-KEQWFGNRWHEGYR
14-mer (human)	H-KEQWFGNRWHEGYR-OH
DOTA CD44v6-14 [daa ₇]	DOTA-keqwfGNRWHEGYr
DOTA CD44v6-14 [r ¹⁴]	DOTA-KEQWFGNRWHEGYR
cycl. 8mer	cy[KGNRWHEG]
cycl 5mer	DOTA-cy[KRWHE]
Myr-Y-cycl 8mer	Myr-Y-cy[KGNRWHEG]

10

Table 4: overview of tested peptide derivatives

Animals treated with the CD44v6 14mer, the PEGylated 14mer (PEG 840 and PEG 2000), 14mers containing D-amino acid modifications as well as cyclic peptides (including a myristoylated cyclic peptide) showed a significant reduction in tumor growth compared to the control peptide. The myristoylated linear 14mer showed no inhibitory effect on tumor growth. The strongest reduction of tumor size was achieved with the PEG2000 14mer, the v6 14mer, the cyclic 8mer and the cyclic 5mer (Figure 23A) compared to the control animals (note here the strong visible vascularization of the primary tumor (Figure 24A)). These four compounds also showed a low variability in the individual tumor size within the group (Figure 23B).

Analysis of the liver showed a decrease of metastatic spreading for PEG840-14mer, PEG2000-14mer v6-14 [daa7] and the myristoylated cyclic 8mer. A complete inhibition of metastasis was observed when animals were treated with the v6 14mer, the v6-14 [r14], the cyclic 8mer and the cyclic 5mer (Figure 24A, B).

This side-by-side comparison confirmed the efficacy of the v6 14mer but revealed the v6-14 [r14], the cyclic 8mer and the cyclic 5mer as promising modifications of the linear peptide sequence that might show an increased in vivo stability.

5 4. Evaluation for other cancer types

4.1 Colon cancer

4.1.1 Background

10 Mutation and inactivation of the adenomatous polyposis coli (Apc) tumor suppressor gene is an early event in the development of colorectal cancer. Inactivation of Apc occurs in approximately 80% of adenomatous polyps and it is also responsible for the genetic disorder familial adenomatous polyposis (FAP), which leads to the formation of adenomatous polyps in the small and large intestine that results in colon cancer. In order to study this hereditary disease the APC/Min/+ mouse model was generated.

15 These genetically manipulated mice can develop up to 100 polyps in the small intestine in addition to colon tumors. Crosses of these mice with CD44 knockout mice reduced the number of adenomas drastically, suggesting a role of CD44 in colorectal carcinogenesis. This could be further confirmed by the elimination of CD44v6 specifically in the intestine of APC/Min/+ mice by shRNA that also resulted

20 in a reduction of adenomas.

4.1.2 Preliminary data

The effect of the v6 14mer was tested in the the APC/Min/+ model model using 18 week old mice. The animals received an i.p. injection of 20 µg of v6 14mer

25 (mouse 14mer) or control peptide (rat 14mer) three times per week for 6 weeks. Animals (three per group) were sacrificed at the end of the experiment and the duodenum was analyzed for the total number of tumors (Figure 25).

The treatment with the v6 14mer led to a strong decrease of the tumor size in the

30 duodenum. Of note is that this decrease is more pronounced in case of small tumors

(0-1mm>1-2mm>2-5mm). The tumor number for tumors bigger than 5 mm is higher for the v6 14mer.

These data reveal a contribution of CD44 in tumor initiation.

5

4.2 Breast Cancer

4.2.1 Background

Different members of the ErbB family of RTKs are either overexpressed or mutated in a wide variety of cancers. For example, high ErbB1 expression was found in a majority of carcinomas including mammary carcinomas and amplification of the ErbB2 gene can be found in 20-30% of metastatic breast cancer lesions. The tumorigenicity of these breast cancer types depends on the constitutive activation of the ErbB receptors.

It was shown that CD44v6 enables the activation of the ErbB receptors through EGF in several breast cancer cell lines. CD44v6 could therefore play a role in breast cancer types that are dependent on EGF-based ErbB activation for their tumorigenicity. And indeed, the evaluation of 100 primary invasive breast tumors and 18 lymph node metastases connected the expression of CD44v6 with tumorigenicity. The presence of these CD44 variants correlates with poor survival. Two more recent studies came to similar results.

However, there are conflicting data concerning the role of CD44v6 in breast cancer. A study, evaluating 109 patients with stage II breast neoplasia for example did not associate CD44v6 expression with disease free survival or overall survival at all. An explanation for the conflicting studies could be that CD44v6 does not act as a co-receptor for all breast cancer types. In cancer types where the activation of the ErbB-receptors is mediated by TGF- α for example, CD44v6 would not be needed as a co-receptor as it could be shown that activation via TGF- α is independent of CD44v6.

30

4.2.2 Effect of the CD44v6 14mer

The effect of the CD44v6 14mer was tested in a syngeneic mouse model by orthotopic injection of the murine mammary carcinoma cells 4T1. 4T1 cells were originally derived from a spontaneously arising BALB/c mammary tumor. When
5 introduced orthotopically, 4T1 cells grow rapidly at the primary site and form metastases in the lung and the lymph nodes within a period of 3-6 weeks. The tumor growth and metastatic spreading of 4T1 cells in BALB/c mice very closely mimics human breast cancer stage IV.

- 10 The 4T1 cells were injected orthotopically into the 6th mammary fat pad. Two groups of 5 animals each with the implanted tumor cells were then used for the experiment. One group was treated with the v6 14mer peptide, one with a control (rat) peptide. The injection of the peptide was performed 3 times per week and planned for three to four weeks but had to be stopped after two weeks as the control
15 group suffered from large tumors.

- Analysis of the primary tumors revealed a significant decrease of tumor size in the v6 14mer treated group (Figure 26A). Immunofluorescent analysis (anti-CD34 staining) to monitor tumor vascularization showed that in the v6 14mer treated group
20 the total number of blood vessels was significantly reduced (Figure 26B). A difference between the v6 14mer and the control peptide treated animals could also be seen in the case of metastatic spreading. The macroscopic analysis of the lungs revealed that all mice treated with the control peptide developed metastases. The average number of metastases was 6 metastases per lung. In the v6 peptide treated
25 group three animals showed no macroscopic metastases at all, whereas two animals had only one metastase. Therefore, the treatment with the v6 peptide drastically reduces the formation of metastases in the lung (Figure 26C).

In conclusion the preliminary data for colon cancer and for breast cancer revealed, that the v6 14mer is as well a promising tool for other indications than pancreatic cancer.

- 5 Example 5 - CD44v6 peptide functionalized nanoparticles selectively bind on the tumor cells

Material and Methods

- 10 The human adenocarcinoma cell line HT29, a gift of A. Zweibaum (Institut National de la Sante et de la Recherche Medicale, France) was grown in DMEM (Invitrogen, Karlsruhe, Germany) supplemented with 10% FCS (PAA, Cölbe, Germany). The rat pancreatic carcinoma cells BSp73AS10 (AS10) and its transfectant BSp73ASs6 (ASs6) (paper vero) were grown in RPMI (Invitrogen) plus 10% FCS. Hydrophobic
15 CdTe quantum dots with emission at 550 nm (particles molar weight is 32000 g/mol) were obtained from PlasmaChem GmbH (Germany). Alpha-(9-fluorenylmethyloxy-carbonyl)amino-omega-carboxy poly(ethylene glycol) (PEG-MW 3.000 Dalton) and alpha-methoxy-omega-carboxylic acid poly(ethylene glycol) (PEG-MW 2.000 Dalton) were obtained from IRIS Biotech GmbH (Germany). Palmitic acid (99%)
20 and poly(ethylene glycol) methyl ether (MW 2000 g/mol) were obtained from Sigma-Aldrich (Germany).

Instrumentation

- 25 Dynamic light scattering was performed on a Malvern Zetasizer Nano ZS (Malvern, Germany). Quantum dots nanoparticles were diluted with Milli Q water to the concentration of quantum dots at 0.1 mg/ml. 750 µl nanoparticle solution were added in the cuvette, and measured particle size (hydrodynamic diameter) and surface charge (zeta potential).

Quantum dots nanoparticles were characterized by scanning electron microscopy (SEM) using the LEO 1530 Gemini scanning electron microscope (Zeiss, Germany). Samples for SEM were prepared by resuspending the particles in Milli Q water at a concentration of 0.1 mg/ml. Resulting dispersion was dripped onto a silicon wafer and dried in air. The particles were then sputter coated with gold for 20 s using a Cressington 108 auto sputter coater before SEM measurement.

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry was performed on a 4800 MALDI-TOF/TOF mass spectrometer (Applied Biosystems, Framingham, MA, USA). Peak lists were generated using Data Explorer Software 4.0 (Applied Biosystems). 2,5-Dihydroxybenzoic acid (DHB, 10 mg in 1 ml tetrahydrofuran containing 0.1% trifluoroacetic acid) was used as a matrix for PEGylated peptides and peptide-PEG-palmitic amide. 10 µl PEGylated peptides and peptide-PEG-palmitic amide (1 mg in 1 ml Milli Q) were mixed with 10 µl matrix, and 1 µl mixed solution was spotted on a stainless steel MALDI carrier.

Preparation of Nanoparticles

Different density of CD44v6 peptide functionalized quantum dots nanoparticles were prepared as follows: (a) PEG-QDs were prepared by mixing 300 µl PEG2000-palmitic ester (4 mg/ml in chloroform) and 30 µl QD (10 mg/ml in chloroform) in 4 ml vials. After vortex for 20 s, the mixed solution was in the open vial for overnight to make sure the chloroform was evaporated. 300 µl PBS buffer (pH 7.4) were added to the 4 ml vial, and vortex for 30 s to form nanoparticles. (b) 10% v6-PEG-QDs were prepared by mixing 270 µl PEG2000-palmitic ester (4 mg/ml in chloroform), 120 µl v6-PEG-palmitic amide (0.5 mg/ml in chloroform) and 30 µl QD (10 mg/ml in chloroform) in 4 ml vials. After vortex for 20 s, the mixed solution was in the open vial for overnight to make sure the chloroform was evaporated. 300 µl PBS buffer (pH 7.4) were added to the 4 ml vial, and vortex for 30 to form nanoparticles. (c) 30% v6-PEG-QDs were prepared by mixing 210 µl PEG2000-

palmitic ester (4 mg/ml in chloroform), 360 μ l v6-PEG-palmitic amide (0.5 mg/ml in chloroform) and 30 μ l QD (10 mg/ml in chloroform) in 4 ml vials. After vortex for 20 s, the mixed solution was in the open vial for overnight to make sure the chloroform was evaporated. 300 μ l PBS buffer (pH 7.4) were added to the 4 ml vial, and vortex for 30 to form nanoparticles.

Western blotting and activation of Met and Erk

ASs6 cells, serum starved for 24 h, were induced with HGF (10 ng/ml) at 37°C for 5 min. Where indicated, the cells were treated with PEGylated or non-PEGylated peptides before induction at 37°C for 10 min (150 nM). Cells were washed with phosphate-buffered saline (PBS) and lysed in boiling sodium dodecyl sulfate (SDS)-sample buffer containing 100mM dithiothreitol (DTT). Subsequently, lysates were subjected to Western blot analysis using antibodies against phosphorylated Mek and Erk (Cell signaling). The Mek and Erk loading control were performed on the same blot, stripped (62.5 mM Tris pH 6.8, 2% SDS, 0.8% DTT) and probed with the Mek and Erk antibodies. Blots were stained using the enhanced chemiluminescence system (Thermo Fisher Scientific).

Scattering assay

Scattering of HT29 cells was determined in 24-well plates. First, 4×10^5 cells were seeded at 37°C; the cells were either left untreated or 48 h later treated with HGF (50 ng/ml) for 24 h or with PEGylated or non-PEGylated peptides (100 ng/ml) prior to HGF induction for 30 min.

Immunofluorescence assay

1×10^4 AS10 and ASs6 cells per well, were seeded on 8- well microscopy chamber slides for 24 h at 37°C. The cells were washed with ice-cold PBS, fixed with

methanol for 15 min at -20°C and again washed three times with PBS. Afterwards, cells were incubated with the indicated modified Quantum Dots for 1 h at room temperature in the dark. Subsequently, cells were stained with 4,6-diamidino-2-phenylindole dihydrochloride (DAPI) in PBS for 30 min at room temperature, washed three times with PBS and embedded with mounting medium. Fluorescence images were obtained using a Leica SPE confocal fluorescence microscope, 63x objective.

Fluorescence activated cell sorting (FACS)

10

ASs6 and AS10 cells cultured in 10 cm petri dishes were harvested at 80% confluency, with 5mM EDTA/PBS. Cells were washed three times with 3% FCS in PBS. Afterwards, cells were incubated with 100ng/ml Quantum Dots in 3% FCS/PBS for 10 minutes. Then, cells were washed 3 times with 3% FCS in PBS, resuspended in PBS without FCS and subjected to FACS measurement.

Results and discussion

Synthesis and characterization of CD44v6 peptides and PEGylated peptides

20

As described above, CD44v6-peptides (CD44v6-pep) can be used to block HGF induced activation of c-Met signaling in a variety of cancer cell lines. Furthermore, these peptides can efficiently block HGF induced cell scattering, a term that describes dissociation and migration of single cells away from cell clusters, in HT29 adenocarcinoma cells. These peptides can efficiently inhibit tumor growth and metastasis; and furthermore eliminate already developed metastases. Altogether, this makes these peptides very promising potential drug candidates for cancer therapy. As CD44v6-peptides specifically bind to CD44v6 expressing cells, these peptides were further functionalized on quantum dots (QDs) surfaces and to show the selective binding of these peptides to tumor cells.

PEGylation is a commonly used technique to increase the stability of peptides, proteins and nanoparticles in vivo. Here, the CD44v6 peptide was PEGylated specifically at the N-terminal. CD44v6 peptides and PEGylated peptides were sequentially synthesized on Wang resin, and purified by preparative-HPLC. The purity of CD44v6 peptides and PEGylated peptides were further analyzed and characterized by analytic-HPLC and MALDI-TOF (Fig. 27).

Blocking assay of CD44v6 peptides and PEGylated peptides

10

Also the blocking ability of the peptides was tested after attachment of a 2000 dalton PEG-chain. The rat pancreatic carcinoma cell line BSp73ASs6, that highly expresses CD44v6, was treated with PEGylated CD44v6 peptides and tested whether this treatment can block HGF induced activation of the RTK Met and its downstream signaling via the Erk pathway. As positive control, non-PEGylated CD44v6 peptides (A = produced by ourselves B= produced by Bachem) was used, as negative controls non-PEGylated or PEGylated control peptides were used where the 3 essential aa were replaced by alanines. Indeed, PEGylation did not affect the functionality of the CD44v6 peptides. Upon treatment of ASs6 cells with PEGylated CD44v6 peptides, HGF completely failed to induce Met-phosphorylation and to activate Erk signaling, whereas PEGylated control peptides showed no effect (Fig. 10). Also HGF induced scattering of HT29 cells was strongly inhibited by pretreatment with PEGylated CD44v6 peptides, but not with PEGylated control peptides (Fig. 11). These results clearly show that PEGylation does not affect the functionality of CD44v6 peptides.

25

Preparation and characterization of CD44v6 peptide functionalized nanoparticles

To functionalize the CD44v6 peptide on QDs, we synthesized the v6-PEG3000-palmitic amide sequentially using solid phase synthesis. The amphiphilic v6-

PEG3000-palmitic amide was further purified by preparative-HPLC and characterized by MALDI-TOF.

Binding assay of CD44v6 peptide functionalized nanoparticles

5

CD44v6 expression often correlates with advanced stages of cancer and goes hand in hand with a poor prognosis in a huge variety of cancer types. Hence, many malignant cancer cells express a high amount of CD44v6 on their surface, designating CD44v6 as important marker to target these cells. Therefore the PEGylated CD44v6 peptides may be used to create multivalent composite nanoparticles that can be used to target and to visualize CD44v6 expression on cells. To this aim, PEGylated CD44v6 peptides were conjugated to Quantum Dots (QDs), small semiconductor nanoparticles, that can be used as bright shining and very stable fluorescent probes. A big advantage of QDs is their multivalency that allows to couple PEGylated CD44v6 peptides in different concentrations to the surface of the Quantum Dots. 3 different types of nanoparticles, one with only PEG chains attached to the Quantum Dots (PEG-QDs) and nanoparticles where 10% and 30% of PEG chains surrounding the Quantum Dots were coupled to CD44v6 peptides (designated as 10%-v6pep-QDs and 30%-v6pep-PEG, respectively) were created. It was tested whether these Quantum Dots can specifically bind to and stain CD44v6 expressing cells and if the CD44v6 peptide concentration influences the binding ability.

We incubated CD44v6 expressing BSp73ASs6 cells (ASs6) or CD44v6 negative BSp73AS10 cells (AS10) with the different Quantum Dots and visualized the staining via immunofluorescence microscopy. QDs surrounded only by PEG chains did neither stain ASs6 nor AS10 cells, excluding unspecific binding of PEGylated QDs to the cells (Fig. 28 A) panel 1). QDs that contain 10% of the PEG chains coupled to CD44v6 peptides also did not stain any of the cell types (Fig. 28 A) panel 2). However, if 30 % of the PEG chains at the QD surface are attached to CD44v6 peptides they could only be detected at the membrane of CD44v6 positive ASs6 cells

but not on CD44v6 negative AS10 cells, showing a specific staining of CD44v6 expressing cells (Fig. 28 A) panel 3). In contrast to this, 50%v6pep-QDs could be detected at the surface of both cell types, irrespective of their CD44v6 expression (data not shown).

5

These results were further analyzed and the percentage of Quantum Dots stained cells of several independent experiments (n=5) be quantified. PEG-QDs did not stain AS10 or ASs6 cells. 10%v6-QDs were found on the surface of only 10% of AS10 or ASs6 cells. However, 90% of ASs6 but only 15 % of AS10 cells were stained with 30%v6-QDs, showing a highly specific binding of these to CD44v6 expressing cells. To further prove the specificity of the 30%-v6pep-QDs AS10 cells were transfected with a mCherry expression vectors and ASs6 cells with expression vectors containing CFP and co-cultured both cell lines. These co-cultured cells were treated with 30%-v6pep-QDs and the staining was visualized by immunofluorescence microscopy. Indeed, 30%-v6pep-QDs only bound to CFP positive and CD44v6 expressing ASs6 cells. mCherry positive AS10 cells remained unstained (Fig. 28 A) panel 4).

15

In addition to immunofluorescence microscopy, the binding of the different CD44v6-peptide coupled Quantum Dots was analysed by fluorescence activated cell sorting analysis (FACS-analysis). According to the microscopy results, we could detect binding of 30%v6pep-QDs only on CD44v6 expressing ASs6 cells (Fig. 28 C). In summary, the data clearly show that PEGylation does not affect the functionality of CD44v6 peptides regarding their blocking effects on HGF induced Met-signaling and cell scattering. These PEGylated peptides can be coupled to Quantum Dots, which provide multifunctional nanoparticles that can be easily used as specific biomarkers to target and stain CD44v6 expressing cancer cells.

20

25

Conclusions

CD44v6 expression often correlates with advanced stages of cancer and bad prognosis. Furthermore, CD44v6 expression can confer metastatic potential to non-metastatic cancer cells, most likely due to its function as co-receptor for c-MET and VEGFR receptor tyrosine kinases. 3 amino acids are essential for the function of CD44v6, namely R-W-H, and small peptides of at least 5 amino acids in length, comprising these 3 amino acids can be used to efficiently block the function of CD44v6. Blocking of CD44v6 results in complete inhibition of HGF induced c-Met signaling or VEGF-A induced VEGFR-2 signaling and in turn in blocking of angiogenesis and metastasis in in vivo mouse and rat models for pancreatic cancer. In addition CD44v6 peptides bind to CD44v6-expressing cells, making it possible to use these peptides as a marker for malignant CD44v6 expressing tumor cells or tissues.

15

In this Example, CD44v6 peptides were PEGylated, the functionality of the PEGylated peptides on the molecular level were confirmed and the PEGylated peptides were coupled to Quantum Dots. These CD44v6-QD nanoparticles specifically bind to and stain CD44v6 expressing cells which was confirmed by immunofluorescence microscopy and FACS-analysis.

20

The coupling of the peptides to the Quantum Dots, has several advantages, on the one hand a bright shining and very stable biomarker for CD44v6 expressing cells was created, which can be used for the detection and better characterization of CD44v6 expressing tumor cells or tissues, or even further to detect CD44v6 expressing metastases in the patient. On the other hand, because of the multivalency there is the possibility to couple other compounds like conventional anti-cancer drugs to the CD44v6-QDs to create multifunctional drugs that specifically target CD44v6 expressing tumor cells.

30

Some of the embodiments described relate to:

1. A compound for use in treating metastasizing cancer in a human being,
5 wherein said compound comprises:
 - a peptide comprising at least the amino acid sequence X_1 -R-W-H- X_5 with X_1 being selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y and X_5 being selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y (SEQ ID No.: 1), or
10 a peptidomimetic thereof, or
 - a peptide comprising at least 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 amino acids of amino acid sequence X_1 - X_2 - X_3 - X_4 - X_5 - X_6 - X_7 -R-W-H- X_{11} - X_{12} - X_{13} - X_{14} , wherein X_1 , X_2 , X_3 , X_4 , X_5 , X_6 , X_7 , X_{11} , X_{12} , X_{13} , or X_{14} is selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or
15 Y (SEQ ID No.: 7), and wherein the peptide comprises at least X_7 -R-W-H- X_{11} of SEQ ID No.: 7, wherein X_7 , and X_{11} is selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y, or a peptidomimetic thereof.
- 20 2. A compound for use of embodiment 1,
wherein said compound comprises:
 - a peptide comprising at least the amino acid sequence X_1 -R-W-H- X_5 , wherein X_1 is selected from the group comprising amino acids with an NH_2 group such as K, R, N, or Q, or amino acids with non-polar side chains such as A,
25 V, L or I and wherein X_5 is selected from the group comprising amino acids with negatively charged side chains such as E or D, or amino acids with non-polar side chains such as A, V, L or I (SEQ ID No. 4) or a peptidomimetic thereof, or

- a peptide comprising at least 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 amino acids of amino acid sequence $X_1-X_2-X_3-X_4-X_5-X_6-X_7-R-W-H-X_{11}-X_{12}-X_{13}-X_{14}$, wherein X_1 is selected from the group comprising amino acids with an NH_2 group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I, wherein X_2 is selected from the group comprising amino acids with negatively charged side chains such as E or D, or amino acids with non-polar side chains such as A, V, L or I, wherein X_3 is selected from the group comprising amino acids with an NH_2 group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I, wherein X_4 is selected from the group comprising amino acids with non-polar or non-charged side changes and aromatic ring structures such as F, W, or Y, or amino acids with non-polar side chains such as A, V, L or I, wherein X_5 is selected from the group comprising amino acids with non-polar or non-charged side changes and aromatic rings structures such as F, W, or Y, or amino acids with non-polar side chains such as A, V, L or I, wherein X_6 is selected from the group comprising G or amino acids with non-polar side chains such as A, V, L or I, wherein X_7 is selected from the group comprising amino acids with an NH_2 group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I, wherein X_{11} is selected from the group comprising amino acids with negatively charged side chains such as E or D, or amino acids with non-polar side chains such as A, V, L or I, wherein X_{12} is selected from the group comprising G or amino acids with non-polar side chains such as A, V, L or I, wherein X_{13} is selected from the group comprising amino acids with non-polar or non-charged side chains and aromatic rings structures such F, W, or Y, or amino acids with non-polar side chains such as A, V, L or I, and wherein X_{14} is selected from the group comprising amino acids with an NH_2 group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I (SEQ ID No. 8), and wherein the peptide comprises at least $X_7-R-W-H-X_{11}$ of SEQ ID No.: 8, , wherein X_7 is selected from the group comprising amino acids with an NH_2

group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I and wherein X_{11} is selected from the group comprising amino acids with negatively charged side chains such as E or D, or amino acids with non-polar side chains such as A, V, L or I, or a peptidomimetic thereof.

5

3. A compound for use of embodiment 2,
wherein said compound comprises:

- a peptide comprising at least the amino acid sequence X_1 -R-W-H- X_5 , wherein X_1 is selected from the group comprising amino acids with an NH_2 group such as K, R, N, or Q, and wherein X_5 is selected from the group comprising amino acids with negatively charged side chains such as E or D, (SEQ ID No. 5) or a peptidomimetic thereof, or
- a peptide comprising at least 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 amino acids of amino acid sequence X_1 - X_2 - X_3 - X_4 - X_5 - X_6 - X_7 -R-W-H- X_{11} - X_{12} - X_{13} - X_{14} , wherein X_1 is selected from the group comprising amino acids with an NH_2 group such as K, R, N, or Q, wherein X_2 is selected from the group comprising amino acids with negatively charged side chains such as E or D, wherein X_3 is selected from the group comprising amino acids with an NH_2 group such as K, R, N, or Q, wherein X_4 is selected from the group comprising amino acids with non-polar or non-charged side changes and aromatic ring structures such as F, W, or Y, wherein X_5 is selected from the group comprising amino acids with non-polar or non-charged side changes and aromatic rings structures such as F, W, or Y, wherein X_6 is selected from the group comprising G or amino acids with non-polar side chains such as A, V, L or I, wherein X_7 is selected from the group comprising amino acids with an NH_2 group such as K, R, N, or Q, wherein X_{11} is selected from the group comprising amino acids with negatively charged side chains such as E or D, wherein X_{12} is selected from the group comprising G or amino acids with non-polar side chains such as A, V, L or I, wherein X_{13} is selected from the group comprising amino acids with non-polar or non-charged side chains and

- aromatic rings structures such F, W, or Y, and wherein X_{14} is selected from the group comprising amino acids with an NH_2 group such as K, R, N, or Q (SEQ ID No. 9), and wherein the peptide comprises at least X_7 -R-W-H- X_{11} of SEQ ID No.: 9, , wherein X_7 is selected from the group comprising amino acids with an NH_2 group such as K, R, N, or Q and wherein X_{11} is selected from the group comprising amino acids with negatively charged side chains such as E or D, or a peptidomimetic thereof.
- 5
4. A compound for use of embodiment 1, 2, or 3
- 10 wherein said compound comprises a peptide comprising, optionally consisting of, the amino acid sequence N-R-W-H-E (SEQ ID No.: 2), amino acid sequence K-E-Q-W-F-G-N-R-W-H-E-G-Y-R (SEQ ID No.: 6), or a peptidomimetic thereof.
5. A compound for use of embodiment 1, 2, 3, or 4,
- 15 wherein said compound is a modified form of said peptide or said peptidomimetic.
6. A compound for use of embodiment 5,
- 20 wherein said compound is a pegylated, hesylated, pasylated, myristoylated, glycosylated, and/or cyclic form of said peptide or peptidomimetic.
7. A compound for use of embodiment 1, 2, 3, 4, or 5,
- 25 wherein the compound is formulated for oral, nasal, or subcutaneous administration.
8. A pharmaceutical composition for use in treating metastasizing cancer in a human being,
- wherein said pharmaceutical composition comprises a compound comprising:
- a peptide comprising at least the amino acid sequence X_1 -R-W-H- X_5 with X_1
- 30 being selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N,

P, Q, R, S, T, V, W, or Y and X₅ being selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y (SEQ ID No.: 1), or a peptidomimetic thereof, or

- 5 • a peptide comprising at least 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 amino acids of amino acid sequence X₁-X₂-X₃-X₄-X₅-X₆-X₇-R-W-H-X₁₁-X₁₂-X₁₃-X₁₄, wherein X₁, X₂, X₃, X₄, X₅, X₆, X₇, X₁₁, X₁₂, X₁₃, or X₁₄ is selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y (SEQ ID No.: 7), and wherein the peptide comprises at least X₇-R-W-H-X₁₁ of SEQ ID No.: 7, wherein X₇, and X₁₁ is selected from the group
10 comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y, or a peptidomimetic thereof.

9. A pharmaceutical composition for use of embodiment 8,

wherein said pharmaceutical composition comprises a compound comprising:

- 15 • a peptide comprising at least the amino acid sequence X₁-R-W-H-X₅, wherein X₁ is selected from the group comprising amino acids with an NH₂ group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I and wherein X₅ is selected from the group comprising amino acids with negatively charged side chains such as E or D, or amino acids with non-
20 polar side chains such as A, V, L or I (SEQ ID No. 4) or a peptidomimetic thereof, or
- 25 • a peptide comprising at least 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 amino acids of amino acid sequence X₁-X₂-X₃-X₄-X₅-X₆-X₇-R-W-H-X₁₁-X₁₂-X₁₃-X₁₄, wherein X₁ is selected from the group comprising amino acids with an NH₂
30 group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I, wherein X₂ is selected from the group comprising amino acids with negatively charged side chains such as E or D, or amino acids with non-polar side chains such as A, V, L or I, wherein X₃ is selected from the group comprising amino acids with an NH₂ group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I, wherein X₄ is

selected from the group comprising amino acids with non-polar or non-charged side chains and aromatic ring structures such as F, W, or Y, or amino acids with non-polar side chains such as A, V, L or I, wherein X₅ is selected from the group comprising amino acids with non-polar or non-charged side chains and aromatic rings structures such as F, W, or Y, or amino acids with non-polar side chains such as A, V, L or I, wherein X₆ is selected from the group comprising G or amino acids with non-polar side chains such as A, V, L or I, wherein X₇ is selected from the group comprising amino acids with an NH₂ group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I, wherein X₁₁ is selected from the group comprising amino acids with negatively charged side chains such as E or D, or amino acids with non-polar side chains such as A, V, L or I, wherein X₁₂ is selected from the group comprising G or amino acids with non-polar side chains such as A, V, L or I, wherein X₁₃ is selected from the group comprising amino acids with non-polar or non-charged side chains and aromatic rings structures such as F, W, or Y, or amino acids with non-polar side chains such as A, V, L or I, and wherein X₁₄ is selected from the group comprising amino acids with an NH₂ group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I (SEQ ID No. 8), and wherein the peptide comprises at least X₇-R-W-H-X₁₁ of SEQ ID No.: 8, , wherein X₇ is selected from the group comprising amino acids with an NH₂ group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I and wherein X₁₁ is selected from the group comprising amino acids with negatively charged side chains such as E or D, or amino acids with non-polar side chains such as A, V, L or I, or a peptidomimetic thereof.

10. A pharmaceutical composition for use of embodiment 9,

wherein said pharmaceutical composition comprises a compound comprising:

- a peptide comprising at least the amino acid sequence X₁-R-W-H-X₅, wherein X₁ is selected from the group comprising amino acids with an NH₂ group

such as K, R, N, or Q, and wherein X₅ is selected from the group comprising amino acids with negatively charged side chains such as E or D, (SEQ ID No. 5) or a peptidomimetic thereof, or

- a peptide comprising at least 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 amino acids of amino acid sequence X₁-X₂-X₃-X₄-X₅-X₆-X₇-R-W-H-X₁₁-X₁₂-X₁₃-X₁₄, wherein X₁ is selected from the group comprising amino acids with an NH₂ group such as K, R, N, or Q, wherein X₂ is selected from the group comprising amino acids with negatively charged side chains such as E or D, wherein X₃ is selected from the group comprising amino acids with an NH₂ group such as K, R, N, or Q, wherein X₄ is selected from the group comprising amino acids with non-polar or non-charged side changes and aromatic ring structures such as F, W, or Y, wherein X₅ is selected from the group comprising amino acids with non-polar or non-charged side changes and aromatic rings structures such as F, W, or Y, wherein X₆ is selected from the group comprising G or amino acids with non-polar side chains such as A, V, L or I, wherein X₇ is selected from the group comprising amino acids with an NH₂ group such as K, R, N, or Q, wherein X₁₁ is selected from the group comprising amino acids with negatively charged side chains such as E or D, wherein X₁₂ is selected from the group comprising G or amino acids with non-polar side chains such as A, V, L or I, wherein X₁₃ is selected from the group comprising amino acids with non-polar or non-charged side chains and aromatic rings structures such F, W, or Y, and wherein X₁₄ is selected from the group comprising amino acids with an NH₂ group such as K, R, N, or Q (SEQ ID No. 9), and wherein the peptide comprises at least X₇-R-W-H-X₁₁ of SEQ ID No.: 9, wherein X₇ is selected from the group comprising amino acids with an NH₂ group such as K, R, N, or Q and wherein X₁₁ is selected from the group comprising amino acids with negatively charged side chains such as E or D, or a peptidomimetic thereof.

11. A pharmaceutical composition for use of embodiment 8, 9, or 10,

wherein said compound comprises a peptide comprising, optionally consisting of, amino acid sequence N-R-W-H-E (SEQ ID No.: 2), amino acid sequence K-E-Q-W-F-G-N-R-W-H-E-G-Y-R (SEQ ID No.: 6), or a peptidomimetic thereof.

- 5 12. A pharmaceutical composition for use of embodiment 8, 9, 10, or 11,
wherein said compound is a modified form of said peptide or said
peptidomimetic.
- 10 13. A pharmaceutical composition for use of embodiment 12,
wherein said compound is a pegylated, hesylated, pasylated, glycosylated,
myristoylated, and/or cyclic form of said peptide or peptidomimetic.
- 15 14. A pharmaceutical composition for use of embodiment 8, 9, 10, 11, 12, or 13,
wherein said pharmaceutical composition comprises a pharmaceutically
acceptable excipient.
- 20 15. A pharmaceutical composition for use of embodiment 8, 9, 10, 11, 12, 13 or 14,
wherein said pharmaceutical composition is formulated for oral, nasal, or
subcutaneous administration.
- 25 16. Use of a compound in the manufacture of a medicament for use in treating
metastasizing cancer in a human being,
wherein said compound comprises:
- a peptide comprising at least the amino acid sequence X₁-R-W-H-X₅ with X₁
being selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N,
P, Q, R, S, T, V, W, or Y and X₅ being selected from the group comprising A,
C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y (SEQ ID No.: 1), or
a peptidomimetic thereof, or
- 30 a peptide comprising at least 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 amino acids of
amino acid sequence X₁- X₂-X₃-X₄-X₅-X₆-X₇-R-W-H-X₁₁-X₁₂-X₁₃-X₁₄, wherein

X₁, X₂, X₃, X₄, X₅, X₆, X₇, X₁₁, X₁₂, X₁₃, or X₁₄ is selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y (SEQ ID No.: 7), and wherein the peptide comprises at least X₇-R-W-H-X₁₁ of SEQ ID No.: 7, wherein X₇, and X₁₁ is selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y, or a peptidomimetic thereof.

17. Use of a compound of embodiment 16,

wherein said compound comprises:

- a peptide comprising at least the amino acid sequence X₁-R-W-H-X₅, wherein X₁ is selected from the group comprising amino acids with an NH₂ group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I and wherein X₅ is selected from the group comprising amino acids with negatively charged side chains such as E or D, or amino acids with non-polar side chains such as A, V, L or I (SEQ ID No. 4) or a peptidomimetic thereof, or
- a peptide comprising at least 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 amino acids of amino acid sequence X₁-X₂-X₃-X₄-X₅-X₆-X₇-R-W-H-X₁₁-X₁₂-X₁₃-X₁₄, wherein X₁ is selected from the group comprising amino acids with an NH₂ group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I, wherein X₂ is selected from the group comprising amino acids with negatively charged side chains such as E or D, or amino acids with non-polar side chains such as A, V, L or I, wherein X₃ is selected from the group comprising amino acids with an NH₂ group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I, wherein X₄ is selected from the group comprising amino acids with non-polar or non-charged side changes and aromatic ring structures such as F, W, or Y, or amino acids with non-polar side chains such as A, V, L or I, wherein X₅ is selected from the group comprising amino acids with non-polar or non-charged side changes and aromatic rings structures such as F, W, or Y, or amino acids with non-polar side chains such as A, V, L or I, wherein X₆ is

5 selected from the group comprising G or amino acids with non-polar side chains such as A, V, L or I, wherein X₇ is selected from the group comprising amino acids with an NH₂ group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I, wherein X₁₁ is selected from the group comprising amino acids with negatively charged side chains such as E or D, or amino acids with non-polar side chains such as A, V, L or I, wherein X₁₂ is selected from the group comprising G or amino acids with non-polar side chains such as A, V, L or I, wherein X₁₃ is selected from the group comprising amino acids with non-polar or non-charged side chains and aromatic rings structures such F, W, or Y, or amino acids with non-polar side chains such as A, V, L or I, and wherein X₁₄ is selected from the group comprising amino acids with an NH₂ group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I (SEQ ID No. 8), and wherein the peptide comprises at least X₇-R-W-H-X₁₁ of SEQ ID No.: 8, ,
10
15 wherein X₇ is selected from the group comprising amino acids with an NH₂ group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I and wherein X₁₁ is selected from the group comprising amino acids with negatively charged side chains such as E or D, or amino acids with non-polar side chains such as A, V, L or I, or a peptidomimetic thereof.

20

18. Use of a compound of embodiment 17,

wherein said compound comprises:

- a peptide comprising at least the amino acid sequence X₁-R-W-H-X₅, wherein X₁ is selected from the group comprising amino acids with an NH₂ group such as K, R, N, or Q, and wherein X₅ is selected from the group comprising amino acids with negatively charged side chains such as E or D, (SEQ ID No. 5) or a peptidomimetic thereof, or
- a peptide comprising at least 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 amino acids of amino acid sequence X₁-X₂-X₃-X₄-X₅-X₆-X₇-R-W-H-X₁₁-X₁₂-X₁₃-X₁₄,
25
30 wherein X₁ is selected from the group comprising amino acids with an NH₂

group such as K, R, N, or Q, wherein X₂ is selected from the group comprising amino acids with negatively charged side chains such as E or D, wherein X₃ is selected from the group comprising amino acids with an NH₂ group such as K, R, N, or Q, wherein X₄ is selected from the group comprising amino acids with non-polar or non-charged side changes and aromatic ring structures such as F, W, or Y, wherein X₅ is selected from the group comprising amino acids with non-polar or non-charged side changes and aromatic rings structures such as F, W, or Y, wherein X₆ is selected from the group comprising G or amino acids with non-polar side chains such as A, V, L or I, wherein X₇ is selected from the group comprising amino acids with an NH₂ group such as K, R, N, or Q, wherein X₁₁ is selected from the group comprising amino acids with negatively charged side chains such as E or D, wherein X₁₂ is selected from the group comprising G or amino acids with non-polar side chains such as A, V, L or I, wherein X₁₃ is selected from the group comprising amino acids with non-polar or non-charged side chains and aromatic rings structures such F, W, or Y, and wherein X₁₄ is selected from the group comprising amino acids with an NH₂ group such as K, R, N, or Q (SEQ ID No. 9), and wherein the peptide comprises at least X₇-R-W-H-X₁₁ of SEQ ID No.: 9, , wherein X₇ is selected from the group comprising amino acids with an NH₂ group such as K, R, N, or Q and wherein X₁₁ is selected from the group comprising amino acids with negatively charged side chains such as E or D, or a peptidomimetic thereof.

19. Use of a compound of embodiment 16, 17, or 18, wherein said compound comprises a peptide comprising, optionally consisting of, amino acid sequence N-R-W-H-E (SEQ ID No.: 2), amino acid sequence K-E-Q-W-F-G-N-R-W-H-E-G-Y-R (SEQ ID No.: 6), or a peptidomimetic thereof.

20. Use of a compound of embodiment 16, 17, 18, or 19,

wherein said compound is a modified form of said peptide or said peptidomimetic.

21. Use of a compound of embodiment 20,

5 wherein said compound is a pegylated, hesylated, pasylated, myristoylated, glycosylated, and/or cyclic form of said peptide or peptidomimetic.

22. Use of a compound of embodiment 16, 17, 18, 19, 20, or 21,

10 wherein said medicament is formulated for oral, nasal, or subcutaneous administration.

23. Method of treating metastasizing cancer in a human being by administering a compound to a human being in need thereof, wherein said compound comprises:

- 15
- a peptide comprising at least the amino acid sequence X_1 -R-W-H- X_5 with X_1 being selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y and X_5 being selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y (SEQ ID No.: 1), or a peptidomimetic thereof, or
 - a peptide comprising at least 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 amino acids of amino acid sequence X_1 - X_2 - X_3 - X_4 - X_5 - X_6 - X_7 -R-W-H- X_{11} - X_{12} - X_{13} - X_{14} ,
20 wherein X_1 , X_2 , X_3 , X_4 , X_5 , X_6 , X_7 , X_{11} , X_{12} , X_{13} , or X_{14} is selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y (SEQ ID No.: 7), and wherein the peptide comprises at least X_7 -R-W-H- X_{11} of SEQ ID No.: 7, wherein X_7 , and X_{11} is selected from the group
25 comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y, or a peptidomimetic thereof.

24. Method of embodiment 23,

wherein said compound comprises:

- 5 • a peptide comprising at least the amino acid sequence X_1 -R-W-H- X_5 , wherein X_1 is selected from the group comprising amino acids with an NH_2 group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I and wherein X_5 is selected from the group comprising amino acids with negatively charged side chains such as E or D, or amino acids with non-polar side chains such as A, V, L or I (SEQ ID No. 4) or a peptidomimetic thereof, or
- 10 • a peptide comprising at least 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 amino acids of amino acid sequence X_1 - X_2 - X_3 - X_4 - X_5 - X_6 - X_7 -R-W-H- X_{11} - X_{12} - X_{13} - X_{14} , wherein X_1 is selected from the group comprising amino acids with an NH_2 group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I, wherein X_2 is selected from the group comprising amino acids with negatively charged side chains such as E or D, or amino acids with non-polar side chains such as A, V, L or I, wherein X_3 is selected from the group comprising amino acids with an NH_2 group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I, wherein X_4 is selected from the group comprising amino acids with non-polar or non-charged side changes and aromatic ring structures such as F, W, or Y, or amino acids with non-polar side chains such as A, V, L or I, wherein X_5 is selected from the group comprising amino acids with non-polar or non-charged side changes and aromatic rings structures such as F, W, or Y, or amino acids with non-polar side chains such as A, V, L or I, wherein X_6 is selected from the group comprising G or amino acids with non-polar side chains such as A, V, L or I, wherein X_7 is selected from the group comprising amino acids with an NH_2 group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I, wherein X_{11} is selected from the group comprising amino acids with negatively charged side chains such as E or D, or amino acids with non-polar side chains such as A, V, L or I, wherein X_{12} is selected from the group comprising G or amino acids with non-polar side chains such as A, V, L or I, wherein X_{13} is selected from the group

comprising amino acids with non-polar or non-charged side chains and aromatic rings structures such F, W, or Y, or amino acids with non-polar side chains such as A, V, L or I, and wherein X_{14} is selected from the group comprising amino acids with an NH_2 group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I (SEQ ID No. 8), and wherein the peptide comprises at least X_7 -R-W-H- X_{11} of SEQ ID No.: 8, wherein X_7 is selected from the group comprising amino acids with an NH_2 group such as K, R, N, or Q, or amino acids with non-polar side chains such as A, V, L or I and wherein X_{11} is selected from the group comprising amino acids with negatively charged side chains such as E or D, or amino acids with non-polar side chains such as A, V, L or I, or a peptidomimetic thereof.

25. Method of embodiment 24,

wherein said compound comprises:

- 15 • a peptide comprising at least the amino acid sequence X_1 -R-W-H- X_5 , wherein X_1 is selected from the group comprising amino acids with an NH_2 group such as K, R, N, or Q, and wherein X_5 is selected from the group comprising amino acids with negatively charged side chains such as E or D, (SEQ ID No. 5) or a peptidomimetic thereof, or
- 20 • a peptide comprising at least 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 amino acids of amino acid sequence X_1 - X_2 - X_3 - X_4 - X_5 - X_6 - X_7 -R-W-H- X_{11} - X_{12} - X_{13} - X_{14} , wherein X_1 is selected from the group comprising amino acids with an NH_2 group such as K, R, N, or Q, wherein X_2 is selected from the group comprising amino acids with negatively charged side chains such as E or D, wherein X_3 is selected from the group comprising amino acids with an NH_2 group such as K, R, N, or Q, wherein X_4 is selected from the group comprising amino acids with non-polar or non-charged side changes and aromatic ring structures such as F, W, or Y, wherein X_5 is selected from the group comprising amino acids with non-polar or non-charged side changes and aromatic rings structures such as F, W, or Y, wherein X_6 is selected from

the group comprising G or amino acids with non-polar side chains such as A, V, L or I, wherein X₇ is selected from the group comprising amino acids with an NH₂ group such as K, R, N, or Q, wherein X₁₁ is selected from the group comprising amino acids with negatively charged side chains such as E or D, wherein X₁₂ is selected from the group comprising G or amino acids with non-polar side chains such as A, V, L or I, wherein X₁₃ is selected from the group comprising amino acids with non-polar or non-charged side chains and aromatic rings structures such F, W, or Y, and wherein X₁₄ is selected from the group comprising amino acids with an NH₂ group such as K, R, N, or Q (SEQ ID No. 9), and wherein the peptide comprises at least X₇-R-W-H-X₁₁ of SEQ ID No.: 9, , wherein X₇ is selected from the group comprising amino acids with an NH₂ group such as K, R, N, or Q and wherein X₁₁ is selected from the group comprising amino acids with negatively charged side chains such as E or D, or a peptidomimetic thereof.

15

26. Method of embodiment 23, 24, or 25,

wherein said compound comprises a peptide comprising, optionally consisting of, amino acid sequence N-R-W-H-E (SEQ ID No.: 2), amino acid sequence K-E-Q-W-F-G-N-R-W-H-E-G-Y-R (SEQ ID No.: 6), or a peptidomimetic thereof.

20

27. Method of embodiment 23, 24, 25, or 26,

wherein said compound is a modified form of said peptide or said peptidomimetic.

28. Method of embodiment 27,

wherein said compound is a pegylated, hesylated, pasylated, myristoylated, glycosylated, and/or cyclic form of said peptide or peptidomimetic.

29. Method of embodiment 23, 24, 25, 26, 27, or 28,

wherein said compound is formulated for oral, nasal, or subcutaneous administration.

30

30. Compound for use of any of embodiments 1, 2, 3, 4, 5, 6 or 7, a pharmaceutical composition for use of any of embodiments 8, 9, 10, 11, 12, 13, 14, or 15, use of any of embodiments 16, 17, 18, 19, 20, 21 or 22 or method of any of
5 embodiments 23, 24, 25, 26, 27, 28, or 29,
 wherein said metastasizing cancer shows expression of CD44v6.
31. Compound for use of any of embodiments 1, 2, 3, 4, 5, 6, 7, or 30, a
 pharmaceutical composition for use of any of embodiments 8, 9, 10, 11, 12, 13,
10 14, 15, or 30, use of any of embodiments 16, 17, 18, 19, 20, 21, 22, or 30, or
 method of any of embodiments 23, 24, 25, 26, 27, 28, 29, or 30,
 wherein said metastasizing cancer is classifiable as Stage III or Stage IV
 according to the TNM anatomic/prognostic group system of the cancer staging
 system of the American Joint Committee on Cancer.
15
32. Compound for use of any of embodiments 1, 2, 3, 4, 5, 6, 7, 30, or 31, a
 pharmaceutical composition for use of any of embodiments 8, 9, 10, 11, 12, 13,
 14, 15, 30, or 31, use of any of embodiments 16, 17, 18, 19, 20, 21, 22, 30, or 31,
 or method of any of embodiments 23, 24, 25, 26, 27, 28, 29, 30, or 31,
20 wherein said metastasizing cancer is classifiable as Stage IV according to the
 TNM anatomic/prognostic group system of the cancer staging system of the
 American Joint Committee on Cancer.
33. Compound for use of any of embodiments 1, 2, 3, 4, 5, 6, 7, 30, 31, or 32, a
25 pharmaceutical composition for use of any of embodiments 8, 9, 10, 11, 12, 13,
 14, 15, 30, 31, or 32, use of any of embodiments 16, 17, 18, 19, 20, 21, 22, 30,
 31, or 32, or method of any of embodiments 23, 24, 25, 26, 27, 28, 29, 30, 31, or
 32,
 wherein said metastasizing cancer is selected from the group comprising
30 metastasizing forms of Hodgkin lymphoma, colorectal cancer, cervical cancer,

lung cancer, skin cancer such as squamous cell cancer or basal cell carcinoma, head and neck cancer, gastric cancer, pancreatic cancer, head and neck squamous cell cancer, or breast cancer.

- 5 34. Compound for use of any of embodiments 1, 2, 3, 4, 5, 6, 7, 30, 31, 32, or 33, a pharmaceutical composition for use of any of embodiments 8, 9, 10, 11, 12, 13, 14, 15, 30, 31, 32, or 33, use of any of embodiments 16, 17, 18, 19, 20, 21, 22, 30, 31, 32 or 33, or method of any of embodiments 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, or 33,
- 10 wherein said metastasizing cancer is selected from the group comprising metastasizing forms of Hodgkin lymphoma, colorectal cancer, cervical cancer, lung cancer, skin cancer such as squamous cell cancer or basal cell carcinoma, head and neck cancer, gastric cancer, pancreatic cancer, or breast cancer, wherein said metastasizing cancer is classifiable as Stage IV according to the TNM
- 15 anatomic/prognostic group system of the cancer staging system of the American Joint Committee on Cancer, and wherein said metastasizing cancer shows expression of CD44v6.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. Method of treating metastasizing cancer in a human being, comprising administering to said human being a compound,
- 5 wherein said compound comprises:
- a peptide comprising at least the amino acid sequence X_1 -R-W-H- X_5 with X_1 being selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y and X_5 being selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y (SEQ ID No.: 1), or
 - 10 a peptidomimetic thereof, or
 - a peptide comprising at least 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 amino acids of amino acid sequence X_1 - X_2 - X_3 - X_4 - X_5 - X_6 - X_7 -R-W-H- X_{11} - X_{12} - X_{13} - X_{14} , wherein X_1 , X_2 , X_3 , X_4 , X_5 , X_6 , X_7 , X_{11} , X_{12} , X_{13} , or X_{14} is selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or
 - 15 Y (SEQ ID No.: 7), and wherein the peptide comprises at least X_7 -R-W-H- X_{11} of SEQ ID No.: 7, wherein X_7 , and X_{11} is selected from the group comprising A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y, or a peptidomimetic thereof.
- 20 2. The method of claim 1,
- wherein said compound comprises:
- a peptide comprising at least the amino acid sequence X_1 -R-W-H- X_5 , wherein X_1 is selected from the group consisting of amino acids with an NH_2 amino acid side group and amino acids with a non-polar side chain, and wherein X_5
 - 25 is selected from the group consisting of amino acids with negatively charged side chains, and amino acids with non-polar side chains (SEQ ID No. 4) or a peptidomimetic thereof, or
 - a peptide comprising at least 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 amino acids of amino acid sequence X_1 - X_2 - X_3 - X_4 - X_5 - X_6 - X_7 -R-W-H- X_{11} - X_{12} - X_{13} - X_{14} ,

wherein X₁ is selected from the group consisting of amino acids with an NH₂ amino acid side group and amino acids with non-polar side chains, wherein X₂ is selected from the group consisting of amino acids with negatively charged side chains and amino acids with non-polar side chains, wherein X₃ is selected from the group consisting of amino acids with an NH₂ amino acid side group and amino acids with non-polar side chains, wherein X₄ is selected from the group consisting of amino acids with non-polar side chains, non-charged side chains, aromatic ring structures and amino acids with non-polar side chains, wherein X₅ is selected from the group consisting of amino acids with non-polar or non-charged side chains and aromatic rings structures and amino acids with non-polar side chains, wherein X₆ is selected from the group consisting of G and amino acids with non-polar side chains, wherein X₇ is selected from the group consisting of amino acids with an NH₂ amino acid side group and amino acids with non-polar side chains, wherein X₁₁ is selected from the group consisting of amino acids with negatively charged side chains and amino acids with non-polar side chains, wherein X₁₂ is selected from the group consisting of G and amino acids with non-polar side chains, wherein X₁₃ is selected from the group consisting of amino acids with non-polar or non-charged side chains and aromatic rings structures and amino acids with non-polar side chains, and wherein X₁₄ is selected from the group consisting of amino acids with an NH₂ amino acid side group and amino acids with non-polar side chains (SEQ ID No. 8), and wherein the peptide comprises at least X₇-R-W-H-X₁₁ of SEQ ID No.: 8, wherein X₇ is selected from the group comprising amino acids with an NH₂ amino acid side group and amino acids with non-polar side chains, and wherein X₁₁ is selected from the group consisting of amino acids with negatively charged side chains and amino acids with non-polar side chains, or a peptidomimetic thereof.

3. The method of claim 2, wherein in the amino acid sequence X₁-R-W-H-X₅, X₁ is selected from the group consisting of amino acids K, R, N, Q, A, V, L and I,

and/or X₅ is selected from the group consisting of amino acids E, D, A, V, L and I,

and/or wherein in the amino acid sequence X₁-X₂-X₃-X₄-X₅-X₆-X₇-R-W-H-X₁₁-X₁₂-

X₁₃-X₁₄, X₁ is selected from the group consisting of amino acids K, R, N, Q, A,

5 V, L and I, and/or X₂ is selected from the group consisting of amino acids E, D, A, V, L and I, and/or X₃ is selected from the group consisting of amino acids K,

R, N, Q, A, V, L and I, and/or X₄ is selected from the group consisting of amino acids F, W, Y, A, V, L and I, and/or X₅ is selected from the group consisting of

amino acids F, W, Y, A, V, L and I, and/or X₆ is selected from the group

10 consisting of amino acids G, A, V, L and I, and/or X₇ is selected from the group

consisting of amino acids K, R, N, Q, A, V, L and I, and/or X₁₁ is selected from

the group consisting of amino acids E, D, A, V, L and I, and/or X₁₂ is selected

from the group consisting of amino acids G, A, V, L and I, and/or X₁₃ is selected

from the group consisting of amino acids F, W, Y, A, V, L and I, and/or X₁₄ is

15 selected from the group consisting of amino acids K, R, N, Q, A, V, L and I.

4. The method of claim 2 or 3,

wherein said compound comprises:

- a peptide comprising at least the amino acid sequence X₁-R-W-H-X₅, wherein

20 X₁ is selected from the group consisting of amino acids with an NH₂ amino acid side group, and wherein X₅ is selected from the group consisting of amino acids with negatively charged side chains, (SEQ ID No. 5) or a peptidomimetic thereof, or

a peptide comprising at least 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 amino acids of

25 amino acid sequence X₁-X₂-X₃-X₄-X₅-X₆-X₇-R-W-H-X₁₁-X₁₂-X₁₃-X₁₄, wherein

X₁ is selected from the group consisting of amino acids with an NH₂ amino acid side group, wherein X₂ is selected from the group consisting of amino acids with negatively charged side chains, wherein X₃ is selected from the group consisting

of amino acids with an NH₂ amino acid side group, wherein X₄ is selected from

30 the group consisting of amino acids with non-polar or non-charged side chains

- and aromatic ring structures, wherein X_5 is selected from the group consisting of amino acids with non-polar or non-charged side chains and aromatic rings structures, wherein X_6 is selected from the group consisting of G or amino acids with non-polar side chains, wherein X_7 is selected from the group consisting of amino acids with an NH_2 amino acid side group, wherein X_{11} is selected from the group consisting of amino acids with negatively charged side chains, wherein X_{12} is selected from the group consisting of G or amino acids with non-polar side chains, wherein X_{13} is selected from the group consisting of amino acids with non-polar or non-charged side chains and aromatic rings structures, and wherein X_{14} is selected from the group consisting of amino acids with an NH_2 amino acid side group (SEQ ID No. 9), and wherein the peptide comprises at least X_7 -R-W-H- X_{11} of SEQ ID No.: 9, wherein X_7 is selected from the group consisting of amino acids with an NH_2 amino acid side group and wherein X_{11} is selected from the group consisting of amino acids with negatively charged side chains, or a peptidomimetic thereof.
5. The method of any one of claims 1, 2, 3 or 4, wherein in the amino acid sequence X_1 -R-W-H- X_5 , X_1 is selected from the group consisting of amino acids K, R, N and Q, and/or X_5 is selected from the group consisting of amino acids E and D; and/or
- wherein in the amino acid sequence X_1 - X_2 - X_3 - X_4 - X_5 - X_6 - X_7 -R-W-H- X_{11} - X_{12} - X_{13} - X_{14} , X_1 is selected from the group consisting of amino acids K, R, N and Q, and/or X_2 is selected from the group consisting of amino acids E and D, and/or X_3 is selected from the group consisting of amino acids K, R, N and Q, and/or X_4 is selected from the group consisting of amino acids F, W and Y, and/or X_5 is selected from the group consisting of amino acids F, W and Y, and/or X_6 is selected from the group consisting of G, A, V, L and I, and/or X_7 is selected from the group consisting of amino acids K, R, N and Q, and/or X_{11} is selected from the group consisting of amino acids E and D, and/or X_{12} is selected from the group consisting of G, A, V, L and I, and/or X_{13} is selected from the group

consisting of amino acids F, W and Y, and/or X_{14} is selected from the group consisting of amino acids K, R, N and Q.

6. The method of any one of claims 1, 2, 3, 4 or 5,
5 wherein said compound comprises a peptide comprising, optionally consisting of, the amino acid sequence N-R-W-H-E (SEQ ID No.: 2), amino acid sequence K-E-Q-W-F-G-N-R-W-H-E-G-Y-R (SEQ ID No.: 6), or a peptidomimetic thereof.
7. The method of any one of claims 1, 2, 3, 4, 5 or 6,
10 wherein said compound is a modified form of said peptide or said peptidomimetic.
8. The method of claim 7,
wherein said compound is a pegylated, hesylated, pasylated, glycosylated,
15 myristoylated and/or cyclic form of said peptide or peptidomimetic.
9. The method of any one of claims 1, 2, 3, 4, 5, 6, 7 or 8,
wherein the compound is formulated for oral, nasal, or subcutaneous
administration.
20
10. The method of any one of claims 1, 2, 3, 4, 5, 6, 7, 8 or 9,
wherein said metastasizing cancer shows expression of CD44v6.
11. The method of any one of claims 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10,
25 wherein said metastasizing cancer is classifiable as Stage III or Stage IV according to the TNM anatomic/prognostic group system of the cancer staging system of the American Joint Committee on Cancer.
12. The method of any one of claims 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11,

wherein said metastasizing cancer is classifiable as Stage IV according to the TNM anatomic/prognostic group system of the cancer staging system of the American Joint Committee on Cancer.

- 5 13. The method of any one of claims 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12, wherein said metastasizing cancer is selected from the group comprising metastasizing forms of Hodgkin lymphoma, colorectal cancer, cervical cancer, lung cancer, skin cancer, head and neck cancer, gastric cancer, pancreatic cancer, head and neck squamous cell cancer, or breast cancer.
- 10 14. The method of any one of claims 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or 13, said metastasizing cancer is selected from the group comprising metastasizing forms of Hodgkin lymphoma, colorectal cancer, cervical cancer, lung cancer, skin cancer, head and neck cancer, gastric cancer, pancreatic cancer, or breast
- 15 cancer, wherein said metastasizing cancer is classifiable as Stage IV according to the TNM anatomic/prognostic group system of the cancer staging system of the American Joint Committee on Cancer, and wherein said metastasizing cancer shows expression of CD44v6.
- 20 15. A use of a compound as defined in any one of claims 1 to 9, in the manufacture of a medicament for use in treating metastasizing cancer in a human being.
16. A use of claim 15, wherein said metastasizing cancer shows expression of CD44v6.
- 25 17. A use of claim 15 or claim 16, wherein said metastasizing cancer is classifiable as Stage III or Stage IV according to the TNM anatomic/prognostic group system of the cancer staging system of the American Joint Committee on Cancer.

18. A use of any one of claims 15, 16, or 17, wherein said metastasizing cancer is classifiable as Stage IV according to the TNM anatomic/prognostic group system of the cancer staging system of the American Joint Committee on Cancer.
- 5
19. A use of any one of claims 15, 16, 17 or 18, wherein said metastasizing cancer is selected from the group comprising metastasizing forms of Hodgkin lymphoma, colorectal cancer, cervical cancer, lung cancer, skin cancer, head and neck cancer, gastric cancer, pancreatic cancer, head and neck squamous cell cancer, or
- 10 breast cancer.
20. A use of any one of claims 15, 16, 17, 18 or 19, wherein said metastasizing cancer is selected from the group comprising metastasizing forms of Hodgkin lymphoma, colorectal cancer, cervical cancer, lung cancer, skin cancer, head and
- 15 neck cancer, gastric cancer, pancreatic cancer, or breast cancer, wherein said metastasizing cancer is classifiable as Stage IV according to the TNM anatomic/prognostic group system of the cancer staging system of the American Joint Committee on Cancer, and wherein said metastasizing cancer shows expression of CD44v6.
- 20
21. The method of claim 13 or claim 14, or the use of claim 19 or claim 20, wherein the skin cancer is squamous cell cancer or basal cell carcinoma.
22. A compound as defined in any one of claims 1 to 9, when used in treating
- 25 metastasizing cancer in a human being.

Figure 1

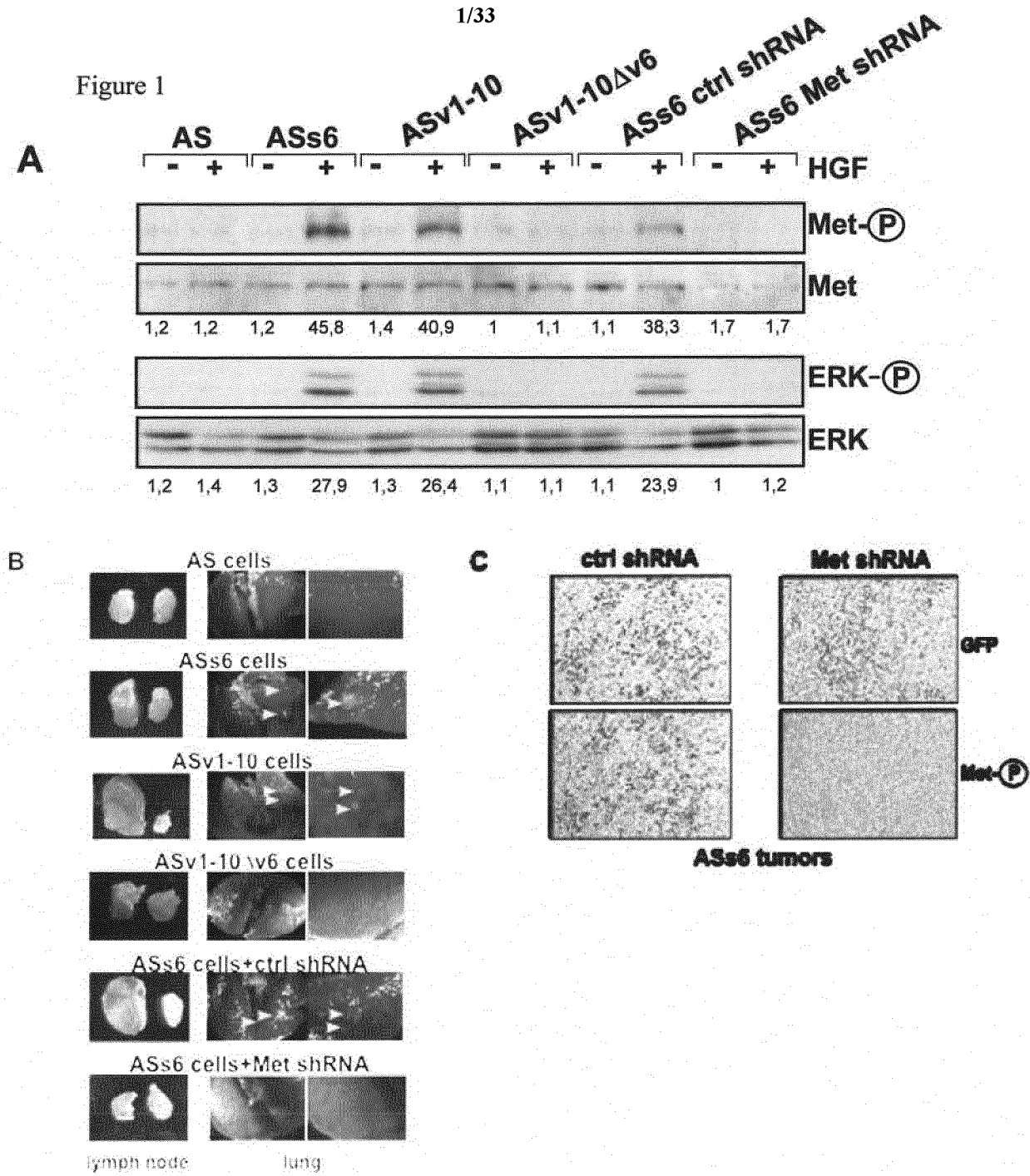


Figure 2

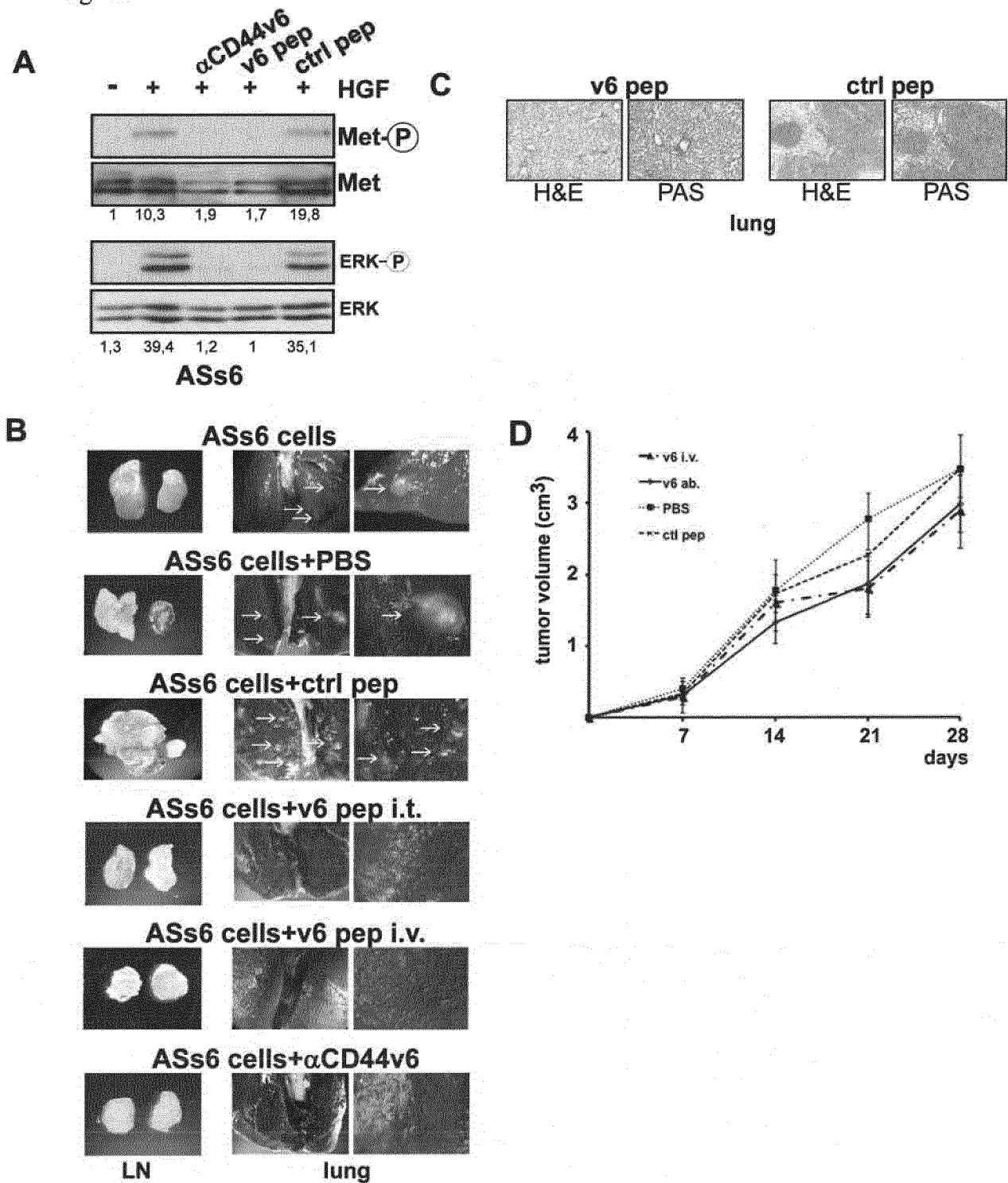
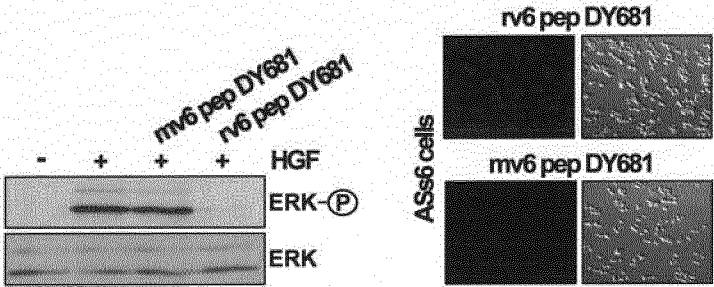
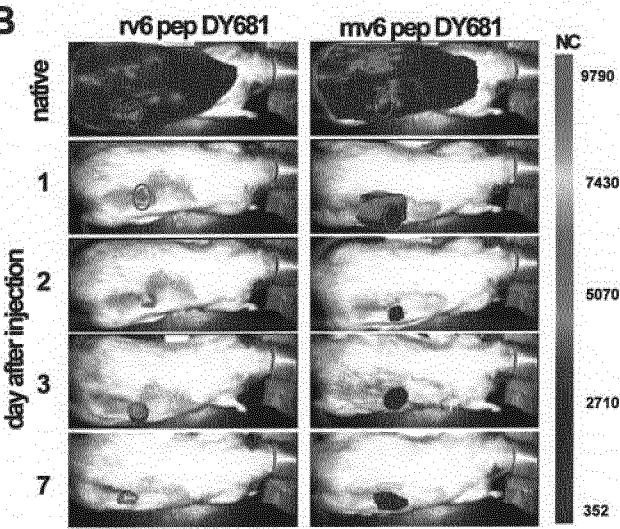


Figure 3

A



B



C

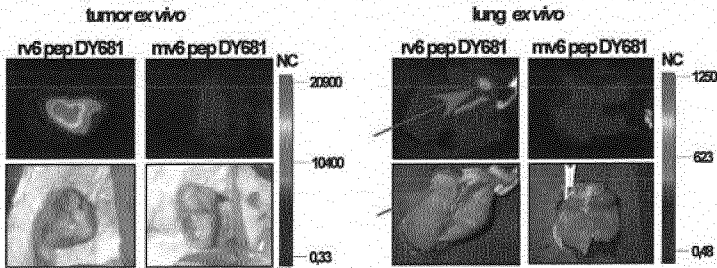
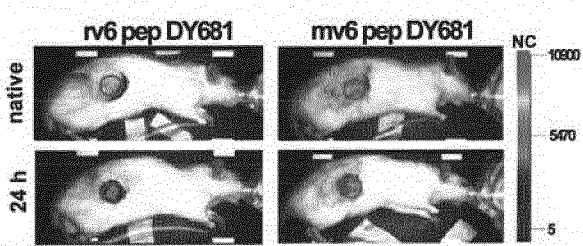


Figure 4

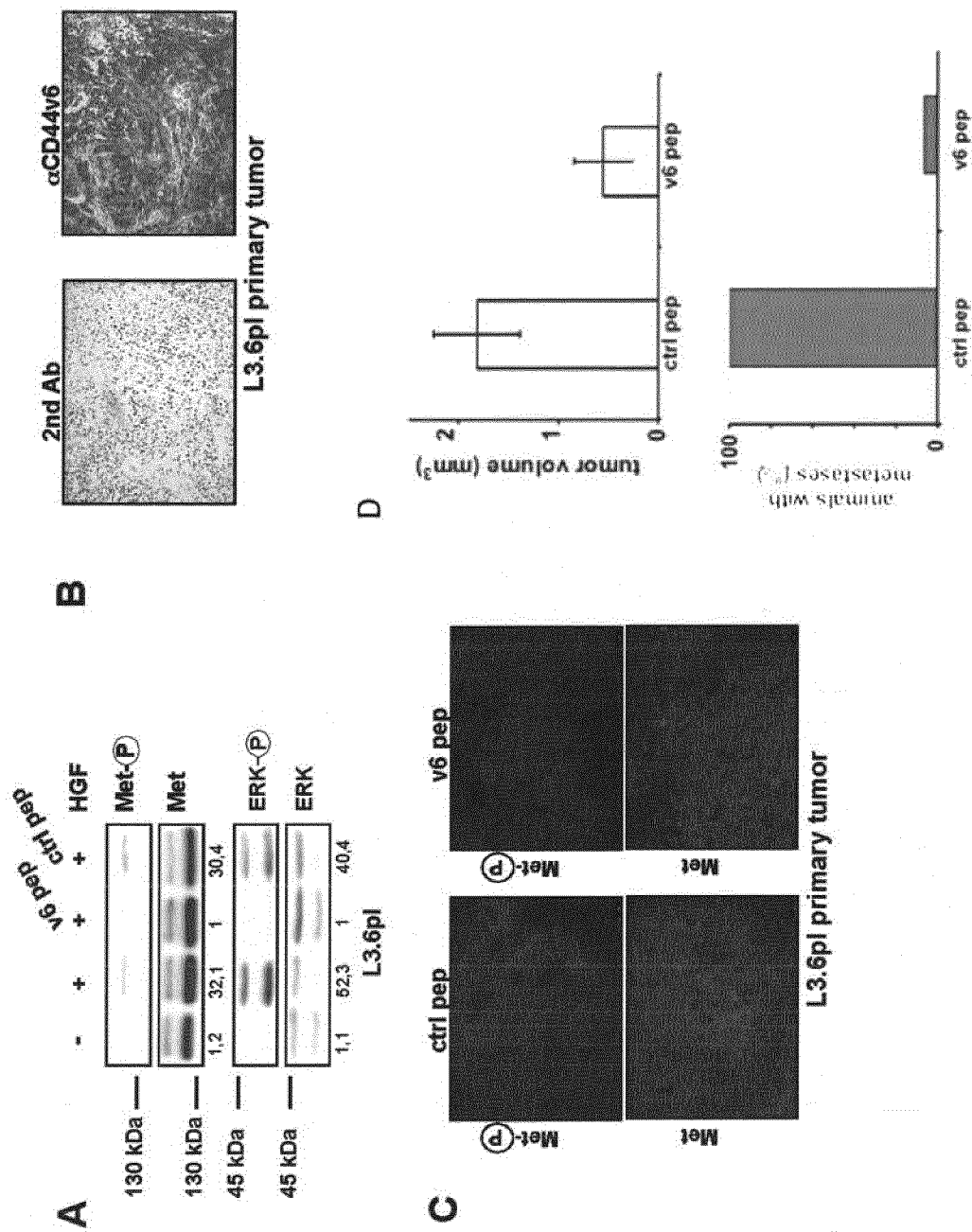


Figure 4 (continued)

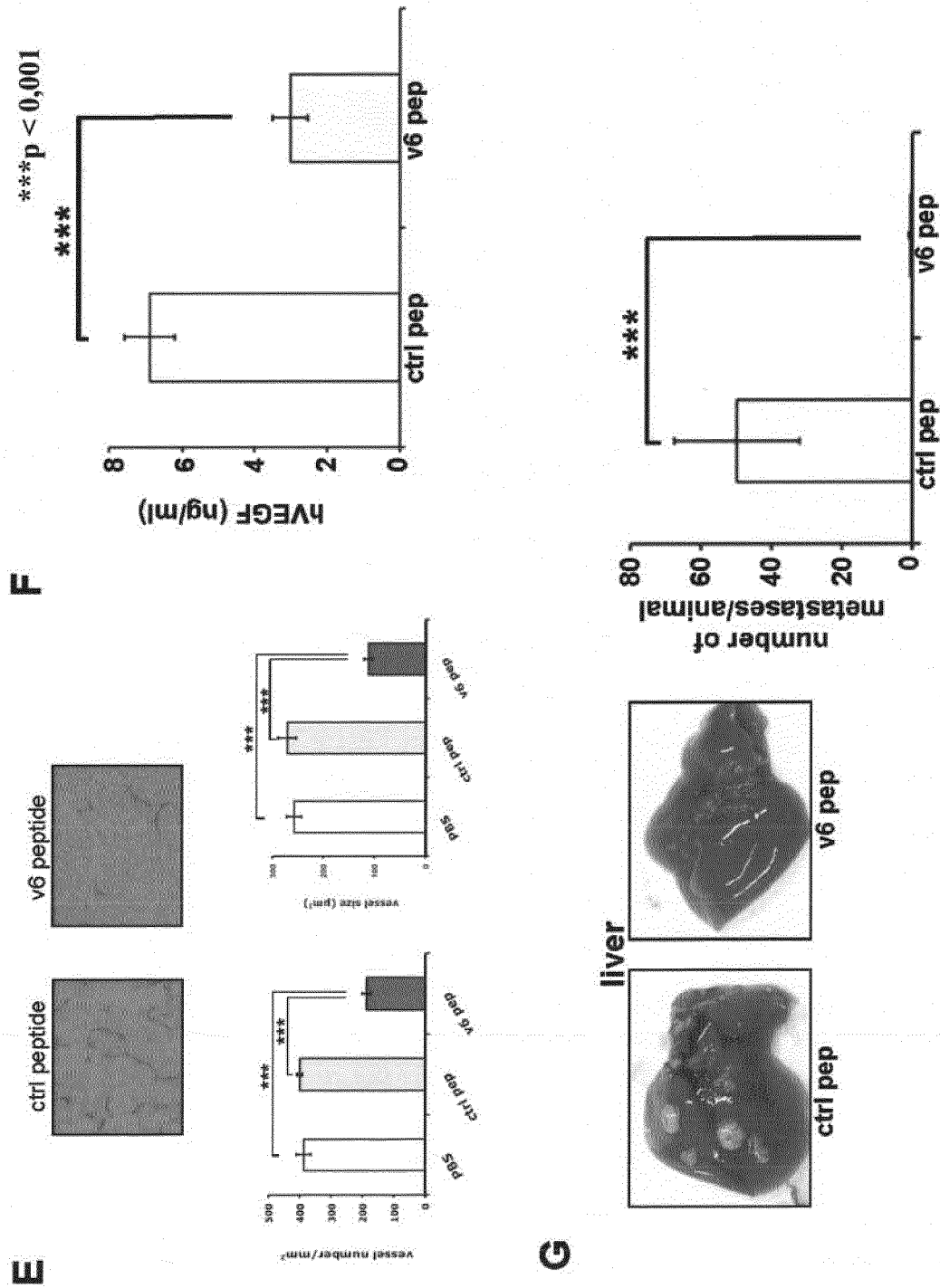


Figure 5

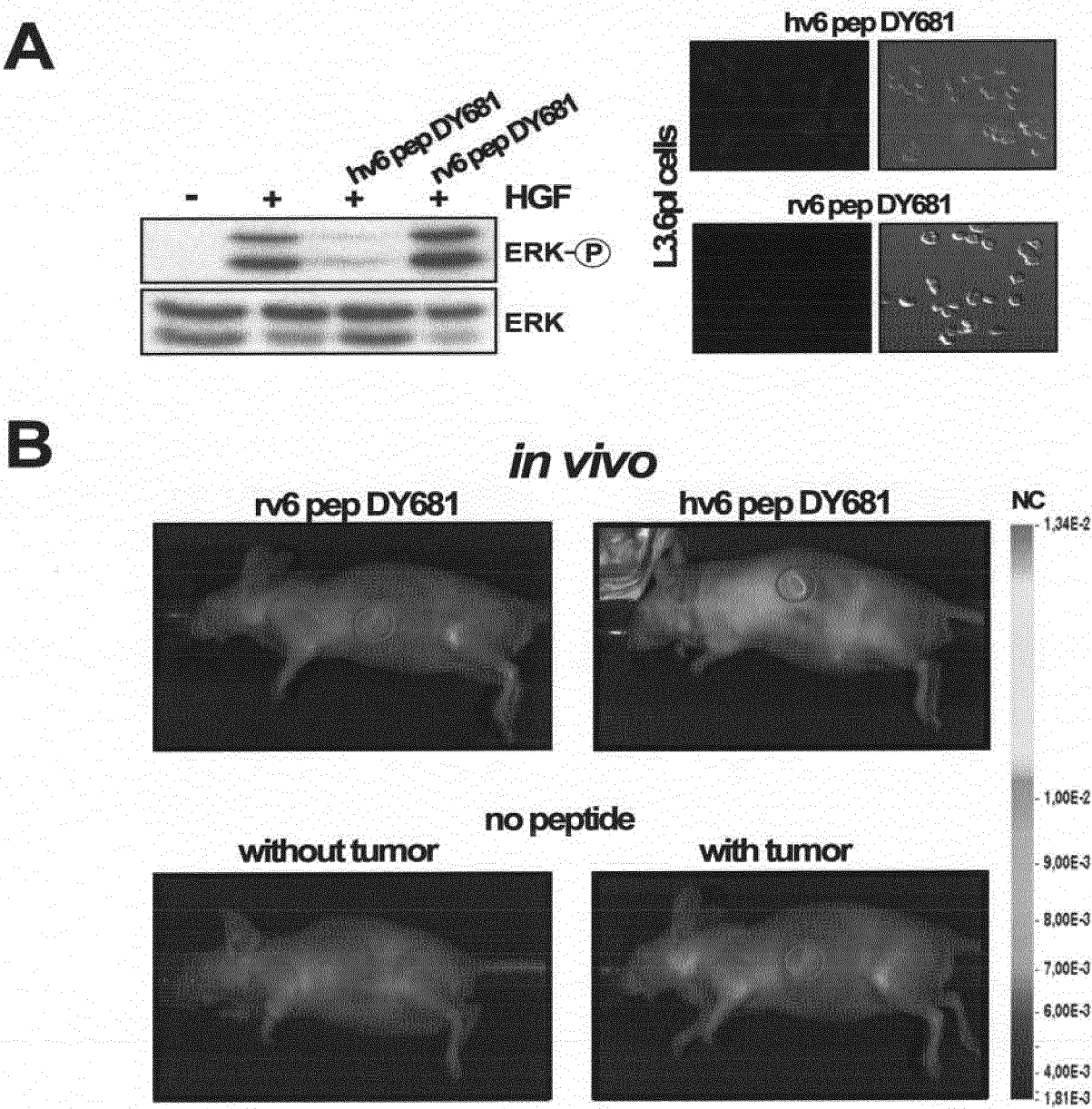


Figure 5 (continued)

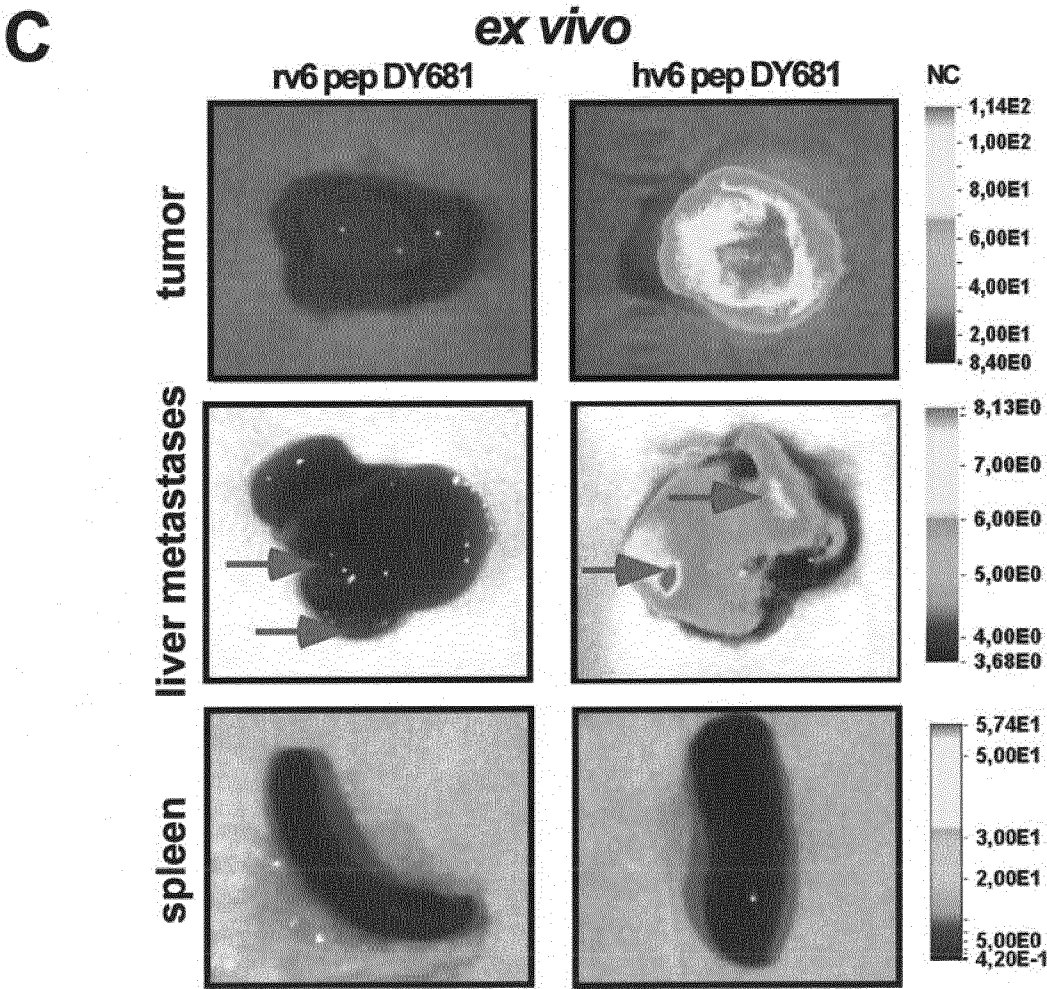


Figure 6

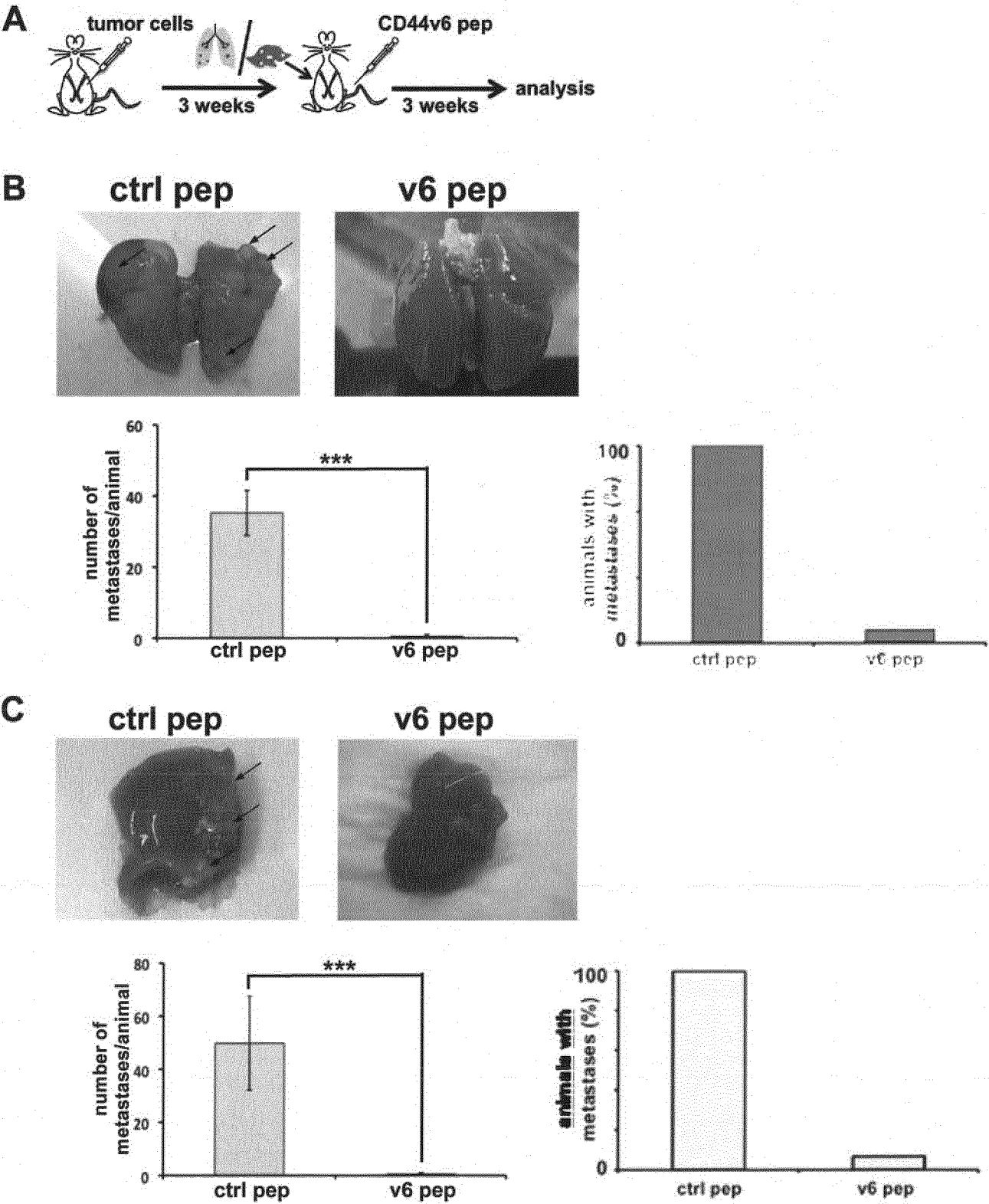


Figure 7

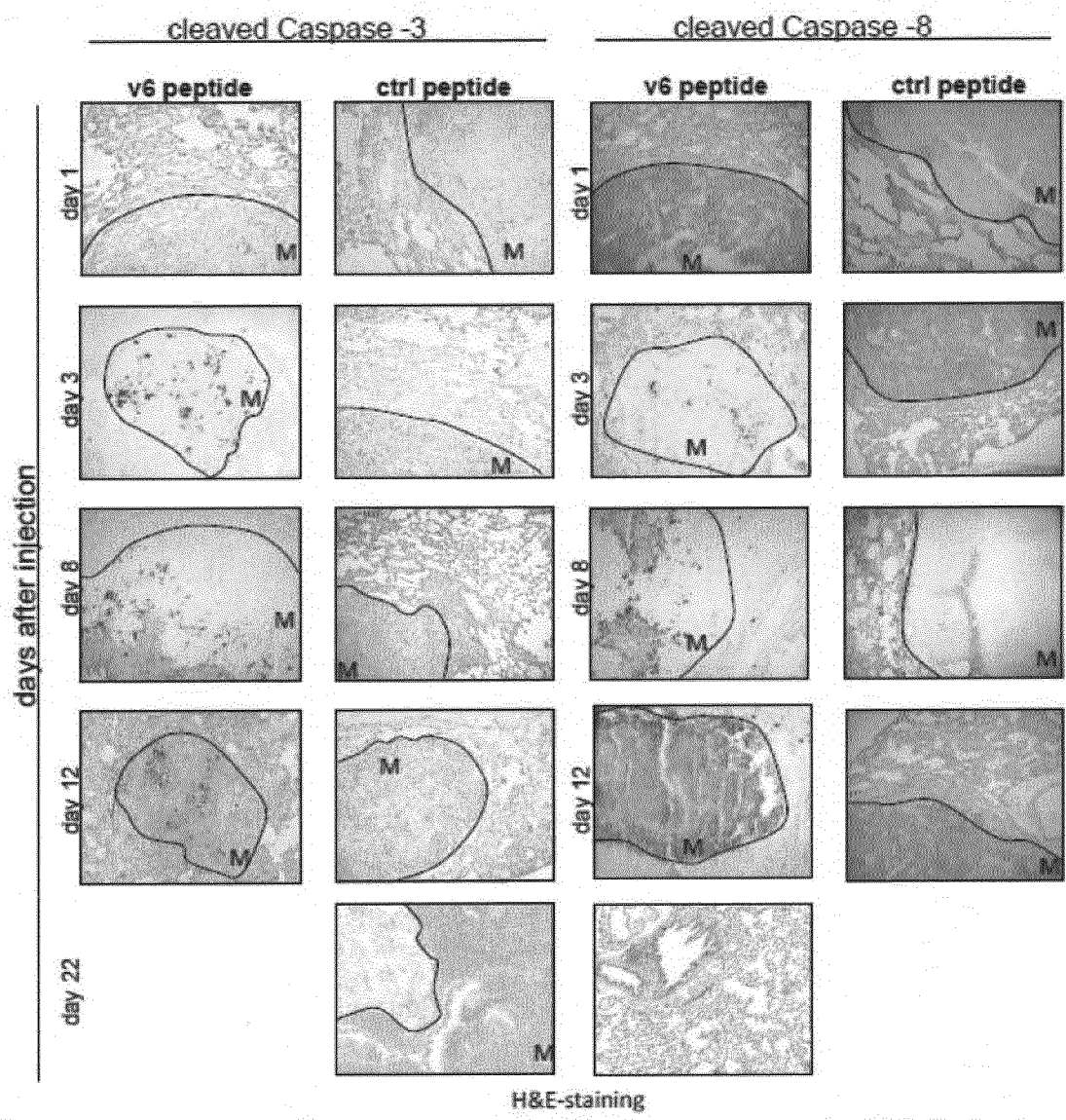


Figure 8

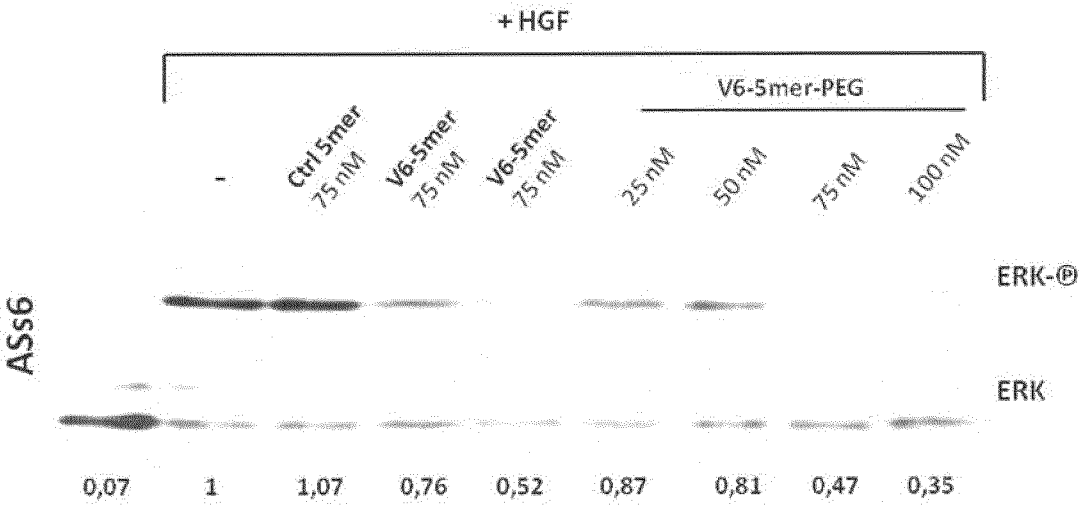
Table 1				
cell line	primary tumor	metastasis lung	metastasis ln	micrometastasis
AS	5/5	0/5	0/5	0/5
ASs6	15/15	15/15	15/15	
ASv1-v10Δv6	10/10	0/10	0/10	0/10
ASv1-v10	10/10	10/10	10/10	
ASs6 ctrl shRNA	10/10	10/10	10/10	
ASs6 Met shRNA	10/10	0/10	0/10	0/10

Table 2				
ASs6 +treatment	primary tumor	metastasis lung	metastasis ln	micrometastasis
CD44v6 Ab	14/14	1/14	1/14	1/14
CD44v6 pep i.t	14/14	0/14	0/14	0/14
CD44v6 pep i.v	15/15	0/15	0/15	0/15
ctrl pep	15/15	15/15	15/15	
PBS	10/10	10/10	10/10	

Table 3				
	BSp73ASs6		L3.6pl	
	primary tumor	lung metastasis	primary tumor	liver metastasis
control (3 weeks after tumor cell injection)	10/10	10/10	10/10	10/10
ctrl peptide treatment (3 more weeks)	15/15	15/15	15/15	15/15
v6 peptide treatment (3 more weeks)	15/15	1/15	15/15	1/15

Figure 9

A



B

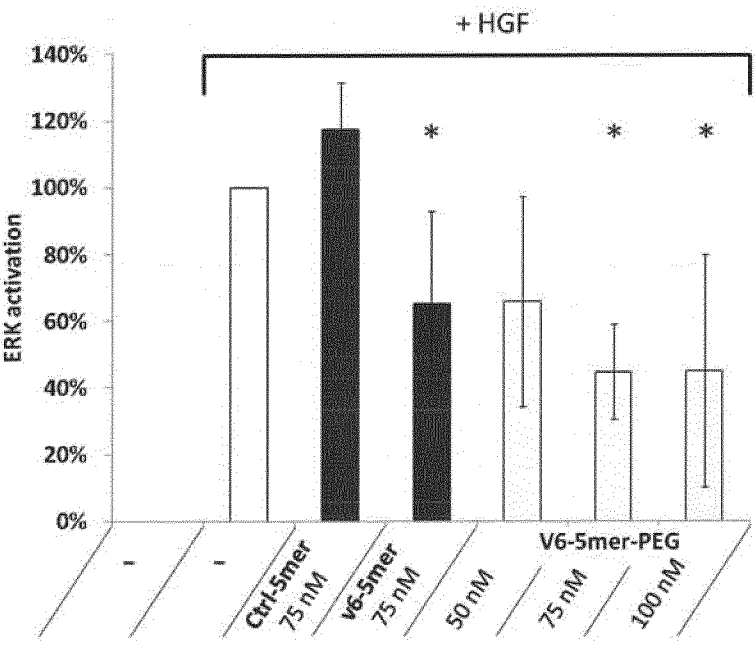


Figure 10

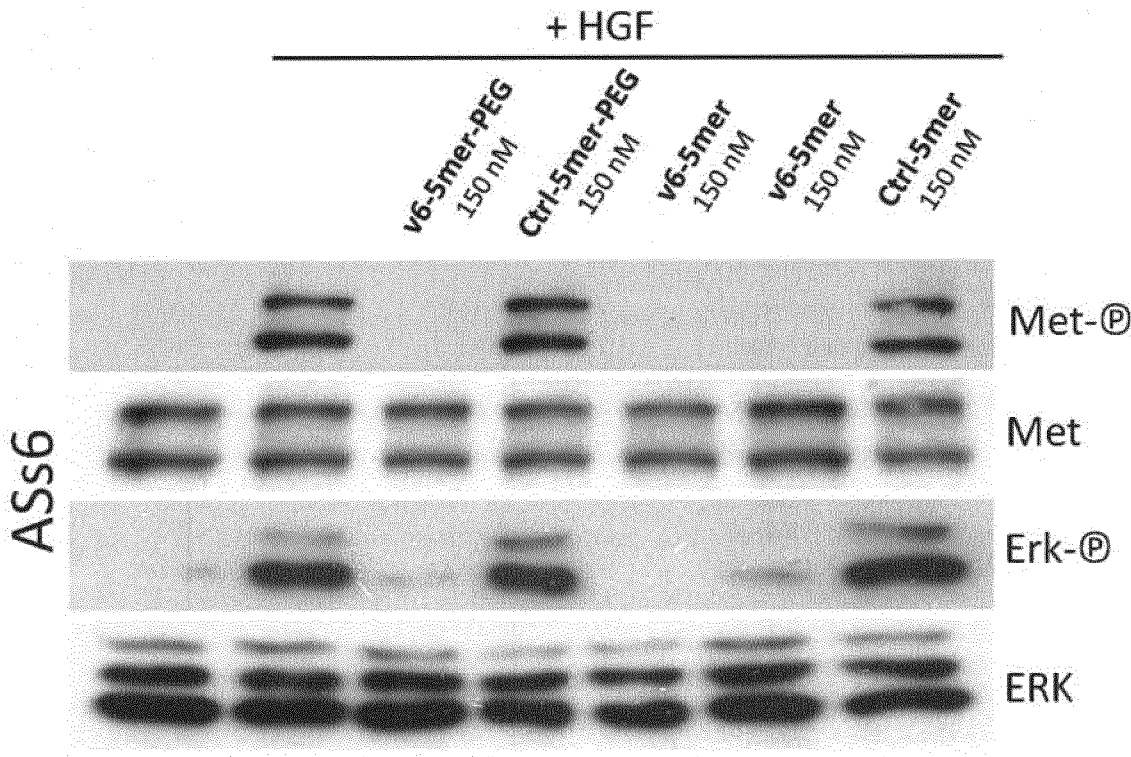
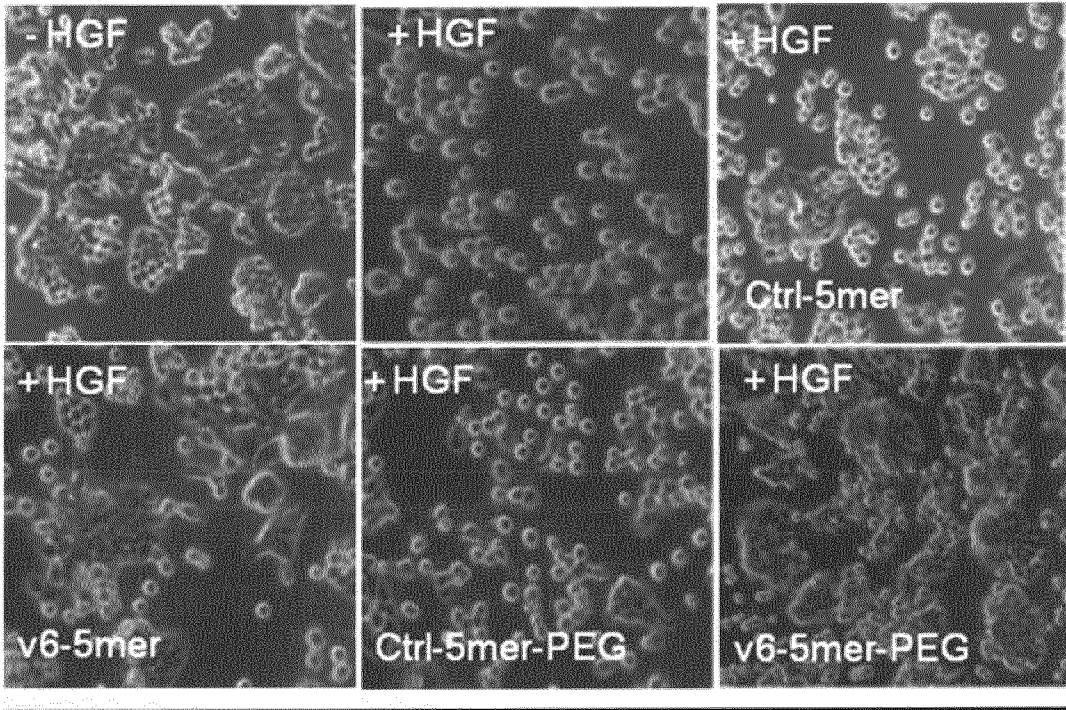


Figure 11



HT29

Figure 12

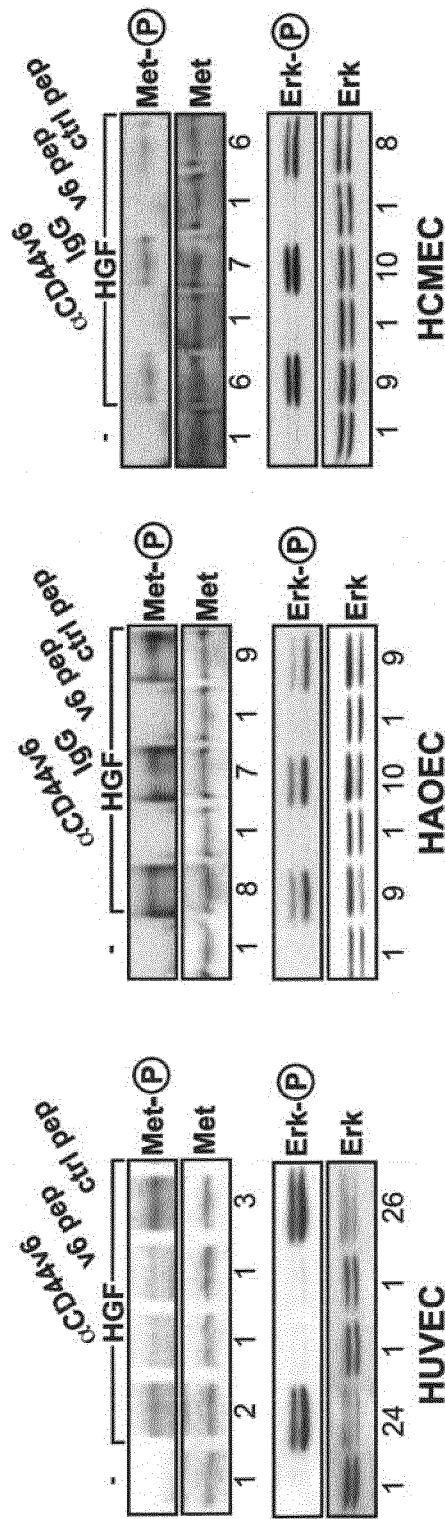


Figure 13

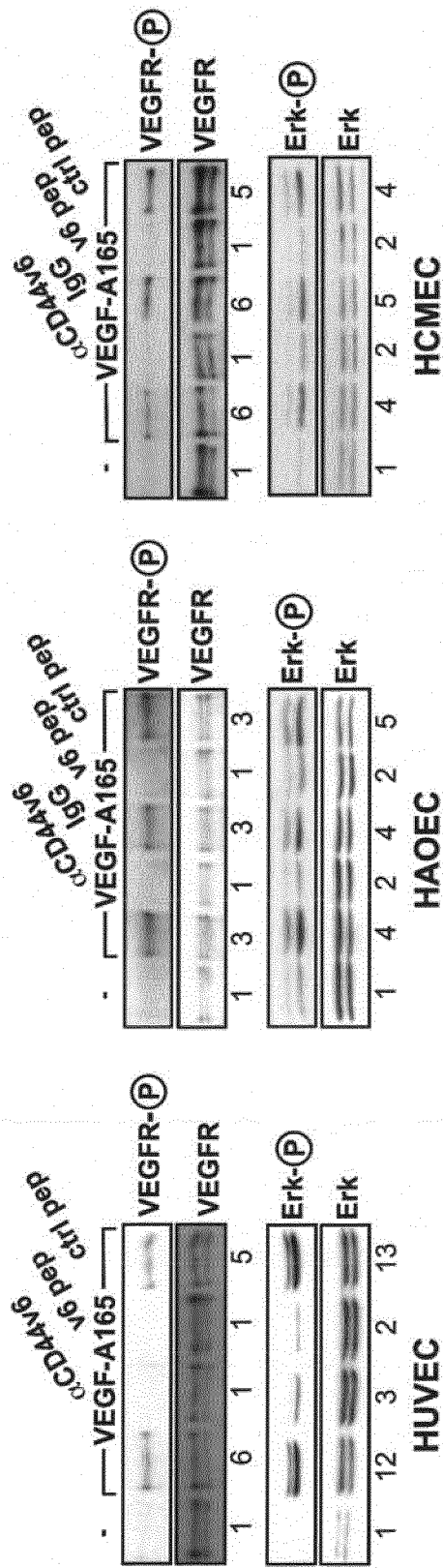
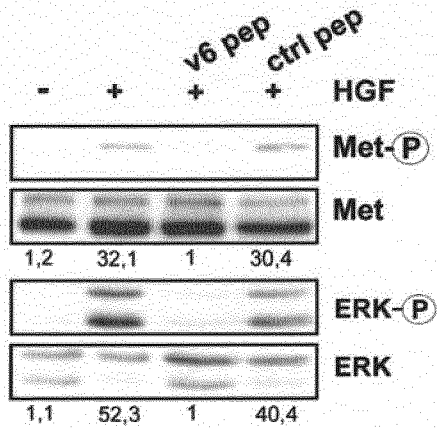
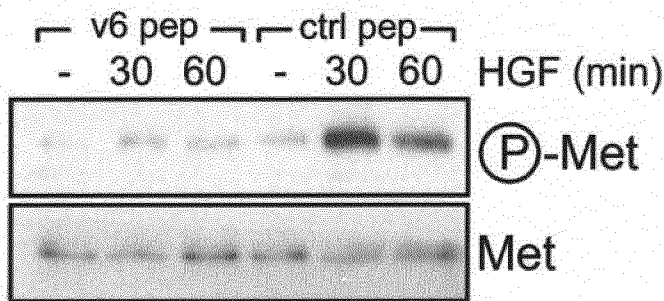


Figure 14

A



B



C

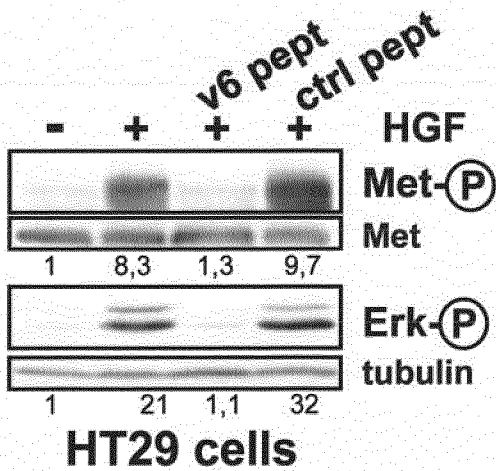


Figure 15

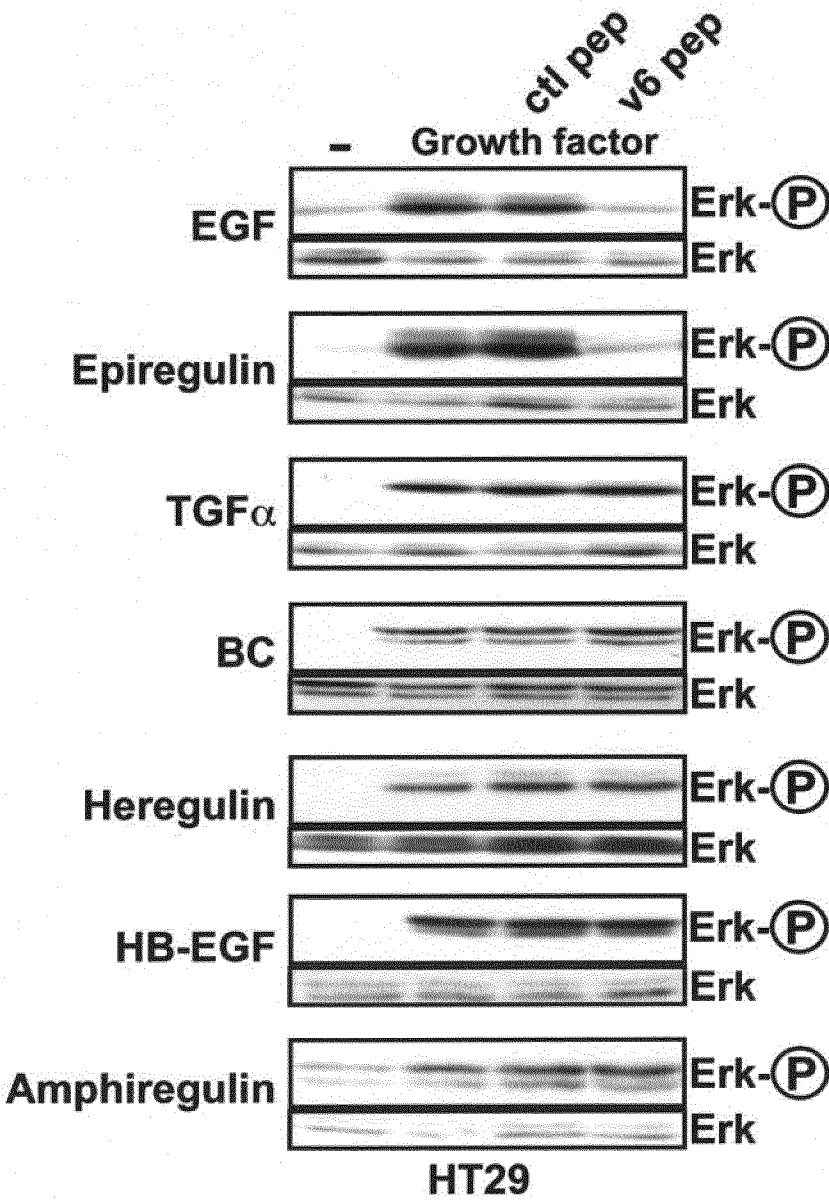


Figure 16

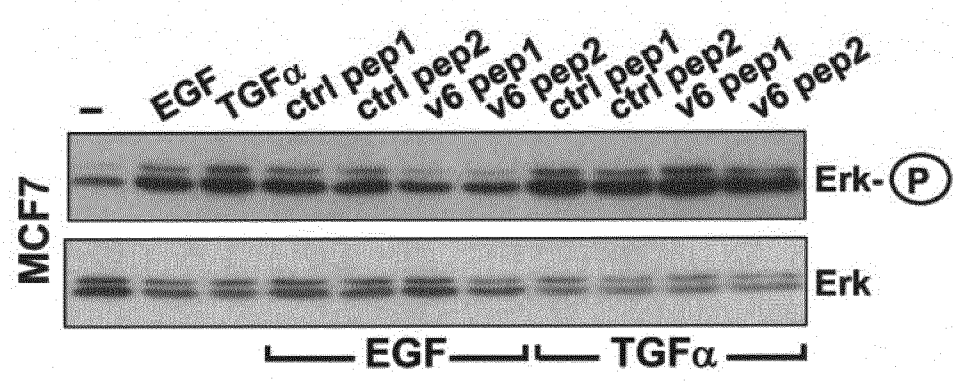


Figure 17

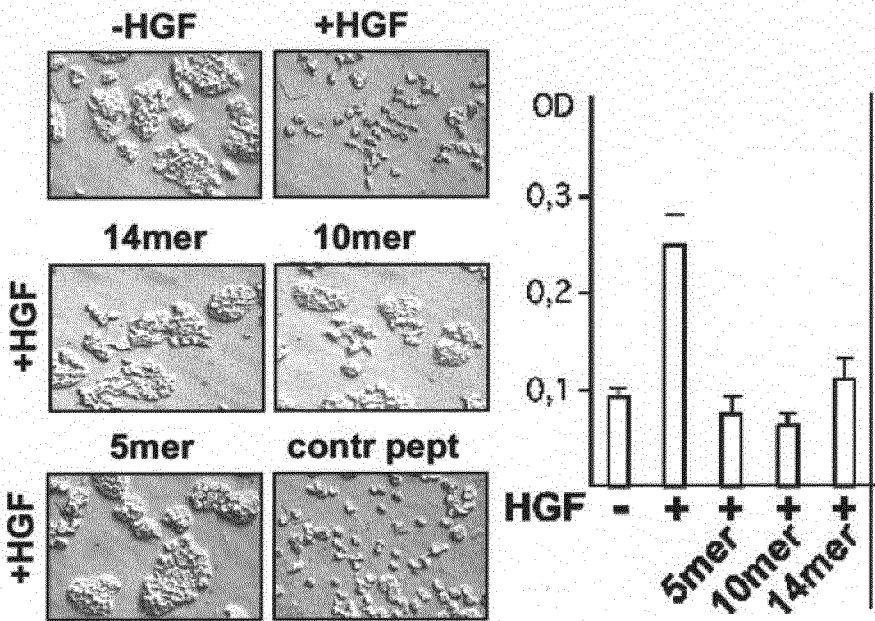


Figure 18

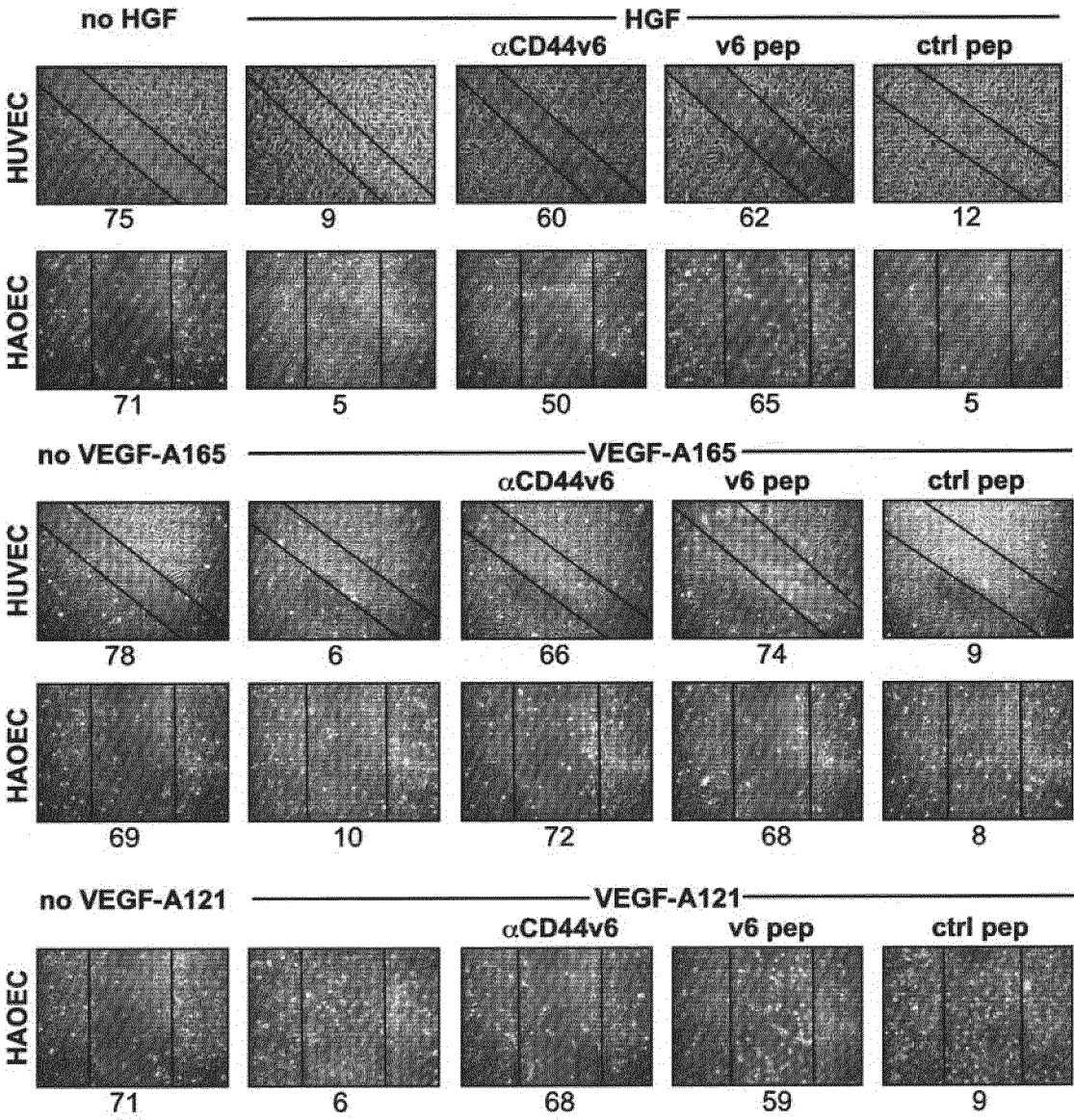


Figure 19

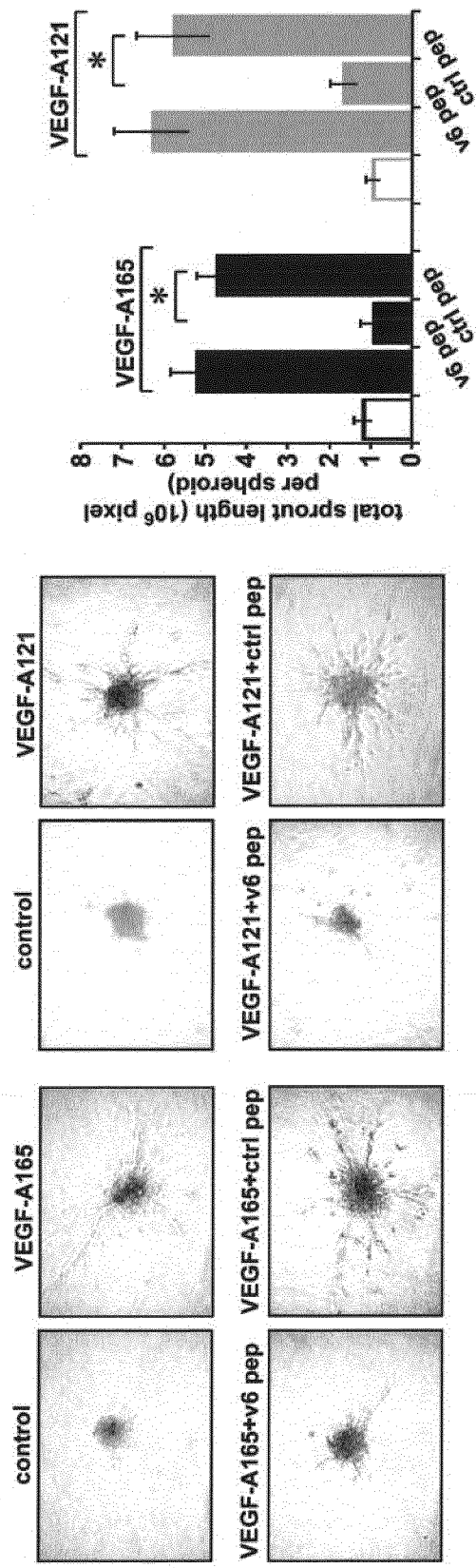


Figure 20

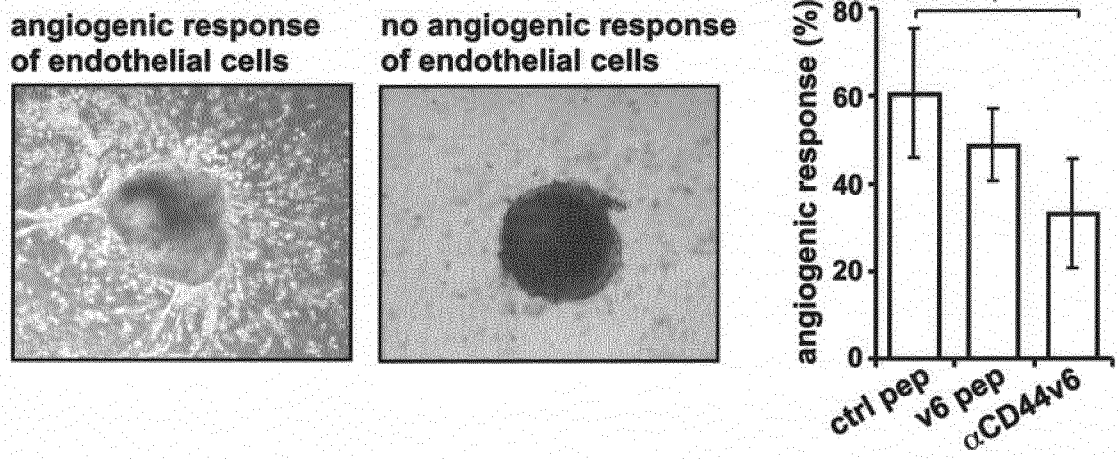


Figure 21

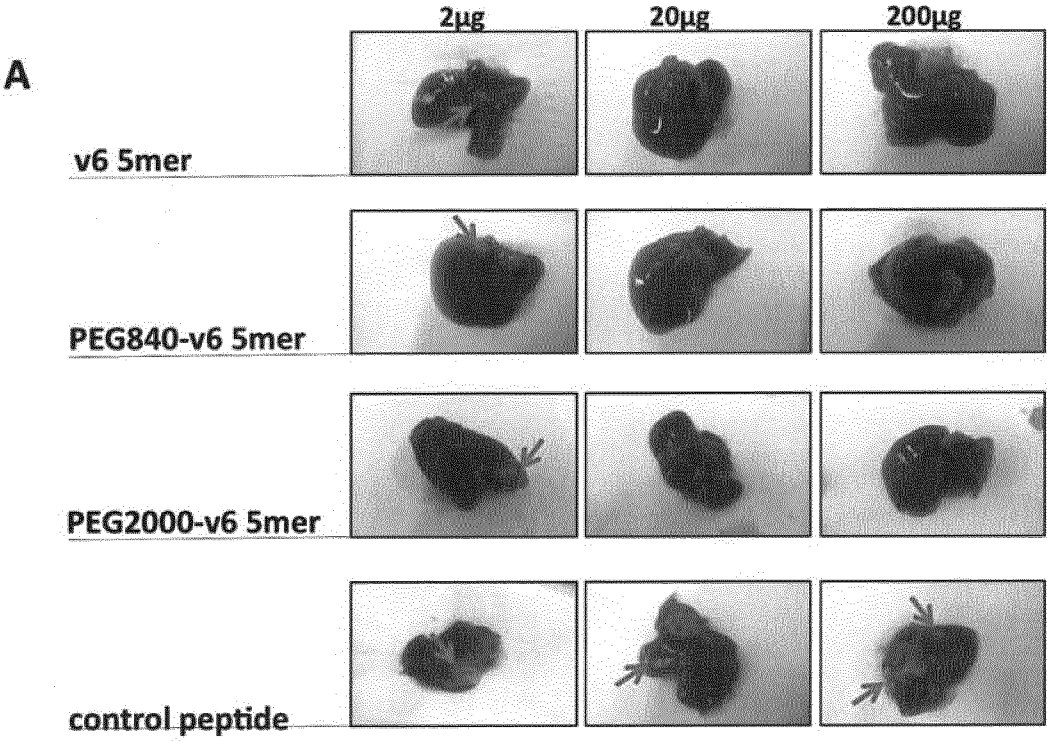
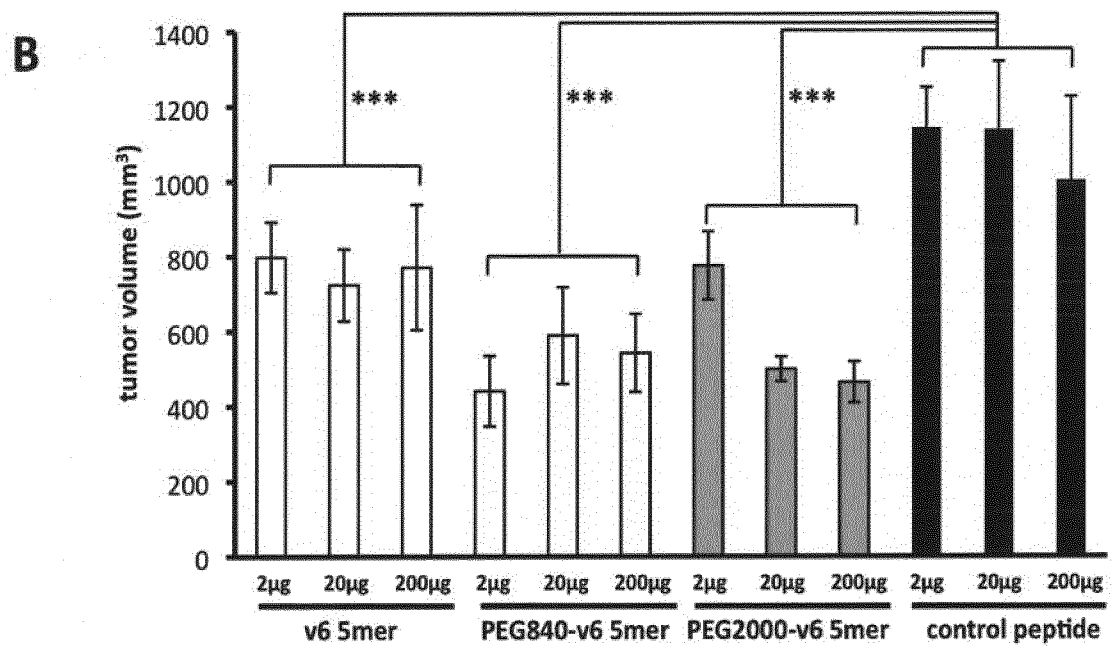


Figure 21 (continued)



C

amount/injection	2µg	20µg	200µg
v6 5mer	3	0	0
PEG840-v6 5mer	5	0	0
PEG2000-v6 5mer	3	0	0
control peptide	10	11	11

Figure 22

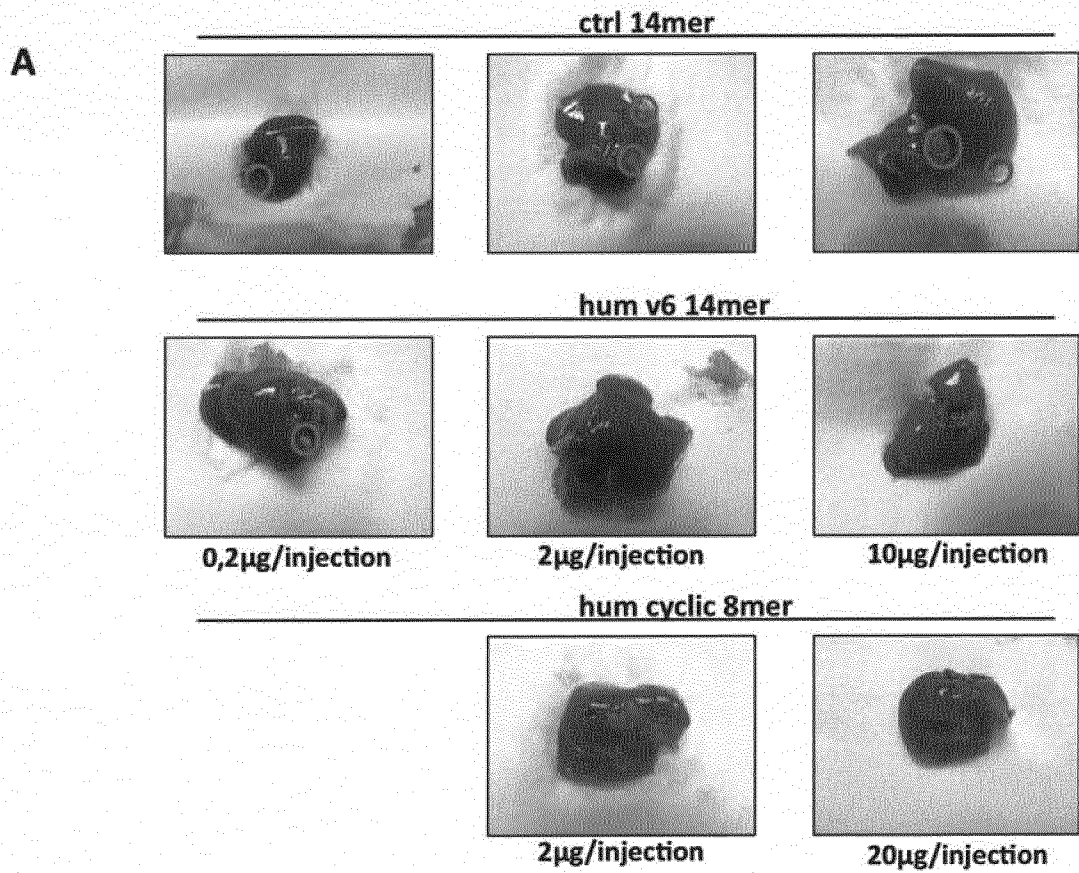


Figure 22 (continued)

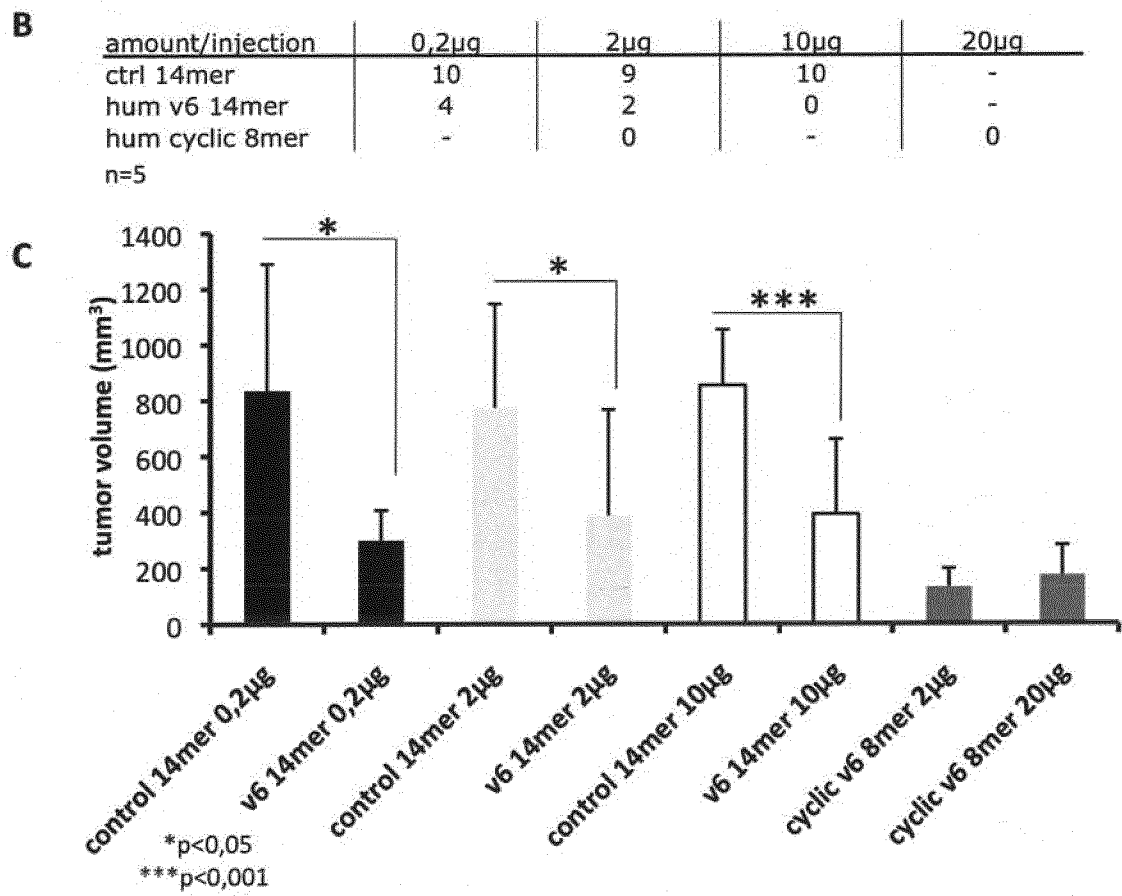


Figure 23

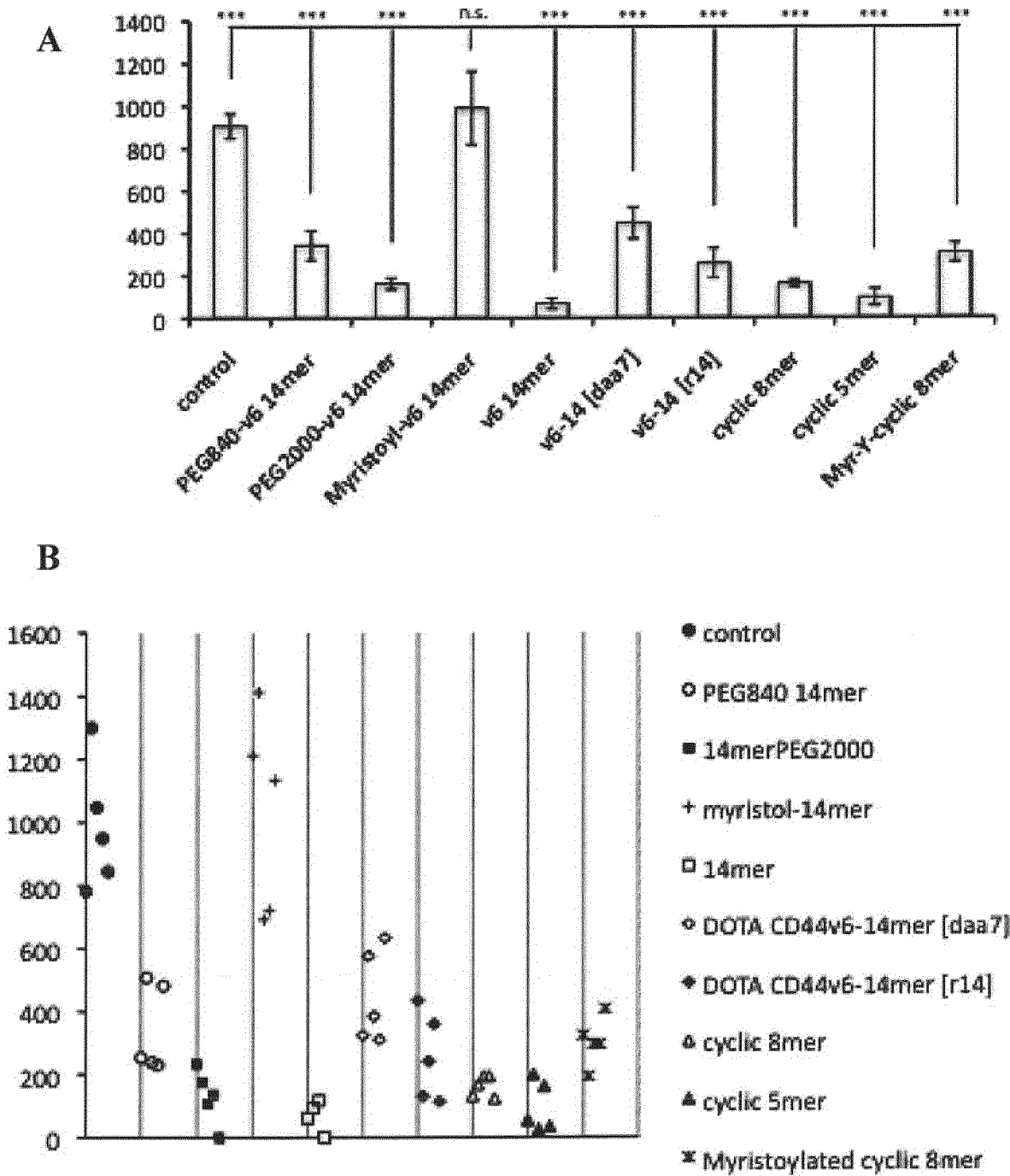
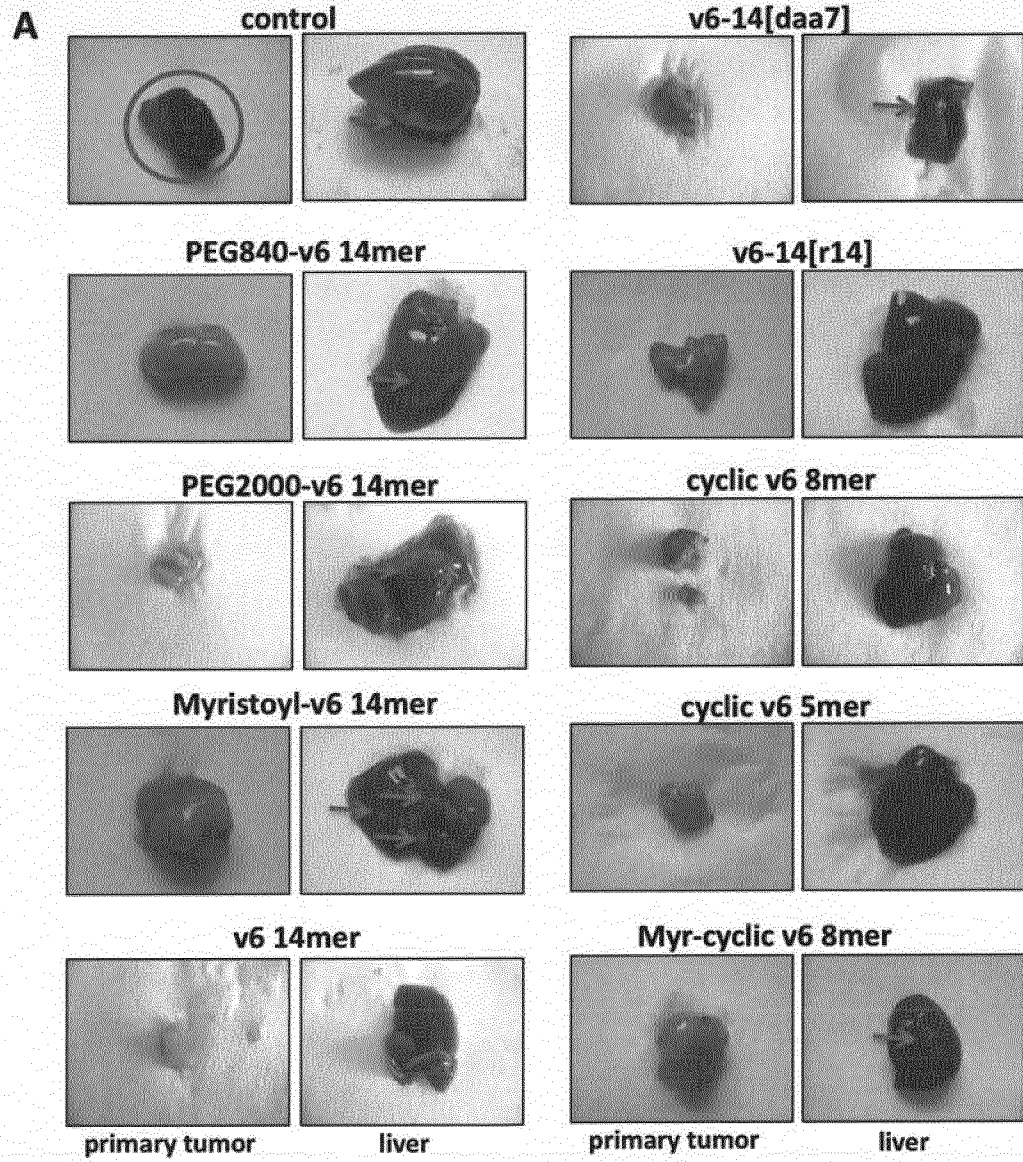


Figure 24



B

group nr.	treatment	animals with metastasis	average nr. of metastases
1	control	5/5	13
2	PEG840-v6 14mer	2/5	5
3	PEG2000-v6 14mer	1/5	2
4	Myristoyl-v6 14mer	5/5	12
5	v6 14mer	0/5	0
6	v6-14 [daa ₇]	1/5	2
7	v6-14 [r ¹⁴]	0/5	0
8	cyclic 8mer	0/5	0
9	cyclic 5mer	0/5	0
10	Myr-Y-cyclic 8mer	3/5	12

Figure 25

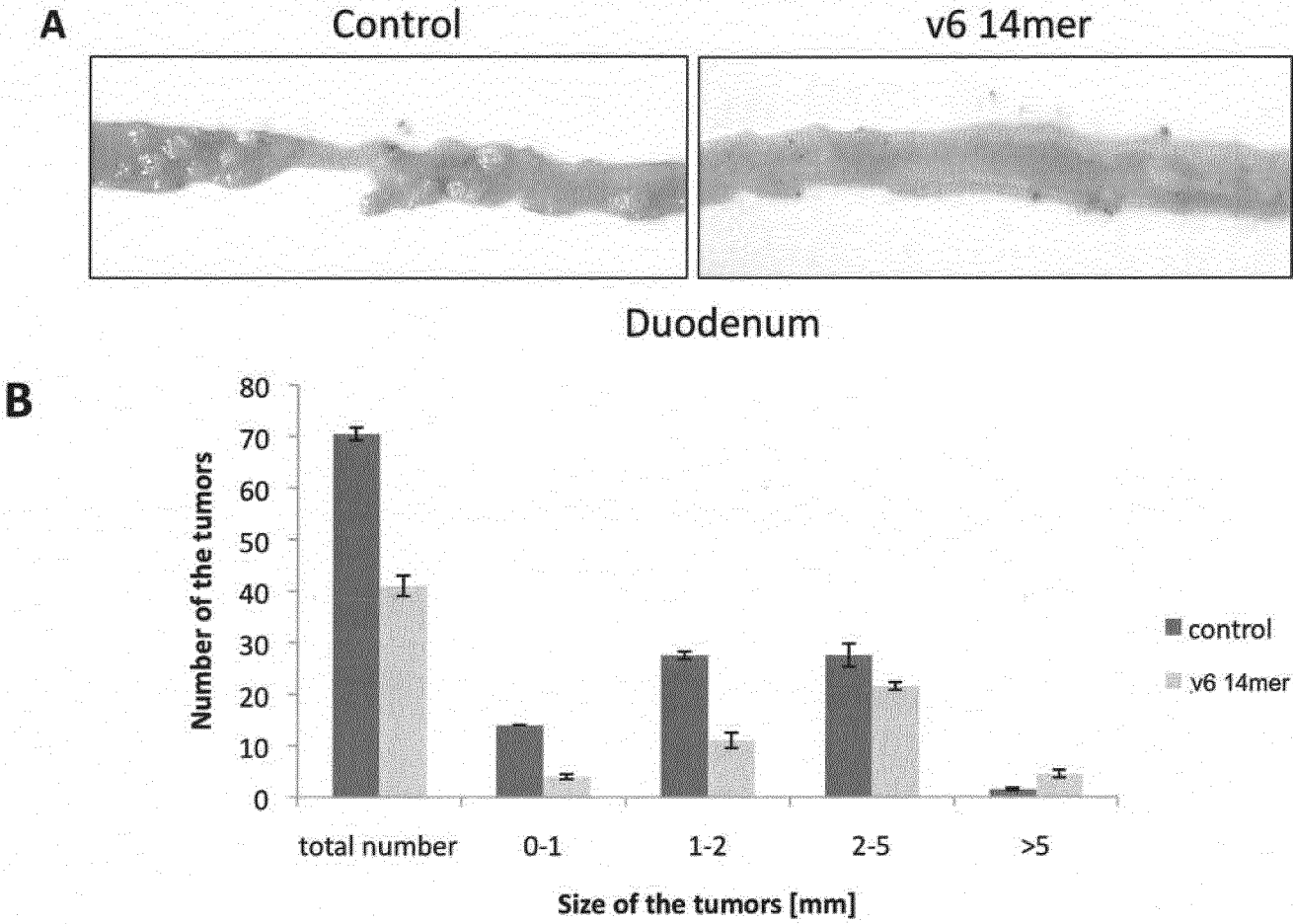


Figure 26

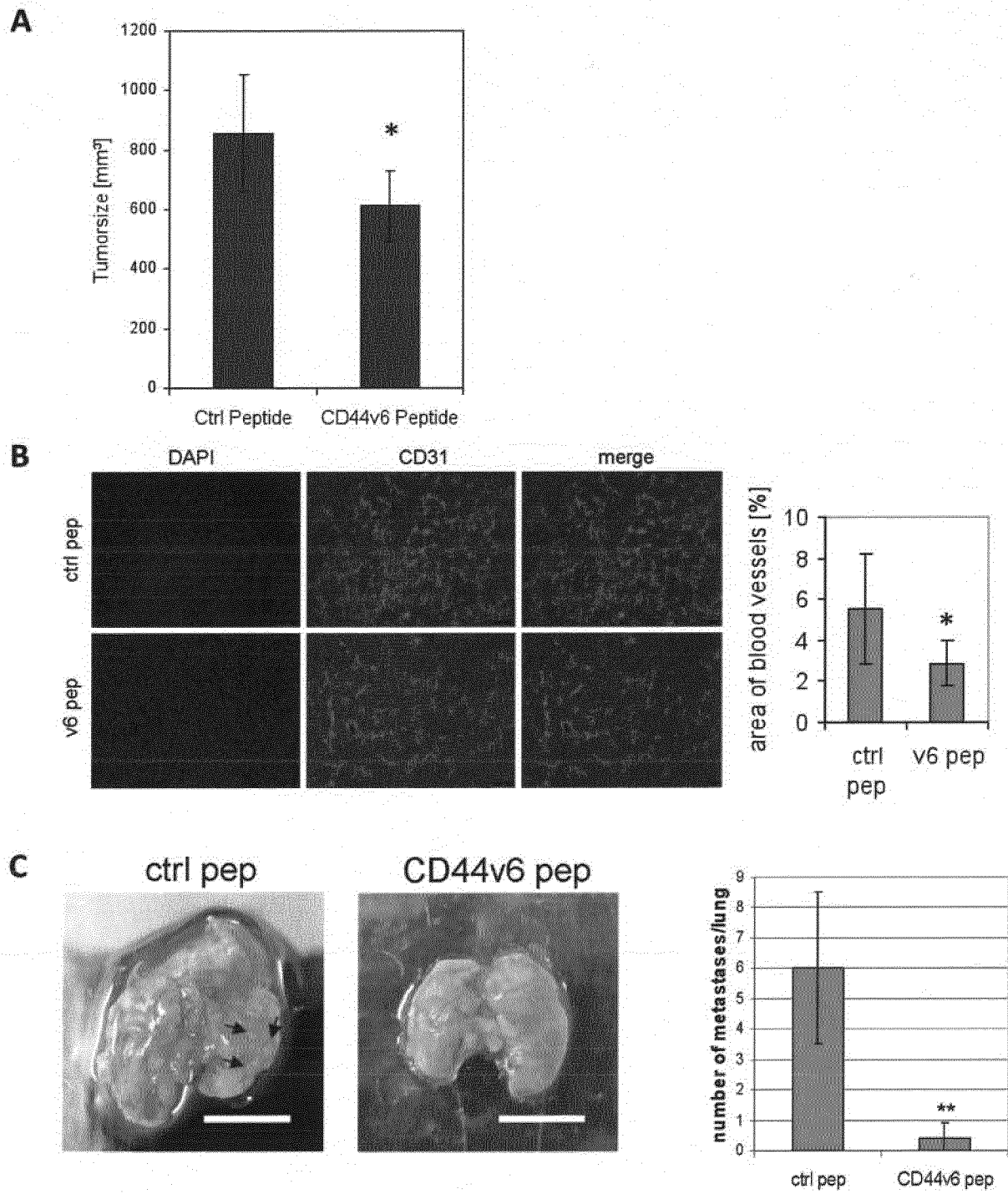
** = $P < 0.005$

Figure 27

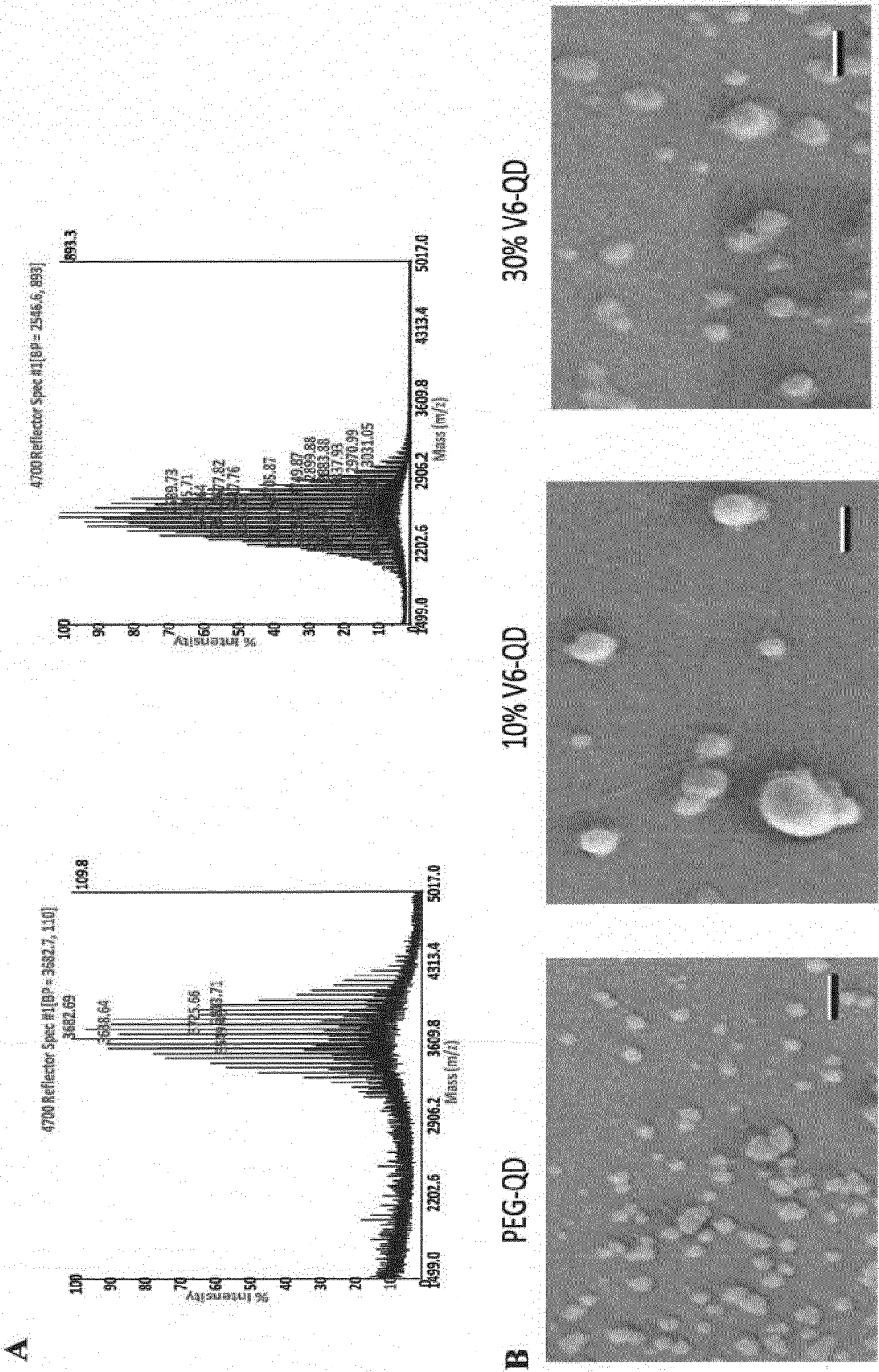


Figure 28

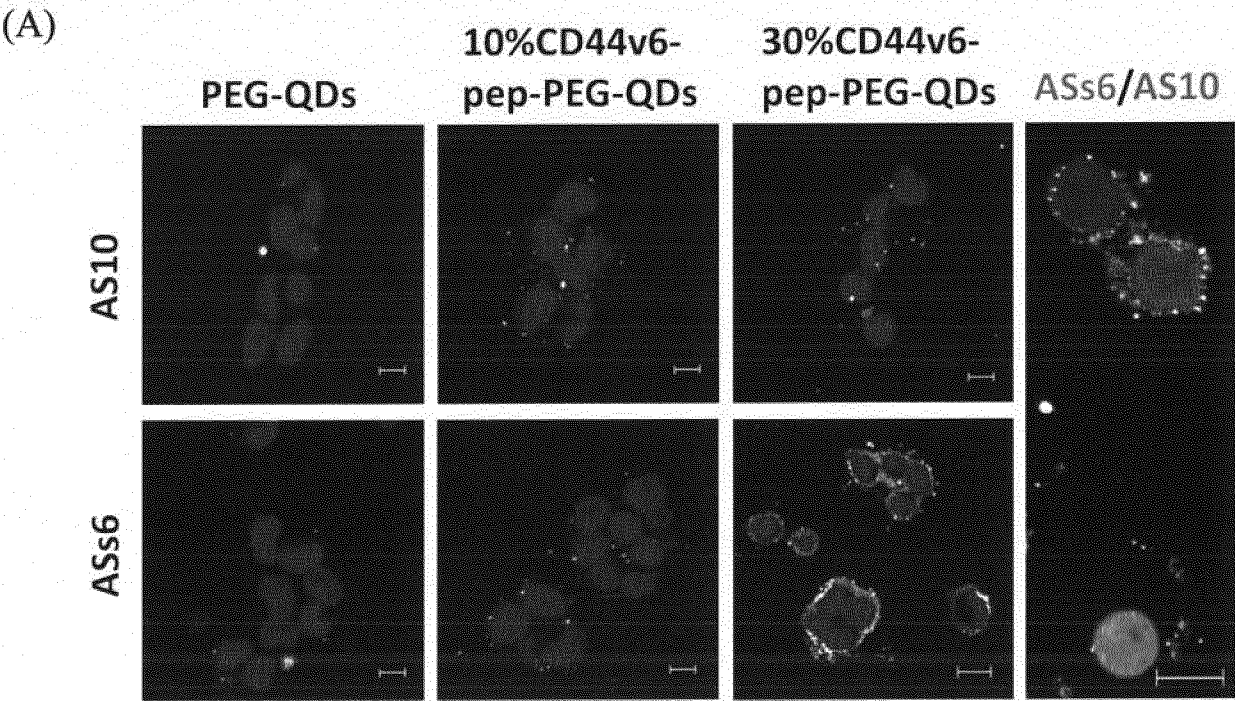
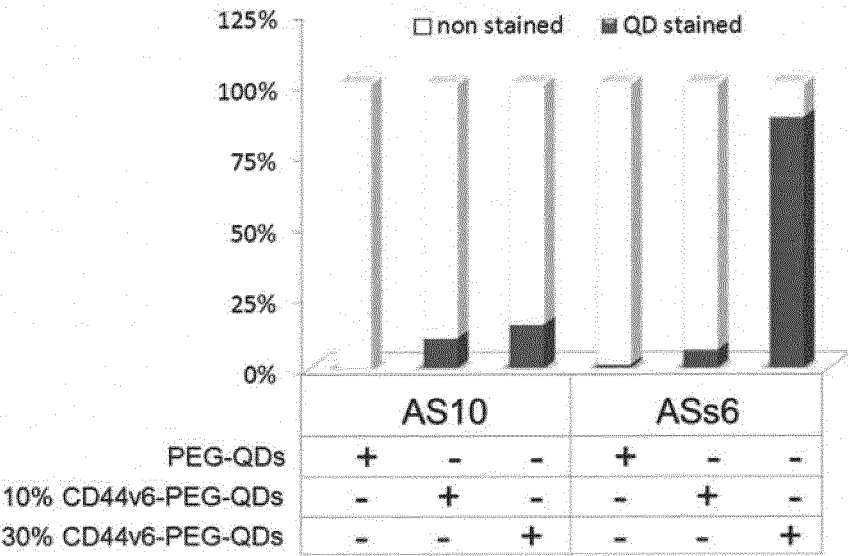
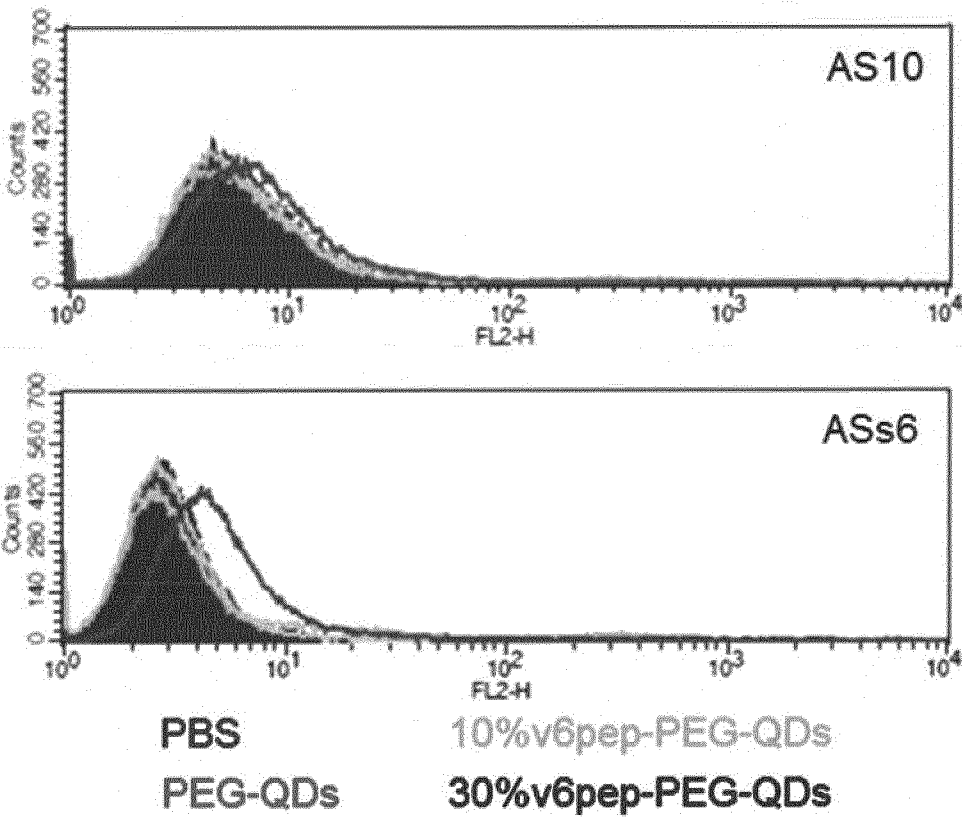


Figure 28 continued)

(B)



C



Page 1 of 15

SEQUENCE LISTING

<110> KARLSRUHER INST. FÜR TECHNOLOGIE, amcure GmbH
 <120> CD44v6-derived peptides for treating metastasizing cancers
 <130> A 13689-WO
 <160> 18
 <170> PatentIn version 3.5
 <210> 1
 <211> 5
 <212> PRT
 <213> Artificial Sequence
 <220>
 <223> peptide
 <220>
 <221> VARIANT
 <222> (1) .. (1)
 <223> /replace="Ala"
 /replace="Cys"
 /replace="Asp"
 /replace="Glu"
 /replace="Phe"
 /replace="Gly"
 /replace="His"
 /replace="Ile"
 /replace="Lys"
 /replace="Leu"
 /replace="Met"
 /replace="Pro"
 /replace="Gln"
 /replace="Arg"
 /replace="Ser"
 /replace="Thr"
 /replace="Val"
 /replace="Trp"
 /replace="Tyr"
 <220>
 <221> VARIANT
 <222> (5) .. (5)
 <223> /replace="Ala"
 /replace="Cys"
 /replace="Asp"
 /replace="Phe"
 /replace="Gly"
 /replace="His"
 /replace="Ile"
 /replace="Lys"
 /replace="Leu"
 /replace="Met"
 /replace="Asn"
 /replace="Pro"
 /replace="Gln"
 /replace="Arg"
 /replace="Ser"
 /replace="Thr"

Page 2 of 15

```

/replace="Val"
/replace="Trp"
/replace="Tyr"

```

<400> 1

```

Asn Arg Trp His Glu
1           5

```

<210> 2

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> peptide

<400> 2

```

Asn Arg Trp His Glu
1           5

```

<210> 3

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> peptide

<400> 3

```

Ala Arg Trp His Ala
1           5

```

<210> 4

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> peptide

<220>

<221> VARIANT

<222> (1) .. (1)

<223> /replace="Lys"

/replace="Arg"

/replace="Gln"

/replace="Ala"

/replace="Val"

/replace="Leu"

/replace="Ile"

<220>

<221> VARIANT

<222> (5) .. (5)

<223> /replace="Asp"

/replace="Ala"

Page 3 of 15

```

/replace="Val"
/replace="Leu"
/replace="Ile"

```

<400> 4

```

Asn Arg Trp His Glu
1          5

```

```

<210> 5
<211> 5
<212> PRT
<213> Artificial Sequence

```

```

<220>
<223> peptide

```

```

<220>
<221> VARIANT
<222> (1) .. (1)
<223> /replace="Lys"
      /replace="Arg"
      /replace="Gln"

```

```

<220>
<221> VARIANT
<222> (5) .. (5)
<223> /replace="Asp"

```

<400> 5

```

Asn Arg Trp His Glu
1          5

```

```

<210> 6
<211> 14
<212> PRT
<213> Artificial Sequence

```

```

<220>
<223> peptide

```

<400> 6

```

Lys Glu Gln Trp Phe Gly Asn Arg Trp His Glu Gly Tyr Arg
1          5          10

```

```

<210> 7
<211> 14
<212> PRT
<213> Artificial Sequence

```

```

<220>
<223> peptide

```

```

<220>
<221> VARIANT
<222> (1) .. (1)

```

<223> /replace="Ala"
 /replace="Cys"
 /replace="Asp"
 /replace="Glu"
 /replace="Phe"
 /replace="Gly"
 /replace="His"
 /replace="Ile"
 /replace="Leu"
 /replace="Met"
 /replace="Asn"
 /replace="Pro"
 /replace="Gln"
 /replace="Arg"
 /replace="Ser"
 /replace="Thr"
 /replace="Val"
 /replace="Trp"
 /replace="Tyr"

<220>

<221> VARIANT

<222> (2) . . (2)

<223> /replace="Ala"
 /replace="Cys"
 /replace="Asp"
 /replace="Phe"
 /replace="Gly"
 /replace="His"
 /replace="Ile"
 /replace="Lys"
 /replace="Leu"
 /replace="Met"
 /replace="Asn"
 /replace="Pro"
 /replace="Gln"
 /replace="Arg"
 /replace="Ser"
 /replace="Thr"
 /replace="Val"
 /replace="Trp"
 /replace="Tyr"

<220>

<221> VARIANT

<222> (3) . . (3)

<223> /replace="Ala"
 /replace="Cys"
 /replace="Asp"
 /replace="Glu"
 /replace="Phe"
 /replace="Gly"
 /replace="His"
 /replace="Ile"
 /replace="Lys"
 /replace="Leu"
 /replace="Met"
 /replace="Asn"
 /replace="Pro"
 /replace="Arg"
 /replace="Ser"
 /replace="Thr"
 /replace="Val"

Page 5 of 15

```

        /replace="Trp"
        /replace="Tyr"

<220>
<221> VARIANT
<222> (4) . . (4)
<223> /replace="Ala"
        /replace="Cys"
        /replace="Asp"
        /replace="Glu"
        /replace="Phe"
        /replace="Gly"
        /replace="His"
        /replace="Ile"
        /replace="Lys"
        /replace="Leu"
        /replace="Met"
        /replace="Asn"
        /replace="Pro"
        /replace="Gln"
        /replace="Arg"
        /replace="Ser"
        /replace="Thr"
        /replace="Val"
        /replace="Tyr"

<220>
<221> VARIANT
<222> (5) . . (5)
<223> /replace="Ala"
        /replace="Cys"
        /replace="Asp"
        /replace="Glu"
        /replace="Gly"
        /replace="His"
        /replace="Ile"
        /replace="Lys"
        /replace="Leu"
        /replace="Met"
        /replace="Asn"
        /replace="Pro"
        /replace="Gln"
        /replace="Arg"
        /replace="Ser"
        /replace="Thr"
        /replace="Val"
        /replace="Trp"
        /replace="Tyr"

<220>
<221> VARIANT
<222> (6) . . (6)
<223> /replace="Ala"
        /replace="Cys"
        /replace="Asp"
        /replace="Glu"
        /replace="Phe"
        /replace="His"
        /replace="Ile"
        /replace="Lys"
        /replace="Leu"
        /replace="Met"
        /replace="Asn"

```

Page 6 of 15

```

/replace="Pro"
/replace="Gln"
/replace="Arg"
/replace="Ser"
/replace="Thr"
/replace="Val"
/replace="Trp"
/replace="Tyr"

<220>
<221> VARIANT
<222> (7) .. (7)
<223> /replace="Ala"
/replace="Cys"
/replace="Asp"
/replace="Glu"
/replace="Phe"
/replace="Gly"
/replace="His"
/replace="Ile"
/replace="Lys"
/replace="Leu"
/replace="Met"
/replace="Pro"
/replace="Gln"
/replace="Arg"
/replace="Ser"
/replace="Thr"
/replace="Val"
/replace="Trp"
/replace="Tyr"

<220>
<221> VARIANT
<222> (11) .. (11)
<223> /replace="Ala"
/replace="Cys"
/replace="Asp"
/replace="Phe"
/replace="Gly"
/replace="His"
/replace="Ile"
/replace="Lys"
/replace="Leu"
/replace="Met"
/replace="Asn"
/replace="Pro"
/replace="Gln"
/replace="Arg"
/replace="Ser"
/replace="Thr"
/replace="Val"
/replace="Trp"
/replace="Tyr"

<220>
<221> VARIANT
<222> (12) .. (12)
<223> /replace="Ala"
/replace="Cys"
/replace="Asp"
/replace="Glu"
/replace="Phe"

```

Page 7 of 15

```

/replace="His"
/replace="Ile"
/replace="Lys"
/replace="Leu"
/replace="Met"
/replace="Asn"
/replace="Pro"
/replace="Gln"
/replace="Arg"
/replace="Ser"
/replace="Thr"
/replace="Val"
/replace="Trp"
/replace="Tyr"

<220>
<221> VARIANT
<222> (13) .. (13)
<223> /replace="Ala"
      /replace="Cys"
      /replace="Asp"
      /replace="Glu"
      /replace="Phe"
      /replace="Gly"
      /replace="His"
      /replace="Ile"
      /replace="Lys"
      /replace="Leu"
      /replace="Met"
      /replace="Asn"
      /replace="Pro"
      /replace="Gln"
      /replace="Arg"
      /replace="Ser"
      /replace="Thr"
      /replace="Val"
      /replace="Trp"

<220>
<221> VARIANT
<222> (14) .. (14)
<223> /replace="Ala"
      /replace="Cys"
      /replace="Asp"
      /replace="Glu"
      /replace="Phe"
      /replace="Gly"
      /replace="His"
      /replace="Ile"
      /replace="Lys"
      /replace="Leu"
      /replace="Met"
      /replace="Asn"
      /replace="Pro"
      /replace="Gln"
      /replace="Ser"
      /replace="Thr"
      /replace="Val"
      /replace="Trp"
      /replace="Tyr"

<400> 7

```

Page 8 of 15

Lys Glu Gln Trp Phe Gly Asn Arg Trp His Glu Gly Tyr Arg
 1 5 10

<210> 8
 <211> 14
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> peptide

<220>
 <221> VARIANT
 <222> (1) .. (1)
 <223> /replace="Arg"
 /replace="Asn"
 /replace="Gln"
 /replace="Ala"
 /replace="Val"
 /replace="Leu"
 /replace="Ile"

<220>
 <221> VARIANT
 <222> (2) .. (2)
 <223> /replace="Asp"
 /replace="Ala"
 /replace="Val"
 /replace="Leu"
 /replace="Ile"

<220>
 <221> VARIANT
 <222> (3) .. (3)
 <223> /replace="Lys"
 /replace="Arg"
 /replace="Asn"
 /replace="Ala"
 /replace="Val"
 /replace="Leu"
 /replace="Ile"

<220>
 <221> VARIANT
 <222> (4) .. (4)
 <223> /replace="Phe"
 /replace="Tyr"
 /replace="Ala"
 /replace="Val"
 /replace="Leu"
 /replace="Ile"

<220>
 <221> VARIANT
 <222> (5) .. (5)
 <223> /replace="Trp"
 /replace="Tyr"
 /replace="Ala"
 /replace="Val"
 /replace="Leu"
 /replace="Ile"

Page 9 of 15

```

<220>
<221> VARIANT
<222> (6) .. (6)
<223> /replace="Ala"
      /replace="Val"
      /replace="Leu"
      /replace="Ile"

```

```

<220>
<221> VARIANT
<222> (7) .. (7)
<223> /replace="Lys"
      /replace="Arg"
      /replace="Gln"
      /replace="Ala"
      /replace="Val"
      /replace="Leu"
      /replace="Ile"

```

```

<220>
<221> VARIANT
<222> (11) .. (11)
<223> /replace="Asp"
      /replace="Ala"
      /replace="Val"
      /replace="Leu"
      /replace="Ile"

```

```

<220>
<221> VARIANT
<222> (12) .. (12)
<223> /replace="Ala"
      /replace="Val"
      /replace="Leu"
      /replace="Ile"

```

```

<220>
<221> VARIANT
<222> (13) .. (13)
<223> /replace="Phe"
      /replace="Trp"
      /replace="Ala"
      /replace="Val"
      /replace="Leu"
      /replace="Ile"

```

```

<220>
<221> VARIANT
<222> (14) .. (14)
<223> /replace="Lys"
      /replace="Asn"
      /replace="Gln"
      /replace="Ala"
      /replace="Val"
      /replace="Leu"
      /replace="Ile"

```

```

<400> 8

```

```

Lys Glu Gln Trp Phe Gly Asn Arg Trp His Glu Gly Tyr Arg
1          5          10

```


Page 10 of 15

<210> 9
 <211> 14
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> peptide

<220>
 <221> VARIANT
 <222> (1) .. (1)
 <223> /replace="Arg"
 /replace="Asn"
 /replace="Gln"

<220>
 <221> VARIANT
 <222> (2) .. (2)
 <223> /replace="Asp"

<220>
 <221> VARIANT
 <222> (3) .. (3)
 <223> /replace="Lys"
 /replace="Arg"
 /replace="Asn"

<220>
 <221> VARIANT
 <222> (4) .. (4)
 <223> /replace="Phe"
 /replace="Tyr"

<220>
 <221> VARIANT
 <222> (5) .. (5)
 <223> /replace="Trp"
 /replace="Tyr"

<220>
 <221> VARIANT
 <222> (6) .. (6)
 <223> /replace="Ala"
 /replace="Val"
 /replace="Leu"
 /replace="Ile"

<220>
 <221> VARIANT
 <222> (7) .. (7)
 <223> /replace="Lys"
 /replace="Arg"
 /replace="Gln"

<220>
 <221> VARIANT
 <222> (11) .. (11)
 <223> /replace="Asp"

<220>
 <221> VARIANT

Page 11 of 15

<222> (12) .. (12)
 <223> /replace="Ala"
 /replace="Val"
 /replace="Leu"
 /replace="Ile"

<220>
 <221> VARIANT
 <222> (13) .. (13)
 <223> /replace="Phe"
 /replace="Trp"

<220>
 <221> VARIANT
 <222> (14) .. (14)
 <223> /replace="Lys"
 /replace="Asn"
 /replace="Gln"

<400> 9

Lys Glu Gln Trp Phe Gly Asn Arg Trp His Glu Gly Tyr Arg
 1 5 10

<210> 10
 <211> 14
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> peptide

<400> 10

Lys Glu Lys Trp Phe Glu Asn Glu Trp Gln Gly Lys Asn Pro
 1 5 10

<210> 11
 <211> 5
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> peptide

<400> 11

Asn Glu Trp Gln Gly
 1 5

<210> 12
 <211> 11
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> peptide

<400> 12

Page 12 of 15

Trp Phe Glu Asn Glu Trp Gln Gly Lys Asn Pro
 1 5 10

<210> 13
 <211> 11
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> peptide

<400> 13

Trp Phe Gln Asn Gly Trp Gln Gly Lys Asn Pro
 1 5 10

<210> 14
 <211> 11
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> peptide

<400> 14

Trp Phe Gly Asn Arg Trp His Glu Gly Tyr Arg
 1 5 10

<210> 15
 <211> 5
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> peptide

<400> 15

Asn Ala Ala Ala Gly
 1 5

<210> 16
 <211> 8
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> peptide

<400> 16

Phe Gly Asn Arg Trp His Glu Gly
 1 5

<210> 17
 <211> 8
 <212> PRT

Page 13 of 15

<213> Artificial Sequence

<220>

<223> peptide

<220>

<221> VARIANT

<222> (1) . . (1)

<223> /replace="Ala"
 /replace="Cys"
 /replace="Asp"
 /replace="Glu"
 /replace="Gly"
 /replace="His"
 /replace="Ile"
 /replace="Lys"
 /replace="Leu"
 /replace="Met"
 /replace="Asn"
 /replace="Pro"
 /replace="Gln"
 /replace="Arg"
 /replace="Ser"
 /replace="Thr"
 /replace="Val"
 /replace="Trp"
 /replace="Tyr"

<220>

<221> VARIANT

<222> (2) . . (2)

<223> /replace="Ala"
 /replace="Cys"
 /replace="Asp"
 /replace="Glu"
 /replace="Phe"
 /replace="His"
 /replace="Ile"
 /replace="Lys"
 /replace="Leu"
 /replace="Met"
 /replace="Asn"
 /replace="Pro"
 /replace="Gln"
 /replace="Arg"
 /replace="Ser"
 /replace="Thr"
 /replace="Val"
 /replace="Trp"
 /replace="Tyr"

<220>

<221> VARIANT

<222> (3) . . (3)

<223> /replace="Ala"
 /replace="Cys"
 /replace="Asp"
 /replace="Glu"
 /replace="Phe"
 /replace="Gly"
 /replace="His"
 /replace="Ile"

Page 14 of 15

```

/replace="Lys"
/replace="Leu"
/replace="Met"
/replace="Pro"
/replace="Gln"
/replace="Arg"
/replace="Ser"
/replace="Thr"
/replace="Val"
/replace="Trp"
/replace="Tyr"

<220>
<221> VARIANT
<222> (7) .. (7)
<223> /replace="Ala"
      /replace="Cys"
      /replace="Asp"
      /replace="Phe"
      /replace="Gly"
      /replace="His"
      /replace="Ile"
      /replace="Lys"
      /replace="Leu"
      /replace="Met"
      /replace="Asn"
      /replace="Pro"
      /replace="Gln"
      /replace="Arg"
      /replace="Ser"
      /replace="Thr"
      /replace="Val"
      /replace="Trp"
      /replace="Tyr"

<220>
<221> VARIANT
<222> (8) .. (8)
<223> /replace="Ala"
      /replace="Cys"
      /replace="Asp"
      /replace="Glu"
      /replace="Phe"
      /replace="His"
      /replace="Ile"
      /replace="Lys"
      /replace="Leu"
      /replace="Met"
      /replace="Asn"
      /replace="Pro"
      /replace="Gln"
      /replace="Arg"
      /replace="Ser"
      /replace="Thr"
      /replace="Val"
      /replace="Trp"
      /replace="Tyr"

<400> 17

Phe Gly Asn Arg Trp His Glu Gly
1          5

```

Page 15 of 15

<210> 18
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> peptide

<400> 18

Lys Gly Asn Arg Trp His Glu Gly
1 5