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(54) Title: MEDICAL USE OF COMBINATIONS OF NON-NATURAL SEMAPHORINS 3 AND ANTIMETABOLITES

(57) Abstract: The invention relates to a combination of an antimetabolite and a mutated Semaphorin 3 or a functional fragment thereof and the medical use of the combination in the treatment of a tumor and/or cancer (in particular a pancreatic tumor and/or cancer). The combination comprises the antimetabolite and the mutated Semaphorin 3 or the functional fragment thereof, wherein the mutated Semaphorin 3 is selected from the group consisting of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C, and Semaphorin 3D, and wherein the mutated Semaphorin 3 or the functional fragment thereof comprises an amino acid sequence CX₁X₂A₃GKD, and wherein the alanine (A₃) is replaced by a hydrophilic amino acid; or a fusion protein comprising the mutated Semaphorin 3 or the functional fragment thereof; or a nucleic acid molecule encoding the same. Further, the present invention relates to a pharmaceutical composition or a kit of parts comprising the combination.



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Medical use of combinations of non-natural Semaphorins 3 and antimetabolites

The present invention relates to a combination of an antimetabolite and a mutated Semaphorin 3 or a functional fragment thereof and the medical use of the combination in the treatment of a tumor and/or cancer. The inventive combination comprises the antimetabolite and the mutated Semaphorin 3 or the functional fragment thereof, wherein the mutated Semaphorin 3 is selected from the group consisting of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C, and Semaphorin 3D, and wherein the mutated Semaphorin 3 or the functional fragment thereof comprises an amino acid sequence $CX_1X_2A_3GKD$, wherein X_1 is an amino acid, which is K or N, X_2 is an amino acid selected from the group of W, M and L, and wherein the alanine (A_3) is replaced by a hydrophilic amino acid; or the inventive combination comprises the antimetabolite and a fusion protein comprising the mutated Semaphorin 3 or the functional fragment thereof; or the inventive combination comprises the antimetabolite and a nucleic acid molecule encoding the mutated Semaphorin 3 or the functional fragment thereof, or the inventive combination comprises the antimetabolite and a nucleic acid molecule encoding the fusion protein. Further, the present invention relates to a pharmaceutical composition or a kit/kit of parts comprising the combination. The present invention further relates to the use of the combination, the kits/kits of parts or the pharmaceutical composition in therapy of a tumor and/or cancer (in particular pancreatic tumors and/or cancer) wherein the survival of a subject is prolonged, the tumor growth is reduced and/or the metastasis, in particular liver metastasis, is reduced compared to a subject being administered the antimetabolite or the mutated Semaphorin 3 alone.

The successful treatment of tumors and/or cancer, in particular pancreatic cancers, is highly desired. Patients suffering from pancreatic tumors or cancers and receiving current therapies have a poor prognosis; see e.g. "Cancer survival for common cancers" <http://www.cancerresearchuk.org/health-professional/cancer-statistics/survival/common-cancers-compared#heading-Zero>; or Quaresma M, Coleman MP, Rachet B. 40-year trends in an index of survival for all cancers combined and survival adjusted for age and sex for each cancer in England and Wales, 1971-2011: a population-based study. *Lancet* 2014 pii: S0140-6736(14)61396-9.

The technical problem underlying the present invention is the provision of means and methods for an improved therapy of tumors and/or cancer, in particular pancreatic tumors and/or cancer.

The technical problem is solved by provision of the embodiments provided herein below and as characterized in the claims.

The present invention relates to a combination comprising:

- a) an antimetabolite; and
- bi) a mutated Semaphorin 3 or a functional fragment thereof, wherein the mutated Semaphorin 3 is selected from the group consisting of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C, and Semaphorin 3D, and wherein the mutated Semaphorin 3 or the functional fragment thereof comprises an amino acid sequence $CX_1X_2A_3GKD$, wherein
 X_1 is an amino acid, which is K or N,
 X_2 is an amino acid selected from the group of W, M and L,
and wherein the alanine (A_3) is replaced by a hydrophilic amino acid; or
- bii) a fusion protein comprising the mutated Semaphorin 3 or the functional fragment thereof; or
- biii) a nucleic acid molecule encoding the mutated Semaphorin 3 or the functional fragment thereof, or a nucleic acid molecule encoding the fusion protein.

The present invention particularly relates to the combination for use in the treatment of tumor and/or cancer (e.g. pancreatic tumor and/or pancreatic cancer), wherein the combination comprises:

- a) an antimetabolite; and
- bi) a mutated Semaphorin 3 or a functional fragment thereof, wherein the mutated Semaphorin 3 is selected from the group consisting of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C, and Semaphorin 3D, and wherein the mutated Semaphorin 3 or the functional fragment thereof comprises an amino acid sequence $CX_1X_2A_3GKD$, wherein
 X_1 is an amino acid, which is K or N,
 X_2 is an amino acid selected from the group of W, M and L,
and wherein the alanine (A_3) is replaced by a hydrophilic amino acid; or
- bii) a fusion protein comprising the mutated Semaphorin 3 or the functional fragment thereof; or
- biii) a nucleic acid molecule encoding the mutated Semaphorin 3 or the functional fragment thereof, or a nucleic acid molecule encoding the fusion protein.

In most preferred aspects, the invention relates to the combination of gemcitabine and the mutated Semaphorin 3 or the functional fragment thereof, for example the fusion protein comprising the mutated Semaphorin 3 or the functional fragment thereof. The fusion protein is also disclosed in WO/2016135130 that is incorporated herein by its entirety. WO/2016135130 discloses in Figure 2A a preferred fusion protein that is incorporated herein.

It was surprisingly found that the combination of the mutated Semaphorin 3 and the antimetabolite, e.g. gemcitabine, demonstrates improved medical effects compared to the mutated Semaphorin 3 or the antimetabolite used as single drugs in *in vivo* cancer models; see e.g. appended Figure 1 A) and B).

The appended examples demonstrate that the combination comprising the non-naturally occurring/artificial/mutated Semaphorins 3 (or their herein described functional fragments and/or fusion proteins comprising said non-naturally occurring/artificial/mutated Semaphorins or said non-naturally occurring/artificial/mutated functional fragments of said Semaphorins) having an amino acid sequence CX₁X₂A₃GKD, wherein the alanine (A₃) is replaced by a hydrophilic amino acid (e.g. lysine), and the antimetabolite, in particular gemcitabine, was significantly more efficient in reducing the tumor growth and the metastatic spread compared to the treatment with the fusion protein or the antimetabolite alone in *in vivo* cancer models (see e.g. Figure 1A and B). The mutated Semaphorin 3, the functional fragments thereof, the fusion protein comprising the mutated Semaphorin, and the nucleic acids molecules encoding the same are disclosed in WO/2016135130 that is incorporated herein by its entirety. In the appended examples, the herein provided combination demonstrates surprising beneficial medical effects. These medical effects are exemplified by Semaphorin 3A comprising the artificial replacement of the (naturally occurring) alanine (A₃) by a hydrophilic amino acid within the consensus motif CX₁X₂A₃GKD of Semaphorin 3 and gemcitabine as an exemplified antimetabolite.

As illustrated in the appended examples, it was unexpectedly found that the inventive combination comprising the mutated Semaphorin 3 and the antimetabolite, in particular gemcitabine, reduces tumor growth by 66% compared to controls (Fig. 1A). The single therapy, wherein either the mutated Semaphorin 3 or the antimetabolite (e.g. gemcitabine) is administered, reduces tumor volume by 50% and 40%, respectively (see e.g. Figure 1B). Figure 1A of the appended examples shows that the combination of the mutated Semaphorin 3 and the antimetabolite (e.g. gemcitabine) has an unexpected synergistic effect on the reduction of tumor growth. Furthermore, it was surprisingly demonstrated herein below that

the combination was significantly more efficient in reducing metastasis formation (in particular liver metastasis) compared to the therapy with the antimetabolite or the mutated Semaphorin 3 alone; see the appended Examples, in particular Figure 1B. The antimetabolite (e.g. gemcitabine) alone does not significantly inhibit metastasis formation as shown in Figure 1B. Therefore, Figure 1B demonstrates that the combination of the mutated Semaphorin 3 with the antimetabolite (e.g. gemcitabine) provides a surprising synergistic effect (i.e. over additive effect) on reducing metastasis. The mutated Semaphorin 3 or the functional fragment thereof normalizes the vasculature (in particular the tumor, tumorous disease, and/or the cancer vasculature). Thus, and without being bound by theory, the mutated Semaphorin 3 or the functional fragment thereof and in particular the constructs disclosed herein and comprising the same may enhance the delivery, availability and/or the efficacy of the antimetabolite (in particular gemcitabine) compared to a monotherapy with such an antimetabolite (i.e. a medical intervention without such mutated Semaphorins 3 / mutated Semaphorin 3 constructs. The results as provided herein and as exemplified in the experimental part of this invention constitute a highly surprising finding and document the over-additive effect of the combination therapy of cancers/comboination therapy of metastasis of this invention.

Accordingly, the appended examples demonstrate that the herein provided combination is an improved, synergistic anti-cancer therapy compared to the single agents. In particular, the application as filed documents that the inventive combination provides beneficial biological effects that allow for an improved anti-cancer/tumor therapy. As demonstrated herein above and below, the inventive combination reduces the tumor growth, prolongs the survival and/or reduces metastasis of subjects suffering from tumor compared to subjects that were treated with the antimetabolite or the mutated Semaphorin 3 alone.

The replacement of alanine (A₃) in the consensus motif CX₁X₂A₃GKD of mouse and/or human Semaphorin 3A, Sema3B, Sema3C and Sema3D renders the wildtype Semaphorin 3A, Sema3B, Sema3C and Sema3D as an improved angiogenesis inhibitor; see also WO/2016135130 that is herein incorporated in its entirety. Accordingly, the combination of the present invention comprises human Semaphorins 3 selected from the group consisting of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D (or functional fragments of these human Semaphorins comprising the consensus motif) comprising the herein defined mutation in alanine (A₃). Most preferably, the present invention relates to mutated human Semaphorin 3A (or functional fragments of said human Semaphorin 3A) comprising the herein defined motif with the herein described replacement of alanine (A₃) in the sequence motif CX₁X₂A₃GKD. Said replacement is a replacement with a hydrophilic amino acid, most preferably a replacement with a lysine (K). The mutated alanine (A₃) is part

of a highly conserved sequence motif $CX_1X_2A_3GKD$ that can be found in mouse and human Sema3A, Sema3B, Sema3C and Sema3D. Consistently, equal beneficial effects that are shown in the appended examples are envisaged from a replacement of said alanine by a hydrophilic amino acid (like K) in (human) Sema3B, Sema3C and/or Sema3D.

Human and mouse Sema3E, Sema3G and Sema3F have a naturally occurring hydrophilic amino acid where alanine A_3 resides in the consensus sequence $CX_1X_2A_3GK$. Hence, they have a hydrophilic amino acid at the position corresponding to the position 106 of Semaphorin 3A as shown in SEQ ID NO: 2. Both Sema3E and Sema3G comprise a lysine and Sema3F comprise a serine at this position. Yet, Sema 3E and Sema 3F failed to show the strong anti-angiogenic properties. The replacement of serine by lysine at the position 107 in Sema3F fails to increase the binding to the Plexin A, B, C, or D receptors. Furthermore, this mutant fails to inhibit EC migration more effectively than its wild type counterpart displaying a serine at the position 107. Moreover, Fc-tagged Semaphorin 3E and Semaphorin 3F fail to inhibit EC migration as strong as the exemplary mutated Semaphorin 3A comprising the inventive replacement of alanine by a hydrophilic amino acid. Consequently, the mutated Semaphorins herein comprised, namely mutated Semaphorin 3A, mutated Semaphorin 3B, mutated Semaphorin 3C, and mutated Semaphorin 3D are superior inhibitors of EC cell motility compared to Semaphorins 3 comprising a naturally occurring hydrophilic amino acid at the position corresponding to position 106 of Semaphorin 3A as given in SEQ ID NO: 2. Accordingly, the present invention does not comprise (human) Sema 3E, Sema 3F and/or Sema 3G.

The replacement of alanine (A_3) by, e.g., lysine in the consensus motif $CX_1X_2A_3GKD$ in Sema 3A/3B/3C or 3D (or in functional fragments thereof) (like, inter alia, in Sema3A the exchange A106K) results in increased inhibition of cancer growth and metastasis formation in two different transgenic mouse models, i.e., spontaneous pancreatic neuroendocrine cancer (RipTag2) and pancreatic ductal adenocarcinoma (PDAC) compared to wild type Semaphorins that are not comprising this mutation; see e.g. WO/2016135130 that is incorporated herein by its entirety. The PDAC mouse that is also used in the appended examples is a model for the frequently and deadly human pancreatic cancer histotype.

In summary, the appended examples demonstrate that the herein provided combination, pharmaceutical composition, or kit of parts allows an improved anti-cancer therapy compared to the single agents. The herein documented medical effects, e.g. the improved reduction of tumor growth, the prolonged survival and/or reduced metastasis provide for an advantageous therapy.

The invention relates to the following items:

1. A combination for use in the treatment of a tumor and/or cancer, wherein the combination comprises:
 - a) an antimetabolite; and
 - bi) a mutated Semaphorin 3 or a functional fragment thereof, wherein the mutated Semaphorin 3 is selected from the group consisting of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C, and Semaphorin 3D, and wherein the mutated Semaphorin 3 or the functional fragment thereof comprises an amino acid sequence $CX_1X_2A_3GKD$, wherein
 X_1 is an amino acid, which is K or N,
 X_2 is an amino acid selected from the group of W, M and L,
and wherein the alanine (A_3) is replaced by a hydrophilic amino acid; or
 - bii) a fusion protein comprising the mutated Semaphorin 3 or the functional fragment thereof; or
 - biii) a nucleic acid molecule encoding the mutated Semaphorin 3 or the functional fragment thereof, or a nucleic acid molecule encoding the fusion protein.
2. The combination of item 1, wherein the mutated Semaphorin 3, the functional fragment thereof, or the fusion protein inhibits angiogenesis and/or functions as vascular normalizing agent.
3. The combination of item 1 or 2, wherein the antimetabolite is selected from gemcitabine, azacytidine, capecitabine, clofarabine, cytarabine, doxifluridine, floxuridine, 5-fluorouracil and pharmaceutically acceptable salts and solvates thereof.
4. The combination according to any one of items 1 to 3, wherein the antimetabolite is selected from gemcitabine and pharmaceutically acceptable salts and solvates thereof.
5. The combination according to any one of items 1 to 4, wherein the tumor or cancer is selected from the group consisting of a tumor, preferably a solid tumor, and a tumorous disease.

6. The combination according to any one of items 1 to 5, wherein the tumor is a solid tumor.
7. The combination according to any one of items 1 to 6, wherein the tumor or cancer is selected from the group consisting of pancreatic cancer, cervical cancer, breast cancer, colon cancer, melanoma, prostate cancer, bladder cancer and tongue cancer.
8. The combination according to any one of items 1 to 7, wherein the tumor or cancer is a pancreatic tumor or pancreatic cancer, in particular wherein the cancer is a metastatic pancreatic cancer.
9. The combination according to any one of items 1 to 8, wherein the combination prolongs the survival of a subject suffering from a tumor or cancer compared to a pharmaceutical composition comprising the mutated Semaphorin 3, the functional fragment thereof, the fusion protein, or the antimetabolite alone.
10. The combination according to any one of items 1 to 9, wherein the combination reduces the tumor growth compared to a pharmaceutical composition comprising the mutated Semaphorin 3, the functional fragment thereof, the fusion protein, or the antimetabolite alone.
11. The combination according to any one of items 1 to 10, wherein the combination reduces metastasis, in particular liver metastasis, compared to a pharmaceutical composition comprising the mutated Semaphorin 3, the functional fragment thereof, the fusion protein, or the antimetabolite alone.
12. The combination according to any one of items 1 to 11, wherein the mutated Semaphorin 3 comprises Semaphorin 3A or a functional fragment thereof and comprises a hydrophilic amino acid at position 106 of SEQ ID NO: 2, and wherein the antimetabolite is gemcitabine.

13. The combination according to any one of items 1 to 12, wherein the mutated Semaphorin 3 comprises SEQ ID NO: 18 or functional fragments thereof, and wherein the antimetabolite is gemcitabine.

14. The combination of any one of items 1 to 13, wherein the mutated Semaphorin 3 or the functional fragment thereof comprises
the hydrophilic amino acid in place of the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2;
the hydrophilic amino acid in place of the alanine corresponding to position 105 of the wild type Semaphorin 3B as shown in SEQ ID NO: 6;
the hydrophilic amino acid in place of the alanine corresponding to position 104 of the wild type Semaphorin 3C as shown in SEQ ID NO: 10; or
the hydrophilic amino acid in place of the alanine corresponding to position 120 of the wild type Semaphorin 3D as shown in SEQ ID NO: 14.

15. The combination of any one of items 1 to 14, wherein the mutated Semaphorin 3 is selected from the group of:
 - (a) a polypeptide that is encoded by a nucleic acid sequence selected from the group of SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 9 and SEQ ID NO: 13, wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding the hydrophilic amino acid, wherein the nucleotides GCA at position 559 to 561 of SEQ ID NO: 5 are replaced by nucleotides encoding the hydrophilic amino acid, wherein the nucleotides GCT at position 872 to 874 of SEQ ID NO: 9 are replaced by nucleotides encoding the hydrophilic amino acid, and wherein the nucleotides GCC at position 398 to 400 of SEQ ID NO: 13 are replaced by nucleotides encoding the hydrophilic amino acid;
 - (b) a polypeptide having the amino acid sequence selected from the group of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 10 and SEQ ID NO: 14, wherein the alanine residue at position 106 of SEQ ID NO: 2, at position 105 of SEQ ID NO: 6, at position 104 of SEQ ID NO: 10 or at position 120 of SEQ ID NO: 14 is replaced by the hydrophilic amino acid;
 - (c) a polypeptide that is encoded by a nucleic acid sequence that hybridizes under

- stringent conditions to the complementary strand of a nucleic acid molecule encoding a polypeptide as defined in (a) or (b);
- (d) a polypeptide that inhibits of angiogenesis and/or functions as a vascular normalizing agent and has at least 55% identity to any one of the polypeptides referred to in (b).
16. The combination of any one of items 1 to 15, wherein the mutated Semaphorin 3 or the functional fragment thereof comprises a hydrophilic amino acid
- (a) at position 106 of SEQ ID NO: 2;
- (b) at position 105 of SEQ ID NO: 6;
- (c) at position 104 of SEQ ID NO: 10; or
- (d) at position 120 of SEQ ID NO: 14.
17. The combination of any one of items 1 to 16, wherein the mutated Semaphorin 3 or the functional fragment thereof comprises a functional sema domain and comprises at least one additional mutation selected from the group consisting of amino acid substitution(s), addition(s), deletions(s) and duplication(s).
18. The combination of any one of items 1 to 17, wherein the hydrophilic amino acid is selected from the group of K, R, N, Q, S,T, E, D, and H.
19. The combination of any one of items 1 to 18, wherein the hydrophilic amino acid is K or R.
20. The combination of item 19, wherein the hydrophilic amino acid replacing the alanine is K.
21. The combination of any one of items 1 to 20, wherein the mutated Semaphorin 3 or the functional fragment thereof comprises one or more of the following sequence(s) as defined in any one of SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47 or SEQ ID NO: 48.

22. The combination of any one of items 1 to 21, wherein the nucleic acid molecule encoding the mutated Semaphorin 3 or the functional fragment thereof comprises:
- (a) the nucleotides from 601 to 1206 of SEQ ID NO: 1 and wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding a hydrophilic amino acid;
 - (b) the nucleotides from 529 to 1137 of SEQ ID NO: 5 and wherein the nucleotides GCA at position 559 to 561 of SEQ ID NO: 5 are replaced by nucleotides encoding a hydrophilic amino acid;
 - (c) the nucleotides from 842 to 1444 of SEQ ID NO: 9 and wherein the nucleotides GCT at position 872 to 874 of SEQ ID NO: 9 are replaced by nucleotides encoding a hydrophilic amino acid; or
 - (d) the nucleotides from 368 to 982 of SEQ ID NO: 13 and wherein the nucleotides GCC at position 398 to 400 of SEQ ID NO: 13 are replaced by nucleotides encoding a hydrophilic amino acid.
23. The combination of any one of items 1 to 22, wherein the mutated Semaphorin 3 or the functional fragment thereof comprises the functional sema domain, wherein the sema domain is selected from an amino acid sequence as shown in:
- (a) SEQ ID NO: 21, wherein the alanine residue corresponding to position 106 of SEQ ID NO: 2 is replaced by a hydrophilic amino acid;
 - (b) SEQ ID NO: 22, wherein the alanine residue corresponding to position 105 of SEQ ID NO: 6 is replaced by a hydrophilic amino acid;
 - (c) SEQ ID NO: 23, wherein the alanine residue corresponding to position 104 of SEQ ID NO: 10 is replaced by a hydrophilic amino acid; or
 - (d) SEQ ID NO: 24, wherein the alanine residue corresponding to position 120 of SEQ ID NO: 14 is replaced by a hydrophilic amino acid.
24. The combination of any one of items 1 to 23, wherein the mutated Semaphorin 3 comprises an amino acid sequence that is selected from the group consisting of SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70 and SEQ ID NO: 72.
25. The combination of any one of items 1 to 24, wherein the fusion protein comprises a

polypeptide as defined in:

- (a) SEQ ID NO: 21, wherein the alanine residue corresponding to position 106 of SEQ ID NO: 2 is replaced by a hydrophilic amino acid;
 - (b) SEQ ID NO: 22, wherein the alanine residue corresponding to position 105 of SEQ ID NO: 6 is replaced by a hydrophilic amino acid;
 - (c) SEQ ID NO: 23, wherein the alanine residue corresponding to position 104 of SEQ ID NO: 10 is replaced by a hydrophilic amino acid; or
 - (d) SEQ ID NO: 24, wherein the alanine residue corresponding to position 120 of SEQ ID NO: 14 is replaced by a hydrophilic amino acid.
26. The combination of any one of items 1 to 25, wherein the fusion protein comprises:
- (a) a stabilizer domain; and/or
 - (b) a dimerization domain.
27. The combination of item 26, wherein
- (a) the stabilizer domain is a Plexin Semaphorin Integrin (PSI) domain, wherein the PSI domain comprises one or more of the following sequences SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47 or SEQ ID NO: 48; and/or
 - (b) the dimerization domain has a dissociation constant K_D in the range of 10^{-5} M to 10^{-6} M with another such dimerization domain and/or wherein the dimerization domain is selected from the group of a C-terminal IgG constant domain, DARPin and leucine zipper.
28. The combination of item 27, wherein the IgG constant domain is IgG1 or IgG3.
29. The combination of any one of items 1 to 28, wherein the nucleic acid molecule encoding the fusion protein comprises a nucleic acid sequence having:
- a nucleic acid sequence spanning from nucleotides 316 to 1959 of SEQ ID NO: 1 and a nucleic acid sequence spanning from nucleotides 295 to 990 of SEQ ID NO: 37, wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding a hydrophilic amino acid;
 - a nucleic acid sequence spanning from nucleotides 247 to 1887 of SEQ ID NO: 5 and a nucleic acid sequence spanning from nucleotides 295 to 990 of

SEQ ID NO: 37, wherein the nucleotides GCA at position 559 to 561 of SEQ ID NO: 5 are replaced by nucleotides encoding a hydrophilic amino acid; a nucleic acid sequence spanning from nucleotides 563 to 2197 of SEQ ID NO: 9 and a nucleic acid sequence spanning from nucleotides 295 to 990 of SEQ ID NO: 37, wherein the nucleotides GCT at position 872 to 874 of SEQ ID NO: 9 are replaced by nucleotides encoding a hydrophilic amino acid; or a nucleic acid sequence spanning from nucleotides 41 to 1735 of SEQ ID NO: 13 and a nucleic acid sequence spanning from nucleotides 295 to 990 of SEQ ID NO: 37, wherein the nucleotides GCC at position 398 to 400 of SEQ ID NO: 13 are replaced by nucleotides encoding a hydrophilic amino acid.

30. The combination of any one of items 1 to 29, wherein the fusion protein comprises an amino acid sequence:
- spanning from amino acid residues 1 to 548 of SEQ ID NO: 2 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 106 of SEQ ID NO: 2 is replaced by a hydrophilic amino acid;
 - spanning from amino acid residues 1 to 547 of SEQ ID NO: 6 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 105 of SEQ ID NO: 6 is replaced by a hydrophilic amino acid;
 - spanning from amino acid residues 1 to 565 of SEQ ID NO: 10 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 104 of SEQ ID NO: 10 is replaced by a hydrophilic amino acid; or
 - spanning from amino acid residues 1 to 545 of SEQ ID NO: 14 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 120 of SEQ ID NO: 14 is replaced by the hydrophilic amino acid.
31. The combination of any one of items 1 to 30, wherein the fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 18, SEQ ID NO: 76, SEQ ID NO: 78 and SEQ ID NO: 79.
32. The combination of any one of items 1 to 31, wherein the antimetabolite and the mutated Semaphorin 3, a functional fragment thereof, the fusion protein, or the nucleic acid molecule are to be administered to a subject in need thereof sequentially.

33. The combination of any one of items 1 to 32, wherein the antimetabolite and the mutated Semaphorin 3, a functional fragment thereof, the fusion protein, or the nucleic acid molecule are to be administered to a subject in need thereof concurrently.
34. The combination of any one of items 1 to 32, wherein the antimetabolite and the mutated Semaphorin 3, a functional fragment thereof, the fusion protein, or the nucleic acid molecule are to be administered to a subject in need thereof via different administration routes.
35. The combination of any one of items 1 to 34, wherein the combination is to be administered parenterally.
36. The combination of any one of items 1 to 35, wherein the antimetabolite is to be administered in a dose of about 100 to 10000 mg/m² body surface, and wherein the mutated Semaphorin 3, the functional fragment thereof, or the fusion protein is to be administered in a dose of about 1 µg protein /kg/day to 15 mg protein /kg/day mg/kg of body weight
37. The combination according to any one of item 1 to 36, wherein the nucleic acid molecule is a vector.
38. The combination according to item 37, wherein the vector is a gene targeting vector or a gene transfer vector.
39. The combination according to item 36 or 37, wherein the vector is an adeno-associated-virus (AAV) vector.
40. The combination according to item 39, wherein the adeno-associated-virus is an AAV8 vector.
41. A kit comprising:
 - a) the antimetabolite as defined in any one of the preceding items; and

- b) the mutated Semaphorin 3 or the functional fragment thereof, the fusion protein comprising the mutated Semaphorin 3 or the functional fragment thereof, or the nucleic acid molecule encoding the mutated Semaphorin 3 or the functional fragment thereof, or the nucleic acid molecule encoding the fusion protein as defined in any one of the preceding items.
42. A pharmaceutical composition comprising:
- a) the antimetabolite as defined in any one of the preceding items; and
- b) the mutated Semaphorin 3 or the functional fragment thereof, the fusion protein comprising the mutated Semaphorin 3 or the functional fragment thereof, or the nucleic acid molecule encoding the mutated Semaphorin 3 or the functional fragment thereof, or the nucleic acid molecule encoding the fusion protein as defined in any one of the preceding items; and
- optionally comprising one or more pharmaceutical excipients.
43. The kit or the pharmaceutical composition of any one of the preceding items for use in medicine.
44. The kit or the pharmaceutical composition of any one of the preceding items for use in the treatment of a tumor, tumorous disease and/or a cancer.
45. The kit or the pharmaceutical composition of any one of the preceding items for use in the treatment of a pancreatic tumor or pancreatic cancer.
46. A method of treatment for a tumor comprising the step of administering to a subject in need of such treatment a pharmaceutical effective amount of the combination or the pharmaceutical composition of any one of the preceding items.

In particular, the invention relates to the following items:

1. A combination for use in the treatment of a tumor and/or cancer, wherein the combination comprises:
- a) an antimetabolite; and

- bi) a mutated Semaphorin 3 or a functional fragment thereof, wherein the mutated Semaphorin 3 is selected from the group consisting of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C, and Semaphorin 3D, and wherein the mutated Semaphorin 3 or the functional fragment thereof comprises an amino acid sequence CX₁X₂A₃GKD, wherein
X₁ is an amino acid, which is K or N,
X₂ is an amino acid selected from the group of W, M and L,
and wherein the alanine (A₃) is replaced by a hydrophilic amino acid; or
- bii) a fusion protein comprising the mutated Semaphorin 3 or the functional fragment thereof; or
- biii) a nucleic acid molecule encoding the mutated Semaphorin 3 or the functional fragment thereof, or a nucleic acid molecule encoding the fusion protein.
2. The combination of item 1, wherein the mutated Semaphorin 3, the functional fragment thereof, or the fusion protein inhibits angiogenesis and/or functions as vascular normalizing agent.
 3. The combination of item 1 or 2, wherein the antimetabolite is selected from gemcitabine, azacytidine, capecitabine, clofarabine, cytarabine, doxifluridine, floxuridine, 5-fluorouracil and pharmaceutically acceptable salts and solvates thereof.
 4. The combination according to any one of items 1 to 3, wherein the antimetabolite is selected from gemcitabine and pharmaceutically acceptable salts and solvates thereof.
 5. The combination according to any one of items 1 to 4, wherein the tumor or cancer is selected from the group consisting of a tumor, preferably a solid tumor, and a tumorous disease, in particular wherein the tumor or cancer is selected from the group consisting of pancreatic cancer, cervical cancer, breast cancer, colon cancer, melanoma, prostate cancer, bladder cancer and tongue cancer.
 6. The combination according to any one of items 1 to 6, wherein the tumor or cancer is a pancreatic tumor or pancreatic cancer, in particular wherein the cancer is a metastatic pancreatic cancer.

7. The combination according to any one of items 1 to 7, wherein the combination prolongs the survival of a subject suffering from a tumor or cancer compared to a pharmaceutical composition comprising the mutated Semaphorin 3, the functional fragment thereof, the fusion protein, or the antimetabolite alone;
wherein the combination reduces the tumor growth compared to a pharmaceutical composition comprising the mutated Semaphorin 3, the functional fragment thereof, the fusion protein, or the antimetabolite alone; and/or
wherein the combination reduces metastasis, in particular liver metastasis, compared to a pharmaceutical composition comprising the mutated Semaphorin 3, the functional fragment thereof, the fusion protein, or the antimetabolite alone.
8. The combination according to any one of items 1 to 7, wherein the mutated Semaphorin 3 comprises Semaphorin 3A or a functional fragment thereof and comprises a hydrophilic amino acid at position 106 of SEQ ID NO: 2 and wherein the antimetabolite is gemcitabine, in particular wherein the mutated Semaphorin 3 comprises SEQ ID NO: 18 or functional fragments thereof and wherein the antimetabolite is gemcitabine.
9. The combination of any one of items 1 to 8, wherein the mutated Semaphorin 3 is selected from the group of:
 - (a) a polypeptide that is encoded by a nucleic acid sequence selected from the group of SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 9 and SEQ ID NO: 13, wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding the hydrophilic amino acid, wherein the nucleotides GCA at position 559 to 561 of SEQ ID NO: 5 are replaced by nucleotides encoding the hydrophilic amino acid, wherein the nucleotides GCT at position 872 to 874 of SEQ ID NO: 9 are replaced by nucleotides encoding the hydrophilic amino acid, and wherein the nucleotides GCC at position 398 to 400 of SEQ ID NO: 13 are replaced by nucleotides encoding the hydrophilic amino acid;
 - (b) a polypeptide having the amino acid sequence selected from the group of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 10 and SEQ ID NO: 14, wherein the

- alanine residue at position 106 of SEQ ID NO: 2, at position 105 of SEQ ID NO: 6, at position 104 of SEQ ID NO: 10 or at position 120 of SEQ ID NO: 14 is replaced by the hydrophilic amino acid;
- (c) a polypeptide that is encoded by a nucleic acid sequence that hybridizes under stringent conditions to the complementary strand of a nucleic acid molecule encoding a polypeptide as defined in (a) or (b);
 - (d) a polypeptide that has at least 55% identity to any one of the polypeptides referred to in (b).
10. The combination of any one of items 1 to 9, wherein the hydrophilic amino acid is selected from the group of K, R, N, Q, S,T, E, D, and H, preferably wherein the hydrophilic amino acid is K or R, and most preferably wherein the hydrophilic amino acid replacing the alanine is K.
11. The combination of any one of items 1 to 10, wherein the fusion protein comprises a polypeptide as defined in:
- (a) SEQ ID NO: 21, wherein the alanine residue corresponding to position 106 of SEQ ID NO: 2 is replaced by a hydrophilic amino acid;
 - (b) SEQ ID NO: 22, wherein the alanine residue corresponding to position 105 of SEQ ID NO: 6 is replaced by a hydrophilic amino acid;
 - (c) SEQ ID NO: 23, wherein the alanine residue corresponding to position 104 of SEQ ID NO: 10 is replaced by a hydrophilic amino acid; or
 - (d) SEQ ID NO: 24, wherein the alanine residue corresponding to position 120 of SEQ ID NO: 14 is replaced by a hydrophilic amino acid.
12. The combination of any one of items 1 to 11, wherein the fusion protein comprises:
- (a) a stabilizer domain; and/or
 - (b) a dimerization domain.
13. The combination of any one of items 1 to 12, wherein the fusion protein comprises an amino acid sequence:

spanning from amino acid residues 1 to 548 of SEQ ID NO: 2 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 106 of SEQ ID NO: 2 is replaced by a hydrophilic amino acid;

spanning from amino acid residues 1 to 547 of SEQ ID NO: 6 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 105 of SEQ ID NO: 6 is replaced by a hydrophilic amino acid;

spanning from amino acid residues 1 to 565 of SEQ ID NO: 10 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 104 of SEQ ID NO: 10 is replaced by a hydrophilic amino acid; or

spanning from amino acid residues 1 to 545 of SEQ ID NO: 14 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 120 of SEQ ID NO: 14 is replaced by the hydrophilic amino acid.

14. The combination of any one of items 1 to 14, wherein the antimetabolite and the mutated Semaphorin 3, a functional fragment thereof, the fusion protein, or the nucleic acid molecule are to be administered to a subject in need thereof sequentially, concurrently, or via different administration routes.

15. A kit comprising:
 - a) the antimetabolite as defined in any one of the preceding claims; and
 - b) the mutated Semaphorin 3 or the functional fragment thereof, the fusion protein comprising the mutated Semaphorin 3 or the functional fragment thereof, or the nucleic acid molecule encoding the mutated Semaphorin 3 or the functional fragment thereof, or the nucleic acid molecule encoding the fusion protein as defined in any one of the preceding claims; ora pharmaceutical composition comprising:
 - a) the antimetabolite as defined in any one of the preceding claims; and
 - b) the mutated Semaphorin 3 or the functional fragment thereof, the fusion protein comprising the mutated Semaphorin 3 or the functional fragment thereof, or the nucleic acid molecule encoding the mutated Semaphorin 3 or the functional fragment thereof, or the nucleic acid molecule encoding the fusion protein as defined in any one of the preceding claims; andoptionally comprising one or more pharmaceutical excipients.

As used herein, the term “combination” refers to a combined occurrence of at least two components, namely,

- (i) the antimetabolite and the mutated Semaphorin 3 or the functional fragment thereof;
- (ii) the antimetabolite and the fusion protein comprising the mutated Semaphorin 3 or the functional fragment thereof;
- (iii) the antimetabolite and the nucleic acid molecule encoding the mutated Semaphorin 3 or the functional fragment thereof; or
- (iv) the antimetabolite and the nucleic acid molecule encoding the fusion protein or the fragment thereof. The combination may occur either as one composition, comprising the antimetabolite and the mutated Semaphorin 3 or the functional fragment thereof, the fusion protein comprising the mutated Semaphorin 3 or the functional fragment thereof, the nucleic acid molecule encoding the mutated Semaphorin 3 or the functional fragment thereof, or the nucleic acid molecule encoding the fusion protein or the fragment thereof in one and the same mixture (e.g. in a pharmaceutical composition), or may occur as a kit of parts, wherein the at least two components form different parts of such a kit of parts. In other words, the term “pharmaceutical composition” in context of this medical invention of a combination therapy approach of cancer treatment/medical intervention of metastatic events refers to one mixture comprising the at least two components, (I) the antimetabolite and (II) the mutated Semaphorin 3/the mutated Semaphorin 3 constructs and/or the mutated Semaphorin 3 fusion proteins. Mutated Semaphorin 3, mutated Semaphorin 3 constructs and/or the mutated Semaphorin 3 fusion proteins to be employed in context of this invention as described herein and in WO/2016135130 (incorporated by reference). Accordingly, the present invention provides for the medical/therapeutic use of a combination of (an) antimetabolite(s) and the mutated Semaphorin 3, mutated Semaphorin 3 constructs and/or the mutated Semaphorin 3 fusion proteins as disclosed herein and in WO/2016135130 (incorporated by reference).

The inventive combination may be presented for use in the form of a pharmaceutical formulation. The individual components of such combinations may be administered either sequentially or simultaneously/concomitantly in separate or combined pharmaceutical formulations by any convenient route. The pharmaceutical composition may further comprise one or more (pharmaceutical) excipients. The term “kit of parts” is herein used exchangeable with the term “kit”. These terms refer to a kit comprising the at least two components as different parts and may comprise further one or more (pharmaceutical) excipients. All the definitions of the antimetabolite and the mutated Semaphorin 3 or the functional fragment thereof; the antimetabolite and the fusion protein comprising the mutated Semaphorin 3 or the functional fragment thereof; the antimetabolite and the nucleic acid molecule encoding the mutated Semaphorin 3 or the functional fragment thereof; or the antimetabolite and the

nucleic acid molecule encoding the fusion protein or the fragment thereof in relation to the inventive combination also apply to the pharmaceutical composition or the kit of parts.

As demonstrated in the appended examples, the inventive combination has beneficial effects and functions. For example the combination inhibits angiogenesis and/or functions as vascular normalizing agent. In particular, the inventive combination further reduces the tumor growth, further prolongs the survival and/or further reduces metastasis of subjects suffering from tumor compared to subjects that were treated with the antimetabolite or the mutated Semaphorin 3 alone. The administration of the antimetabolite and the polypeptide (e.g. the mutated Semaphorin 3 or the functional fragment thereof; or the fusion protein comprising the mutated Semaphorin 3 or the functional fragment thereof) and/or the nucleic acid molecule encoding the mutated Semaphorin 3 or the functional fragment thereof, or the fusion protein may occur either simultaneously or timely staggered, either at the same site of administration or at different sites of administration.

As indicated above and as demonstrated below, the combination prolongs the survival of a subject suffering from a tumor compared to a pharmaceutical composition comprising the mutated Semaphorin 3, the functional fragment thereof, the fusion protein, or the antimetabolite alone. The combination also reduces the tumor growth compared to a pharmaceutical composition comprising the mutated Semaphorin 3, the functional fragment thereof, the fusion protein, or the antimetabolite alone. In particular aspects of the invention, the combination reduces metastasis compared to a pharmaceutical composition comprising the mutated Semaphorin 3, the functional fragment thereof, the fusion protein, or the antimetabolite alone. In particular, the appended examples document that the combination reduces liver metastasis compared to a pharmaceutical composition comprising the mutated Semaphorin 3, the functional fragment thereof, the fusion protein, or the antimetabolite alone. In other words, the combination provided herein has an increased anti-metastatic effect (in particular in metastatic pancreatic cancer) compared to a pharmaceutical composition comprising the mutated Semaphorin 3, the functional fragment thereof, the fusion protein, or the antimetabolite alone. In particular, it is meant that the combination further prolongs the survival, further reduces the tumor growth, further reduces the metastasis compared to the pharmaceutical composition comprising the mutated Semaphorin 3, the functional fragment thereof, the fusion protein, or the antimetabolite alone. The skilled person is well aware how to determine these medical effects. The appended examples disclose exemplary methods how these medical effects can be determined.

The term “compared to a pharmaceutical composition comprising the mutated Semaphorin 3, the functional fragment thereof, the fusion protein, or the antimetabolite alone” refers to the

comparison of the medical effects observed in a subject or a cohort of subjects that were treated with the single components, e.g. the antimetabolite alone, the mutated Semaphorin 3 or the functional fragment alone, or the fusion protein or the functional fragment alone. The subject(s) receiving the single therapy (reference subjects) should suffer from the same disease or share the same disease state as the subject(s) receiving the inventive combination. The appended examples document that the inventive combination was surprisingly superior compared to the single therapies.

In particular, the inventive combination, the pharmaceutical composition, or the kit of parts comprise the antimetabolite as described herein and the mutated Semaphorin 3A or the functional fragment thereof, or the fusion protein comprising the mutated Semaphorin 3A or functional fragment(s) thereof comprising the herein described mutation, or the nucleic acid molecules encoding the same.

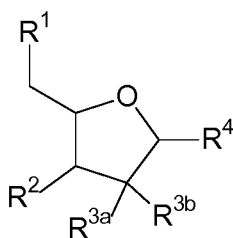
Further, the inventive combination, the pharmaceutical composition, or the kit of parts comprise the antimetabolite as described herein, in particular gemcitabine, and the mutated Semaphorin 3A or the functional fragment thereof, or the fusion protein or the functional fragment thereof comprising a hydrophilic amino acid in place of the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2. As described below, the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 may also be referred to as A₃ in the amino acid sequence CX₁X₂A₃GKD.

In particular aspects, the inventive combination, the pharmaceutical composition, or the kit of parts comprises the antimetabolite as described herein, in particular Gemcitabine, and the mutated Semaphorin 3A or the functional fragment thereof, or the fusion protein or the functional fragment comprising the mutated Semaphorin 3A or the functional fragment thereof, or the nucleic acid molecule encoding the same, wherein the mutated Semaphorin 3, the functional fragment of the mutated Semaphorin 3A, the fusion protein or the functional fragment of the fusion protein comprises a hydrophilic amino acid at position 106 of SEQ ID NO: 2.

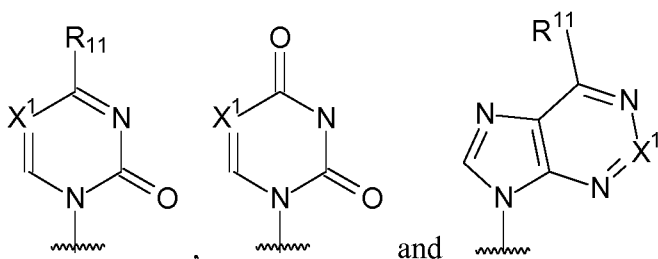
In particular preferred aspects, the combination, the pharmaceutical composition, or the kit of parts provided herein comprises the antimetabolite as described herein, in particular gemcitabine, and the fusion protein or the functional fragment thereof comprising the mutated Semaphorin 3A or the functional fragment of the mutated Semaphorin 3A, or the nucleic acid molecule encoding the same, wherein the fusion protein comprises SEQ ID NO: 18. The fusion protein may also comprise a functional fragment of SEQ ID NO: 18, wherein the functional fragment comprises the mutation a position 106 or the corresponding position.

The combination, the pharmaceutical composition or the kit of parts of the present invention which is preferably for use in the treatment of a tumor and/or cancer (in particular pancreatic cancer and/or tumor) contains one or more antimetabolites. Antimetabolites are similar to natural chemicals in a normal biochemical reaction in cells but different enough to interfere with the normal division and functions of cells. Antimetabolites are usually structural analogs of naturally occurring nucleosides that are essential for cell growth and division. Antimetabolites typically interfere with DNA synthesis by competing with the natural substrate for the active site on an essential enzyme or receptor. Thus, they typically have a chemical structure in which a furanose or a derivative thereof is linked to a nucleotide or a derivative thereof, although some antimetabolites such as 5-fluorouracil contain only the nucleotide or a derivative thereof. Either the furanose or the nucleotide or both contain(s) at least one modification compared to natural nucleosides that results in impaired DNA replication and thereby reduced cell proliferation.

A wide range of antimetabolites known to date have the following structure (A) or are pharmaceutically acceptable salts thereof:



wherein R¹, R², R^{3a} and R^{3b} are each independently selected from -H, -OH and -F, and R⁴ is a substituent selected from the following formulae (A1) to (A3):



wherein X¹ is selected from N, C-Cl and C-F and R¹¹ is selected from -NH₂ and NH(C=O)-O-C₄₋₈-alkyl.

Antimetabolites which have a structure such as structure (A) in which the sugar moiety is

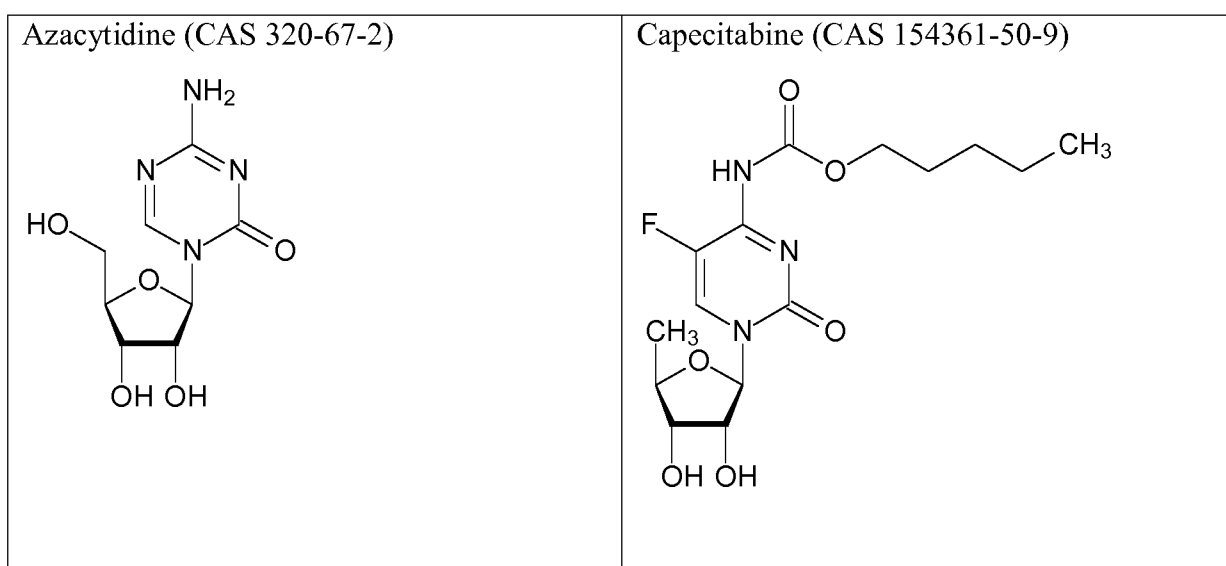
absent, are also known. One example thereof is 5-fluorouracil.

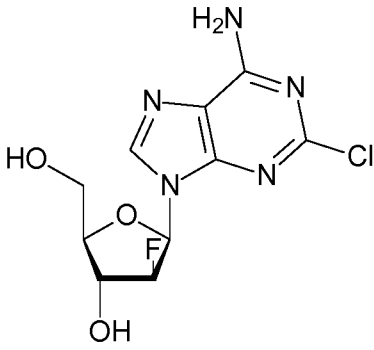
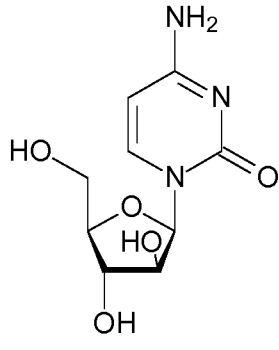
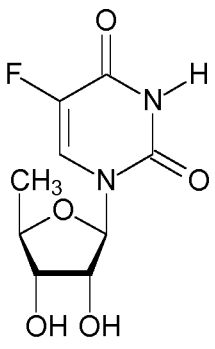
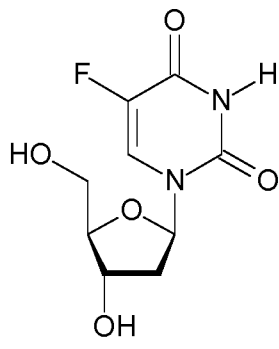
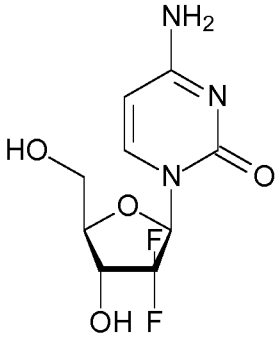
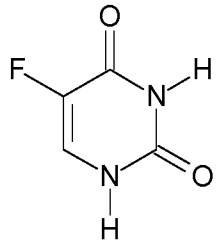
Specific examples of antimetabolites include Azacitidine, Azathioprine, Capecitabine, Cladribine, Clofarabine, Cytarabine, Decitabine, Doxifluridine, Floxuridine, Fludarabine, 5-Fluorouracil, Hydroxyurea, Gemcitabine, Methotrexate, Mercaptopurine, Nelarabine, Pemetrexed, Pentostatine, Pralatrexate, Raltitrexed, Tegafur, Thioguanine, Trimetrexate and pharmaceutically acceptable salts thereof.

The antimetabolites to be used in the present invention are preferably one or more selected from azacytidine, capecitabine, clofarabine, cytarabine, doxifluridine, floxuridine, gemcitabine, 5-fluorouracil and pharmaceutically acceptable salts thereof.

The present inventors have surprisingly found that gemcitabine and antimetabolites having similar mechanisms of action, such as azacytidine, capecitabine, clofarabine, cytarabine, doxifluridine, floxuridine and 5-fluorouracil, lead to surprisingly superior effects in combination with the mutated semaphorins described herein, in particular the mutated Semaphorin 3A.

The structures of these preferred antimetabolites can be depicted as follows:



<p>Clofarabine (CAS 123318-82-1)</p> 	<p>Cytarabine (CAS 147-94-4)</p> 
<p>Doxifluridine (CAS 3094-09-5)</p> 	<p>Floxuridine (CAS 50-91-9)</p> 
<p>Gemcitabine (CAS 95058-81-4)</p> 	<p>5-Fluorouracil (CAS 51-21-8)</p> 

and any pharmaceutically acceptable salts and solvates thereof.

It has been found that the combination of 5-fluorouracil and the mutated Semaphorin 3A is particularly useful in the treatment of colon cancer. Accordingly, the inventive combination, the pharmaceutical composition or the kit may comprise 5-fluorouracil and the mutated

Semaphorin 3A or the functional fragment thereof, or the fusion protein or the functional fragment comprising the mutated Semaphorin 3A or the functional fragment thereof, or the nucleic acid molecule encoding the same. Such combination, such pharmaceutical composition, or such kit may be used in the therapy of colon cancer.

Most preferably, the antimetabolite comprises gemcitabine and/or a pharmaceutically acceptable salt and/or a solvate thereof. Accordingly, the combination, the pharmaceutical composition, or the kit of parts provided herein comprises most preferably gemcitabine and/or a pharmaceutically acceptable salt and/or a solvate thereof. The combination, the pharmaceutical composition, or the kit of parts comprising gemcitabine and/or a pharmaceutically acceptable salt and/or a solvate thereof are preferably used in the therapy as provided herein, in particular in the treatment of a pancreatic cancer and/or tumor.

In the present invention, any pharmaceutically acceptable salt forms of the antimetabolite(s) may be used, e.g., by protonation of an atom carrying an electron lone pair which is susceptible to protonation, such as an amino group, with an inorganic or organic acid, or as a salt of an acid group (such as a carboxylic acid group) with a physiologically acceptable cation. Exemplary base addition salts comprise, for example: alkali metal salts such as sodium or potassium salts; alkaline earth metal salts such as calcium or magnesium salts; zinc salts; ammonium salts; aliphatic amine salts such as trimethylamine, triethylamine, dicyclohexylamine, ethanolamine, diethanolamine, triethanolamine, procaine salts, meglumine salts, ethylenediamine salts, or choline salts; aralkyl amine salts such as N,N dibenzylethylenediamine salts, benzathine salts, benethamine salts; heterocyclic aromatic amine salts such as pyridine salts, picoline salts, quinoline salts or isoquinoline salts; quaternary ammonium salts such as tetramethylammonium salts, tetraethylammonium salts, benzyltrimethylammonium salts, benzyltriethylammonium salts, benzyltributylammonium salts, methyltrioctylammonium salts or tetrabutylammonium salts; and basic amino acid salts such as arginine salts, lysine salts, or histidine salts. Exemplary acid addition salts comprise, for example: mineral acid salts such as hydrochloride, hydrobromide, hydroiodide, sulfate salts (such as, e.g., sulfate or hydrogensulfate salts), nitrate salts, phosphate salts (such as, e.g., phosphate, hydrogenphosphate, or dihydrogenphosphate salts), carbonate salts, hydrogencarbonate salts, perchlorate salts, borate salts, or thiocyanate salts; organic acid salts such as acetate, propionate, butyrate, pentanoate, hexanoate, heptanoate, octanoate, cyclopentanepropionate, decanoate, undecanoate, oleate, stearate, lactate, maleate, oxalate, fumarate, tartrate, malate, citrate, succinate, adipate, gluconate, glycolate, nicotinate, benzoate, salicylate, ascorbate, pamoate (embonate), camphorate, glucoheptanoate, or pivalate salts; sulfonate salts such as methanesulfonate (mesylate), ethanesulfonate (esylate), 2 hydroxyethanesulfonate (isethionate), benzenesulfonate (besylate), p-toluenesulfonate

(tosylate), 2-naphthalenesulfonate (napsylate), 3-phenylsulfonate, or camphorsulfonate salts; glycerophosphate salts; and acidic amino acid salts such as aspartate or glutamate salts. Preferred pharmaceutically acceptable salts of the antimetabolites include a hydrochloride salt, a hydrobromide salt, a mesylate salt, a sulfate salt, a tartrate salt, a fumarate salt, an acetate salt, a citrate salt, and a phosphate salt. A particularly preferred pharmaceutically acceptable salt of the antimetabolite is a hydrochloride salt. Accordingly, it is preferred that the antimetabolite, including any one of the specific antimetabolites described herein, is in the form of a hydrochloride salt, a hydrobromide salt, a mesylate salt, a sulfate salt, a tartrate salt, a fumarate salt, an acetate salt, a citrate salt, or a phosphate salt, and it is particularly preferred that the antimetabolite is in the form of a hydrochloride salt.

Moreover, the antimetabolites may be used in any solvated form, including, e.g., solvates with water, for example hydrates, or with organic solvents such as, e.g., methanol, ethanol or acetonitrile, i.e., as a methanolate, ethanolate or acetonitrilate, respectively, or in the form of any polymorph. It is to be understood that such solvates of the antimetabolites also include solvates of pharmaceutically acceptable salts of the antimetabolites.

Furthermore, the antimetabolites may exist in the form of different isomers, in particular stereoisomers (including, e.g., geometric isomers (or cis/trans isomers), enantiomers and diastereomers) or tautomers. All such isomers of the antimetabolites are contemplated as being part of the present invention, either in admixture or in pure or substantially pure form. As for stereoisomers, the invention embraces the isolated optical isomers of the compounds according to the invention as well as any mixtures thereof (including, in particular, racemic mixtures/racemates). The racemates can be resolved by physical methods, such as, e.g., fractional crystallization, separation or crystallization of diastereomeric derivatives, or separation by chiral column chromatography. The individual optical isomers can also be obtained from the racemates via salt formation with an optically active acid followed by crystallization. Furthermore, any tautomers of the antimetabolites may be used.

As used herein, the term “mutated Semaphorin 3” is exchangeable used with “genetically modified Semaphorin 3”, “non-naturally occurring Semaphorin 3” or “non-natural Semaphorin 3”. These terms refer to a polypeptide or a functional fragment thereof, wherein the polypeptide is selected from the group consisting of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C, and Semaphorin 3D, and wherein the polypeptide or the functional fragment thereof comprises an amino acid sequence CX₁X₂A₃GKD, wherein

X₁ is an amino acid, which is K or N,

X₂ is an amino acid selected from the group of W, M and L,

and wherein the alanine (A₃) is replaced by a hydrophilic amino acid. The polypeptide or the functional fragment thereof has a function as documented in the appended examples and/or as disclosed in WO/2016135130. For example, the polypeptide or the functional fragment thereof is an inhibitor of tumor and/or cancer growth, inhibits angiogenesis and/or normalizes the vasculature as described herein. The mutated Semaphorin 3 as used herein may preferably be a functional fragment thereof and comprising the amino acid sequence CX₁X₂A₃GKD, wherein the alanine (A₃) is replaced by a hydrophilic amino acid. In particular, the polypeptide refers to a fusion protein comprising the herein described mutation. The mutated Semaphorin 3 or the functional fragment thereof may also be referred to as mutated Semaphorin 3 or the functional fragment thereof comprising a hydrophilic amino acid in place of the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2; comprising a hydrophilic amino acid in place of the alanine corresponding to position 105 of the wild type Semaphorin 3B as shown in SEQ ID NO: 6; comprising a hydrophilic amino acid in place of the alanine corresponding to position 104 of the wild type Semaphorin 3C as shown in SEQ ID NO: 10; or comprising a hydrophilic amino acid in place of the alanine corresponding to position 120 of the wild type Semaphorin 3D as shown in SEQ ID NO: 14.

In particular, the mutated Semaphorin 3 is a mutated Semaphorin 3A comprising a replacement of the alanine by a hydrophilic amino acid at the position that by comparison of homology corresponds to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2. In other words, the alanine (A₃) in the consensus motif CX₁X₂A₃GKD (also shown in SEQ ID NO: 73) in the Semaphorin 3 proteins is mutated to a hydrophilic amino acid (table 1). Accordingly, the mutated Semaphorin 3A comprises an amino acid sequence, wherein the alanine at the position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 or 4 is replaced by a hydrophilic amino acid. The mutated Semaphorin 3B comprises an amino acid sequence, wherein the alanine at the position 105 of the wild type Semaphorin 3B as shown in SEQ ID NO: 6 or 8 is replaced by a hydrophilic amino acid. The mutated Semaphorin 3C comprises an amino acid sequence, wherein the alanine at the position 104 of the wild type Semaphorin 3C as shown in SEQ ID NO: 10 or 12 is replaced by a hydrophilic amino acid. The mutated Semaphorin 3D comprises an amino acid sequence, wherein the alanine at the position 120 of the wild type Semaphorin 3D as shown in SEQ ID NO: 14 or 16 is replaced by a hydrophilic amino acid. Said hydrophilic amino acid can be, e.g., a lysine, arginine, asparagine, glutamine, serine, threonine, glutamic acid, aspartic acid or histidine, more preferably a lysine or arginine, most preferably a lysine. It is also envisaged herein that the hydrophilic amino acid can be a non-proteinogenic or a non-standard α -amino acid (such as, e.g., ornithine and citrulline). It is shown herein above and in the appended examples that the replacement of the alanine by a hydrophilic amino acid results in an increased inhibition of cancer growth and metastasis formation. In general, the mutation to a hydrophilic amino acid

in the Semaphorins 3 results in a high binding affinity to the Plexin receptors. The increased binding of the mutated Semaphorin 3 results in an increased activation of the Plexin receptors and their downstream signaling. Plexin receptors are crucial in the control of integrin activation, cell adhesion and migration on or towards ECM proteins, which are key aspects in cancer cell progression and metastasis. Accordingly, the mutated Semaphorin 3 of the invention can be employed as inhibitor of angiogenesis and as a vascular normalizing agent.

Table 1. The mutated Semaphorin 3 comprises an amino acid sequence CX₁X₂A₃GKD, wherein the alanine A₃ is replaced by a hydrophilic amino acid. The amino acids that correspond to X₁ and X₂ are indicated. The positions of the amino acids of the wild type Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D as shown in SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14 and 16 are indicated.

C	X ₁	X ₂	A ₃	G	K	D	Semaphorin 3 and SEQ ID NO:
C 103	K 104	W 105	A 106	G 107	K 108	D 109	Human Semaphorin 3A SEQ ID NO:2
C 102	N 103	W 104	A 105	G 106	K 107	D 108	Human Semaphorin 3B SEQ ID NO:6
C 101	K 102	M 103	A 104	G 105	K 106	D 107	Human Semaphorin 3C SEQ ID NO:10
C 117	K 118	L 119	A 120	G 121	K 122	D 123	Human Semaphorin 3D SEQ ID NO:14
C 103	K 104	W 105	A 106	G 107	K 108	D 109	Mouse Semaphorin 3A SEQ ID NO:4
C 102	N 103	W 104	A 105	G 106	K 107	D 108	Mouse Semaphorin 3B SEQ ID NO:8
C 101	K 102	M 103	A 104	G 105	K 106	D 107	Mouse Semaphorin 3C SEQ ID NO:12
C 117	K 118	L 119	A 120	G 121	K 122	D 123	Mouse Semaphorin 3D SEQ ID NO:16

In particular, the invention relates to mutated Semaphorin 3 proteins which mean that the mutated Semaphorins are selected from the group of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D. Likewise, if reference is made to mutated Semaphorin 3 or mutated Semaphorin 3 proteins in the context of the present invention, this is intended to refer to mutated Semaphorin 3A, mutated Semaphorin 3B, mutated Semaphorin 3C and

mutated Semaphorin 3D. In most preferred aspects of the invention the mutated Semaphorin 3 is mutated Semaphorin 3A. It is understood herein that the mutated Semaphorin 3 is not Semaphorin 3E, Semaphorin 3F or Semaphorin 3G. Furthermore, it is herein understood that mutated Semaphorin 3 according to the invention does not include Semaphorin 3B isoform X2, e.g. from *Equus przewalskii*, or Semaphorin 3B isoform X6, e.g., from *Panthera tigris altaica*. Such Semaphorins do not comprise the amino acid sequence CX₁X₂A₃GKD, wherein the alanine A₃ is replaced by a hydrophilic amino acid. Furthermore, the mutated Semaphorin 3 according to the invention functions as an inhibitor of angiogenesis and/or as a vascular normalizing agent.

The terms “Semaphorin 3A”, “Semaphorin 3B”, “Semaphorin 3C”, and “Semaphorin 3D”, “Sema3A”, “Sema3B”, “Sema3C” and “Sema3D”, SEMA3A”, “SEMA3B”, “SEMA3C” and “SEMA3D” as used herein refer primarily to a protein. “Sema3A”, “Sema3B”, “Sema3C” and “Sema3D” as defined herein and to be used in accordance with the present invention are preferably human “Sema3A”, “Sema3B”, “Sema3C” and “Sema3D”. “Semaphorin 3A”, “Semaphorin 3B”, “Semaphorin 3C”, and “Semaphorin 3D” as defined herein and to be used in accordance with the present invention are preferably human “Sema3A”, “Sema3B”, “Sema3C” and “Sema3D”. “SEMA3A”, “SEMA3B”, “SEMA3C” and “SEMA3D” as defined herein and to be used in accordance with the present invention are preferably human “Sema3A”, “Sema3B”, “Sema3C” and “Sema3D”.

Sema3A A106K or Semaphorin 3A A106K is also designated herein and in the appended examples as (Fc-tagged) Sema3A A106K ΔIg-b.

The amino acid sequences and encoding nucleotide sequences of wild-type Semaphorin 3 are well known in the art. Nucleic acid sequences can be retrieved in public databases like NCBI using the following accession numbers (the following sequences have been retrieved from the NCBI database):

The definitions and explanations in relation to the sequences disclosed in WO/2016135130 are incorporated herein by reference.

Homo sapiens SEMA3A, >gi|100913215|ref|NM_006080.2| corresponding to SEQ ID NO: 1; Mus musculus Sema3A, >gi|340523098|ref|NM_009152.4|) corresponding to SEQ ID NO: 3; Homo sapiens SEMA3B, >gi|586798179|ref|NM_001290060.1| corresponding to SEQ ID NO: 5; Mus musculus Sema3B, >gi|615276319|ref|NM_001042779.2| corresponding to SEQ ID NO: 7; Homo sapiens SEMA3C >gi|335057525|ref|NM_006379.3| corresponding to SEQ ID NO: 9; Mus musculus Sema3C, >gi|118130842|ref|NM_013657.5| corresponding to SEQ ID NO: 11; Homo sapiens SEMA3D, >gi|41406085|ref|NM_152754.2|

corresponding to SEQ ID NO: 13; or *Mus musculus* Sema3D >gi| 282847343|ref| NM_028882.4| corresponding to SEQ ID NO: 15.

SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13 or SEQ ID NO: 15 encode wild type full length Semaphorin 3 proteins.

Corresponding amino acid sequences can be retrieved in public databases like NCBI. The following sequences have been retrieved from the NCBI database.

Homo sapiens SEMA3A, |ref|NP_006071.1| corresponding to SEQ ID NO: 2;
Mus musculus Sema3A, |ref|NP_033178.2| corresponding to SEQ ID NO: 4;
Homo sapiens SEMA3B, |ref|NP_001276989.1| corresponding to SEQ ID NO: 6;
Mus musculus Sema3B, |ref|NP_001036244.1| corresponding to SEQ ID NO: 8;
Homo sapiens SEMA3C |ref|NP_006370.1| corresponding to SEQ ID NO: 10;
Mus musculus Sema3C, |ref|NP_038685.3| corresponding to SEQ ID NO: 12;
Homo sapiens SEMA3D, |ref|NP_689967.2| corresponding to SEQ ID NO: 14; or
Mus musculus Sema3D, |ref|NP_083158.3| corresponding to SEQ ID NO: 16.

SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 or SEQ ID NO: 16 comprise amino acid sequences of wild type full length Semaphorin 3 proteins.

Amino acid sequences of Semaphorin 3 of the invention can also be obtained from Uniprot, e.g. for mouse and human Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D.

In an embodiment of the invention, the combination comprises the mutated Semaphorin 3 or fragment thereof, wherein the mutated Semaphorin 3 or said fragment thereof is referred to a mutated Semaphorin 3 or said fragment comprising an amino acid sequence, wherein the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 or the alanine at the position which corresponds in other Semaphorins of the Semaphorin 3 proteins by comparison of homology to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by a hydrophilic amino acid, wherein said Semaphorin 3 is selected from the group of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D.

As explained above and as illustrated in Table 1, the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 refers to the specific alanine of

Semaphorin 3A at position 106 of SEQ ID NO: 2 or the specific alanine amino acid residue in a known wild-type sequence of Semaphorin 3A, 3B, 3C or 3D, preferably Semaphorin 3A, corresponding to said specific alanine of Semaphorin 3A at position 106 of SEQ ID NO: 2. It also means a specific amino acid residue in a known wild-type sequence e.g. Semaphorin 3A, 3B, 3C or 3D that is homologous to said specific alanine at position 106 of SEQ ID NO: 2, at position 105 of SEQ ID NO: 6, at position 104 of SEQ ID NO: 10 or at position 120 of SEQ ID NO: 14. Exemplary homologous amino acids residues can be alanine, valine, isoleucine, leucine, methionine, phenylalanine, tyrosine or tryptophan. Most preferably, the specific amino acid residue is alanine.

The corresponding amino acid residue in other wild-type sequences at the corresponding position can be selected preferably by standard homology screenings or PCR-mediated screening techniques for related sequences as described below. The alanine or the corresponding alanine is replaced by or changed to a hydrophilic amino acid in the mutated Semaphorin 3 according to the invention.

As mentioned and explained herein, the alanine at the position which corresponds in other Semaphorin 3 proteins by comparison of homology to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 refers to the specific alanine amino acid residue in a known wild-type sequence of Semaphorin 3B, 3C or 3D that corresponds to said specific alanine of said Semaphorin 3A at position 106 given in SEQ ID NO: 2. The corresponding alanine is replaced by a hydrophilic amino acid in mutated Semaphorin 3B, 3C or 3D. In other words, the alanine corresponding to position 105 of the wild type Semaphorin 3B as shown in SEQ ID NO: 6, the alanine corresponding to position 104 of the wild type Semaphorin 3C as shown in SEQ ID NO: 10; or the alanine corresponding to position 120 of the wild type Semaphorin 3D as shown in SEQ ID NO: 14 is replaced by the hydrophilic amino acid.

The alanine corresponding to position 105 of the wild type Semaphorin 3B as shown in SEQ ID NO: 6 refers to the specific alanine of Semaphorin 3B at position 105 of SEQ ID NO: 6 or the specific alanine amino acid residue in a known wild-type sequence of Semaphorin 3B corresponding to said specific alanine of Semaphorin 3B at position 105 of SEQ ID NO: 6.

The alanine corresponding to position 104 of the wild type Semaphorin 3C as shown in SEQ ID NO: 10 refers to the specific alanine of Semaphorin 3C at position 104 of SEQ ID NO: 10 or the specific alanine amino acid residue in a known wild-type sequence of Semaphorin 3C corresponding to said specific alanine of Semaphorin 3C at position 104 of SEQ ID NO: 10.

The alanine corresponding to position 120 of the wild type Semaphorin 3D as shown in SEQ ID NO: 14 refers to the specific alanine of Semaphorin 3D at position 120 of SEQ ID NO: 14 or the specific alanine amino acid residue in a known wild-type sequence of Semaphorin 3D corresponding to said specific alanine of Semaphorin 3D at position 120 of SEQ ID NO: 14.

As mentioned and detailed herein, the corresponding amino acid residue at the corresponding position can be selected preferably by comparison of homology. Homology among polypeptides or nucleotide sequences is typically inferred from their sequence similarity. Alignments of multiple sequences can herein be used to indicate which regions or specific amino acids of each sequence are homologous. The amino acid sequences of Semaphorin 3A, B, C and D can be used as (a) reference sequences. The homology exist preferably over a stretch of amino acids, e.g. 10, more preferably 20, more preferably 30, more preferably 50, or more preferably 100 amino acid residues, or most preferably the homology exist over the whole amino acid stretch. An illustrative amino acid sequence alignment of exemplary amino acid stretches of Semaphorin 3 proteins is shown in Table 1. The corresponding alanine is most preferably A₃ in the amino acid sequence CX₁X₂A₃GKD comprised in the mutated Semaphorin 3. Thus, the mutation can also be identified with the help of the amino acid sequence CX₁X₂A₃GKD.

As described herein, the combination comprises the mutated Semaphorin 3 or the functional fragment thereof that comprises a hydrophilic amino acid in place of the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 or comprises a hydrophilic amino acid in place of the alanine at the position which corresponds in other Semaphorin 3 proteins by comparison of homology to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 and wherein said Semaphorin 3 is selected from the group of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D. In other words, the mutated Semaphorin 3 or the functional fragment thereof comprises a hydrophilic amino acid in place of the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 or comprises a hydrophilic amino acid in place of the alanine at the position which corresponds in Semaphorin 3 B, C or D by comparison of homology to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2; and wherein said Semaphorin 3 is selected from the group of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D. In other words, the other Semaphorins are Semaphorin 3B, 3C and 3D.

In other words, the mutated Semaphorin 3 or the functional fragment thereof wherein said Semaphorin 3 is selected from the group consisting of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D and wherein said mutated Semaphorin 3 comprises a hydrophilic amino acid in place of the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2;
a hydrophilic amino acid in place of the alanine corresponding to position 105 of the wild type Semaphorin 3B as shown in SEQ ID NO: 6;
a hydrophilic amino acid in place of the alanine corresponding to position 104 of the wild type Semaphorin 3C as shown in SEQ ID NO: 10; or

a hydrophilic amino acid in place of the alanine corresponding to position 120 of the wild type Semaphorin 3D as shown in SEQ ID NO: 14.

In most preferred embodiments of the invention, the combination comprises the mutated Semaphorin 3 or the functional fragment thereof comprising a hydrophilic amino acid in place of the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2, and wherein said Semaphorin 3 is Semaphorin 3A. In other words, the combination comprises the mutated Semaphorin 3A or a functional fragment thereof, wherein said mutated Semaphorin 3A comprises a hydrophilic amino acid in place of the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2.

The replacement of the alanine (A_3) in the amino acid sequence $CX_1X_2A_3GKD$ renders Semaphorin 3A, B, C or D polypeptides to angiogenesis inhibitors and/or to vascular normalizing agents. Therefore, in the amino acid sequence $CX_1X_2A_3GKD$ comprised in the mutated Semaphorins, the alanine (A_3) is mutated to the hydrophilic amino acid, i.e., the present invention relates to a mutated Semaphorin 3 or a functional fragment thereof wherein said mutated Semaphorin 3 is selected from the group consisting of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D and wherein said mutated Semaphorin 3 or said functional fragment thereof comprises an amino acid sequence $CX_1X_2A_3GKD$, wherein

X_1 is an amino acid, which is K or N,

X_2 is an amino acid selected from the group of W, M and L,

and wherein the alanine (A_3) is replaced by said hydrophilic amino acid.

The following description refers to all different embodiments. The following relates to the herein provided non-naturally occurring/artificial/mutated Semaphorin 3 proteins or their herein described functional fragments or the herein described functional sema domains and/or fusion proteins/polypeptides comprising said non-naturally occurring/artificial/mutated Semaphorin 3 proteins or said non-naturally occurring/artificial/mutated functional fragments or said functional sema domains of the mutated Semaphorin 3 proteins that function as documented in the appended examples or in WO/2016135130. The non-naturally occurring/artificial/mutated Semaphorin 3 proteins or their herein described functional fragments or the herein described functional sema domains and/or fusion proteins/polypeptides comprising said non-naturally occurring/artificial/mutated Semaphorin 3 proteins or said non-naturally occurring/artificial/mutated functional fragments or said functional sema domains of the mutated Semaphorin 3 proteins inhibit angiogenesis and/or function as a vascular normalizing agent. In other words, these proteins or polypeptides can function as angiogenesis inhibitors. In addition, the non-naturally occurring/artificial/mutated

Semaphorin 3 proteins or their herein described functional fragments or the herein described functional sema domains and/or fusion proteins/polypeptides comprising said non-naturally occurring/artificial/mutated Semaphorin 3 proteins or said non-naturally occurring/artificial/mutated functional fragments or said functional sema domains of the mutated Semaphorin 3 proteins inhibit tumor and/or cancer growth.

The mutated Semaphorin 3 or the functional fragments thereof or the functional sema domains and/or fusion proteins/polypeptides comprising the mutated Semaphorin 3 or the mutated functional fragments or the functional sema domains of the mutated Semaphorin 3 proteins can be stronger angiogenesis inhibitors and reduce further the tumor/cancer growth compared to non-mutated (e.g. wildtype) Semaphorins 3 or functional fragments thereof. An inhibitor of angiogenesis prevents the formation of new blood vessels, thereby stopping or slowing the growth or spread of tumors. The skilled person is well aware to determine whether a compound inhibits angiogenesis. In addition, the skilled person is well aware how to determine the quality and quantity of such an effect. Exemplary methods are disclosed in WO/2016135130 that are incorporated herein by reference. The appended examples also show exemplary methods for evaluating the anti-angiogenic activity and/or the vascular normalization. For example, an angiogenesis inhibitor inhibits the motility of endothelial cells. The mutated Semaphorins 3 or the functional fragments thereof, or the fusion proteins comprising the mutated Semaphorins 3 reduce the blood vessel area, normalize cancer blood vessels, i.e., increase the pericyte coverage, enhance the perfusion of cancer blood vessels and/or reduce the tissue hypoxia. Accordingly, the polypeptide and/or the nucleic sequences encoding the polypeptides described herein relate to a direct and/or an indirect inhibitor of angiogenesis.

A vascular normalizing agent normalizes cancer, tumor and/or tumorous disease blood vessel, i.e., increases the pericyte coverage, enhances the perfusion of cancer blood vessels, reduces tissue hypoxia and/or improves drug delivery to cancer. Accordingly, the amino acid sequences and/or nucleic sequences of the present invention relate to a direct and/or indirect vascular normalizing agent. A vascular normalizing agent normalizes the vasculature, in particular the cancer vasculature, more preferably the pancreatic cancer vasculature. The term "normalizes" means that the vasculature after the treatment with the herein described means is more similar to the vasculature of a group of healthy subjects.

Inhibitors of angiogenesis may also bind to receptors on the surface of cells, such as ECs and/or to other proteins in the downstream signaling pathways, blocking their activities. The mutated Semaphorin 3, the functional fragment thereof, the functional sema domain, the fusion protein or the polypeptide described herein binds to the corresponding Plexin receptor, e.g. type A Plexins such as Plexin A4 or Plexin A2, with a high affinity, i.e. displaying dissociation constant K_D in the very-low-nanomolar/sub-nanomolar range. Accordingly, the polypeptide and/or nucleic sequences encoding the polypeptide comprised in the inventive

combination can inhibit directly and/or indirectly angiogenesis. In particular, the mutated Semaphorin 3, the functional fragment thereof or the fusion protein comprised in the inventive combination has an affinity to its Plexin receptor with a dissociation constant K_D lower than 6 nM. In preferred aspects, the mutated Semaphorin 3, the functional fragment thereof or the fusion protein/ has an affinity to its Plexin receptor with a dissociation constant K_D lower than 4 nM. In even more preferred aspects, the mutated Semaphorin 3, the functional fragment thereof or the fusion protein/polypeptide has an affinity to its Plexin receptor with a dissociation constant K_D lower than 2 nM. In most preferred aspects, the mutated Semaphorin 3, the functional fragment thereof or the fusion protein/polypeptide has an affinity to its Plexin receptor with a dissociation constant K_D lower than 1 nM. The dissociation constant K_D can be measured by standard methods known in the art, such as an assay that is evident from the appended examples. The mutated Semaphorin 3A binds to Plexin A4 receptor, with a high affinity, i.e. displaying dissociation constant K_D in the very-low-nanomolar/sub-nanomolar range. Furthermore, the mutated Semaphorin 3B binds to Plexin A2 receptor, with a high affinity, i.e. displaying dissociation constant K_D in the very-low-nanomolar/sub-nanomolar range.

Further, the mutated Semaphorin 3, the functional fragment thereof, the functional sema domain or the fusion protein/polypeptide comprised in the inventive combination inhibits Rap1 GTP loading (by about 65%). Accordingly, the amino acid sequences and/or nucleic sequences described herein mediate downstream signaling pathways relevant in angiogenesis. Therefore, the mutated Semaphorin 3, the functional fragment thereof or the fusion protein/polypeptide comprised in the inventive combination inhibits directly and/or indirectly angiogenesis, as shown in the appended examples. In particular, the mutated Semaphorin 3, the functional fragment thereof or the fusion protein/polypeptide comprised in the inventive combination inhibits Rap1 GTP loading by at least 50%, 55%, or 65% as disclosed in WO/2016135130 incorporated herein by reference. The inhibition of Rap1 GTP loading can be measured by standard methods known in the art and exemplified in WO/2016135130 incorporated herein by reference. Further, the mutated Semaphorin 3, the functional fragment thereof, the functional sema domain or the fusion protein/polypeptide comprised in the inventive combination activates ERK 1/2 phosphorylation (by about 3.9 fold). In particular, the mutated Semaphorin 3, the functional fragment thereof or the fusion protein/polypeptide comprised in the inventive combination activates ERK 1/2 phosphorylation by at least 2.5 fold, 3.0 fold, 3.5 fold, or 4.9 fold as disclosed in WO/2016135130 incorporated herein by reference.. The activation of ERK 1/2 phosphorylation can be measured by standard methods known in the art, such as an assay shown in WO/2016135130 incorporated herein by reference.

Further, the mutated Semaphorin 3, the functional fragment thereof, the functional sema domain, the fusion protein or the polypeptide comprised in the inventive combination inhibits the motility of cells, such as ECs (by about 46%). Accordingly, the mutated Semaphorin 3 of the invention, the functional fragment thereof and the fusion protein/polypeptide comprised in the inventive combination are (superior) inhibitors of the motility of cells and/or inhibitors of the metastatic dissemination of cancer cells. Thus, the mutated Semaphorin 3, the functional fragment thereof or the fusion protein/polypeptide comprised in the inventive combination inhibits metastatic dissemination of cancer cells. In particular, the mutated Semaphorin 3, the functional fragment thereof or the fusion protein/polypeptide comprised in the inventive combination inhibits the motility of cells such as endothelial cells by at least 30%. In preferred aspects, the mutated Semaphorin 3, the functional fragment thereof or the fusion protein/polypeptide comprised in the inventive combination inhibits the motility of cells such as endothelial cells by at least 35%, 40%, or 45% as disclosed in WO/2016135130 incorporated herein by reference..The motility of cells can be measured by standard methods known in the art, such as an assay that is evident from WO/2016135130 incorporated herein by reference.

In a further embodiment the inventive combination comprises a nucleic acid molecule encoding the mutated Semaphorin 3 or the functional fragment thereof, or a nucleic acid molecule encoding the fusion protein as disclosed in WO/2016135130 incorporated herein by reference. The nucleic acid molecule may also be referred to as a nucleic acid molecule encoding a polypeptide comprising an amino acid sequence of a mutated Semaphorin 3 or a functional fragment thereof, wherein the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 or the alanine at the position which corresponds in other Semaphorin 3 proteins by comparison of homology to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by a hydrophilic amino acid, wherein said Semaphorin 3 is selected from the group of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D.

The inventive combination comprises the non-naturally occurring/artificial/mutated Semaphorin 3 proteins or their herein described functional fragments and/or fusion proteins/polypeptides comprising said non-naturally occurring/artificial/mutated Semaphorin 3 proteins or said non-naturally occurring/artificial/mutated functional fragments or said functional sema domains of said mutated Semaphorin 3 proteins or the polypeptides inhibit angiogenesis. The polypeptides may also be vascular normalizing agents. In other words the polypeptides comprised in the inventive combination, i.e., the mutated Semaphorin 3 proteins/functional fragments thereof/functional sema domain/fusion polypeptides/proteins as described herein inhibit angiogenesis and/or are vascular normalizing agent, wherein the

vascular normalizing agent normalizes cancer blood vessel, e.g. increases the pericyte coverage, enhances the perfusion of cancer blood vessels, reduces tissue hypoxia and/or improves drug delivery to cancer. Accordingly, the polypeptides and/or the nucleic acid molecules comprised in the inventive combination preferably inhibit angiogenesis, and/or are a direct and/or indirect vascular normalizing agent.

The following description relates to each one of the embodiments of the present invention as described herein above unless explicitly stated otherwise.

The mutated Semaphorin 3 proteins, genetically modified Semaphorin 3 proteins or related polypeptides (functional fragments thereof and/or fusion proteins comprising said mutated Semaphorin 3 proteins or said mutated functional fragments of said Semaphorin 3 proteins having an identity of at least 55 % to the specific Semaphorin 3 proteins provided and defined herein, and the like) inhibit angiogenesis and/or function as vascular normalizing agent. In particular, the above and below described polypeptides or nucleic acid molecules encoding the herein provided polypeptides have a function as described in the appended examples, for example the combination prolongs the survival of a subject suffering from a tumor or cancer compared to a pharmaceutical composition comprising the mutated Semaphorin 3, the functional fragment thereof, the fusion protein, or the antimetabolite alone; reduces the tumor growth compared to a pharmaceutical composition comprising the mutated Semaphorin 3, the functional fragment thereof, the fusion protein, or the antimetabolite alone; and/or reduces metastasis, in particular liver metastasis, compared to a pharmaceutical composition comprising the mutated Semaphorin 3, the functional fragment thereof, the fusion protein, or the antimetabolite alone.

In preferred aspects, the nucleic acid molecule encoding for the mutated Semaphorin 3 is preferably at least 50% homologous/identical to the nucleic acid sequence as shown in SEQ ID NO: 1. It is understood that such nucleic acid sequences can also include orthologous/homologous/identical (and thus related) sequences. More preferably, the nucleic acid sequence encoding the mutated Semaphorin 3 is at least 52%, 53%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% homologous/identical to the nucleic acid sequence as shown in any one of SEQ ID NO: 1, wherein the higher values of sequence identity are preferred.

In certain aspects, the nucleic acid molecule encoding for the mutated Semaphorin 3 is preferably at least 48% homologous/identical to the nucleic acid sequence as shown in SEQ ID NOs: 5. It is understood that such nucleic acid sequences can also include orthologous/homologous/identical (and thus related) sequences. More preferably, the nucleic

acid sequence encoding the mutated Semaphorin 3 is at least 50%, 52%, 53%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% homologous/identical to the nucleic acid sequence as shown in any one of SEQ ID NO: 5, wherein the higher values of sequence identity are preferred.

In certain aspects, the nucleic acid molecule encoding the mutated Semaphorin 3 is preferably at least 55% homologous/identical to the nucleic acid sequence as shown in SEQ ID NO: 9. It is understood that such nucleic acid sequences can also include orthologous/homologous/identical (and thus related) sequences. More preferably, the nucleic acid sequence encoding the mutated Semaphorin 3 is at least 57%, 60%, 63%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% homologous/identical to the nucleic acid sequence as shown in any one of SEQ ID NO: 9, wherein the higher values of sequence identity are preferred.

In certain aspects, the nucleic acid molecule encoding the mutated Semaphorin 3 is preferably at least 45% homologous/identical to the nucleic acid sequence as shown in SEQ ID NO: 13. It is understood that such nucleic acid sequences can also include orthologous/homologous/identical (and thus related) sequences. More preferably, the nucleic acid sequence encoding the mutated Semaphorin 3 is at least 48%, 50%, 53%, , 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% homologous/identical to the nucleic acid sequence as shown in any one of SEQ ID NO: 13, wherein the higher values of sequence identity are preferred.

In certain aspects, the nucleic acid molecule encoding the mutated Semaphorin 3 is preferably at least 55% homologous/identical to the nucleic acid sequence as shown in any one of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 57, 59, 61, 63, 65, 67, 69 or 71. It is understood that such nucleic acid sequences can also include orthologous/homologous/identical (and thus related) sequences. More preferably, the nucleic acid sequence encoding the mutated Semaphorin 3 is at least 56%, 58%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% homologous/identical to the nucleic acid sequence as shown in any one of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 57, 59, 61, 63, 65, 67, 69 or 71, wherein the higher values of sequence identity are preferred. More preferably, the nucleic acid sequence of all aspects encoding the mutated Semaphorin 3 is at least 60% homologous/identical to the nucleic acid sequence as shown in any one of SEQ ID NO: NO: 1, 3, 5, 7, 9, 11, 13, 15, 57, 59, 61, 63, 65, 67, 69 or 71. More preferably, the nucleic acid sequence encoding the mutated Semaphorin 3 is at least 70% homologous/identical to the nucleic acid sequence as shown in any one of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 57, 59, 61, 63, 65, 67, 69 or 71. Even more preferably, the nucleic acid sequence encoding the mutated Semaphorin 3 is at least 80%

homologous/identical to the nucleic acid sequence as shown in any one of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 57, 59, 61, 63, 65, 67, 69 or 71. Most preferably, the nucleic acid sequence encoding the mutated Semaphorin 3 is at least 90% homologous/identical to the nucleic acid sequence as shown in any one of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 57, 59, 61, 63, 65, 67, 69 or 71. The above defined orthologous/homologous/identical sequences can also be encompassed in longer or shorter isoforms, spliced variants and fusion transcripts. The term "orthologous protein" or "orthologous gene" as used herein refers to proteins and genes, respectively, in different species that are similar to each other because they originated from a common ancestor.

Hybridization assays for the characterization of orthologs or other related sequences of known nucleic acid sequences are well known in the art; see e.g. Sambrook, Russell "Molecular Cloning, A Laboratory Manual", Cold Spring Harbor Laboratory, N.Y. (2001); Ausubel, "Current Protocols in Molecular Biology", Green Publishing Associates and Wiley Interscience, N.Y. (1989).

The term "hybridization" or "hybridizing", as used herein in connection with nucleic acids, relates to hybridizations under conditions of any degree of stringency as disclosed in WO/2016135130 incorporated herein by reference. Thus, the definitions and explanations in this regards disclosed in WO/2016135130 are herein incorporated herein by reference.

The explanations and definitions given herein and in WO/2016135130 incorporated in respect of "homology/identity of nucleic acid sequences" apply, mutatis mutandis, to "amino acid sequences" of members of the mutated Semaphorin 3 or the functional fragments thereof or the polypeptide, in particular an amino acid sequence as depicted in SEQ ID NO: 2 (Homo sapiens SEMA3A), SEQ ID NO: 6 (Homo sapiens SEMA3B), SEQ ID NO: 10 (Homo sapiens SEMA3C), SEQ ID NO: 14 (Homo sapiens SEMA3D), SEQ ID NO: 4 (Mus musculus Sema3A), SEQ ID NO: 8 (Mus musculus Sema3B), SEQ ID NO: 12 (Mus musculus Sema3C) and SEQ ID NO: 16 (Mus musculus Sema3D). Exemplary sequences of the mutated Semaphorin 3 proteins comprising a lysine at the position that by comparison of homology corresponds to position 106 of the wild type human Semaphorin 3A as shown in SEQ ID NO: 2 are given in SEQ ID NO: 58, 60, 62, 64, 66, 68, 70 or 72.

The mutated Semaphorin 3 proteins or genetically modified Semaphorin 3 proteins may have at least 55 % homology/identity to a wild type Semaphorin 3 protein/polypeptide as, for example, depicted in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16.

In preferred aspects, the mutated Semaphorin 3 of the invention is preferably at least 50% homologous/identical to the amino acid sequence as shown in SEQ ID NO: 2. It is understood that such amino acid sequences can also include orthologous/homologous/identical (and thus related) sequences. More preferably, the amino acid sequence encoding the mutated Semaphorin 3 is at least 52%, 53%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% homologous/identical to the amino acid sequence as shown in any one of SEQ ID NO: 2, wherein the higher values of sequence identity are preferred.

In certain aspects, the mutated Semaphorin 3 is preferably at least 48% homologous/identical to the amino acid sequence as shown in SEQ ID NO: 6. It is understood that such amino acid sequences can also include orthologous/homologous/identical (and thus related) sequences. More preferably, the amino acid sequence encoding the mutated Semaphorin 3 is at least 50%, 52%, 53%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% homologous/identical to the amino acid sequence as shown in any one of SEQ ID NO: 6, wherein the higher values of sequence identity are preferred.

In certain aspects, the mutated Semaphorin 3 of the invention is preferably at least 55% homologous/identical to the amino acid sequence as shown in SEQ ID NO: 10. It is understood that such amino acid sequences can also include orthologous/homologous/identical (and thus related) sequences. More preferably, the amino acid sequence encoding the mutated Semaphorin 3 is at least 57%, 60%, 63%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% homologous/identical to the amino acid sequence as shown in any one of SEQ ID NO: 10, wherein the higher values of sequence identity are preferred.

In certain aspects, the mutated Semaphorin 3 of the invention is preferably at least 45% homologous/identical to the amino acid sequence as shown in SEQ ID NO: 14. It is understood that such amino acid sequences can also include orthologous/homologous/identical (and thus related) sequences. More preferably, the amino acid sequence encoding the mutated Semaphorin 3 is at least 48%, 50%, 53%, , 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% homologous/identical to the amino acid sequence as shown in any one of SEQ ID NO: 14, wherein the higher values of sequence identity are preferred.

In certain aspects, the mutated Semaphorin 3 is preferably at least 55% homologous/identical to the amino acid sequence as shown in any one of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16. More preferably, the mutated Semaphorin 3 has at least 57%, 60%, 65%, 70%, 75%, 80%,

85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% homology/identity to a wild type Semaphorin 3 protein/polypeptide having the amino acid sequence as, for example, depicted in SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14 and 16, respectively, wherein the higher values are preferred. Most preferably, the mutated Semaphorin 3 has at least 99% homology to a wild type Semaphorin 3 protein/polypeptide having the amino acid sequence as, for example, depicted in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16.

The inventive combination may also comprise amino acid sequences further deviating from the herein described sequences, For example, said deviation may be, for example, the result of amino acid and/or nucleotide substitution(s), deletion(s), addition(s), insertion(s), duplication(s), inversion(s) and/or recombination(s) either alone or in combination. Those deviations may naturally occur or be produced via recombinant DNA techniques well known in the art; see, for example, the techniques described in Sambrook (Molecular Cloning; A Laboratory Manual, 2nd Edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, NY (1989)) and Ausubel, "Current Protocols in Molecular Biology", Green Publishing Associates; and Wiley Interscience, N.Y. (1989). The allelic variations may be naturally occurring allelic variants as well as synthetically produced or genetically engineered variants. The polypeptides, peptides or protein fragments encoded by the various derivatives, allelic variants, homologues or analogues of the above-described nucleic acid molecules encoding mutated Semaphorin 3 and/or the fragment thereof may share specific common characteristics, such as the angiogenesis inhibition, or the herein described functions and activities, or the molecular weight, immunological reactivity, conformation etc., as well as physical properties, such as electrophoretic mobility, chromatographic behavior, sedimentation coefficients, pH optimum, stability, solubility, spectroscopic properties etc.

The terms "complement", "reverse complement" and "reverse sequence" referred to herein are described in the following example: For sequence 5'AGTGAAGT3', the complement is 3'TCACTTCA5', the reverse complement is 3'ACTTCACT5' and the reverse sequence is 5'TGAAGTGA3'.

The mutated Semaphorin 3 or the functional fragment thereof as disclosed in WO/2016135130 are herein incorporated by reference. The mutated Semaphorin 3 or the functional fragment thereof defined herein may e.g. be selected from the group of:

- (a) a polypeptide that is encoded by a nucleic acid sequence selected from the group of SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 9 and SEQ ID NO: 13, wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding said hydrophilic amino acid,

wherein the nucleotides GCA at position 559 to 561 of SEQ ID NO: 5 are replaced by nucleotides encoding said hydrophilic amino acid,
wherein the nucleotides GCT at position 872 to 874 of SEQ ID NO: 9 are replaced by nucleotides encoding said hydrophilic amino acid, and
wherein the nucleotides GCC at position 398 to 400 of SEQ ID NO: 13 are replaced by nucleotides encoding said hydrophilic amino acid;

- (b) a polypeptide having the amino acid sequence selected from the group of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 10 and SEQ ID NO: 14, wherein the alanine residue at position 106 of SEQ ID NO: 2, at position 105 of SEQ ID NO: 6, at position 104 of SEQ ID NO: 10 or at position 120 of SEQ ID NO: 14 is replaced by said hydrophilic amino acid;
- (c) a polypeptide that is encoded by a nucleic acid sequence that hybridizes under stringent conditions to the complementary strand of a nucleic acid molecule encoding a polypeptide as defined in (a) or (b);
- (d) a polypeptide that functions as an inhibitor of angiogenesis and has at least 55% identity to any one of the polypeptides referred to in (b).

In preferred embodiments, the mutated Semaphorin 3A or the functional fragment thereof as defined herein that can be selected from the group of:

- (a) a polypeptide that is encoded by a nucleic acid sequence as shown in SEQ ID NO: 1
wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding said hydrophilic amino acid,
- (b) a polypeptide having the amino acid sequence of SEQ ID NO: 2, wherein the alanine residue at position 106 of SEQ ID NO: 2 is replaced by said hydrophilic amino acid;
- (c) a polypeptide that is encoded by a nucleic acid sequence that hybridizes under stringent conditions to the complementary strand of a nucleic acid molecule encoding a polypeptide as defined in (a) or (b);
- (d) a polypeptide that inhibits angiogenesis and has at least 50% identity to any one of the polypeptides referred to in (b).

In certain aspects, the mutated Semaphorin 3, the functional fragment thereof or the polypeptide comprises the amino acid sequence or the functional fragment thereof selected from the group of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, and SEQ ID NO: 14, wherein the alanine residue corresponding to position 106 of SEQ ID NO: 2; wherein the alanine residue corresponding to position 106 of

SEQ ID NO: 4; wherein the alanine residue corresponding to position 105 of SEQ ID NO: 6; wherein the alanine residue corresponding to position 105 of SEQ ID NO: 8; wherein the alanine residue corresponding to position 104 of SEQ ID NO: 10; wherein the alanine residue corresponding to position 104 of SEQ ID NO: 12; wherein the alanine residue corresponding to position 120 of SEQ ID NO: 14; or wherein the alanine residue corresponding to position 120 of SEQ ID NO: 16 is replaced by an hydrophilic amino acid.

In most preferred embodiments, the mutated Semaphorin 3A, the functional fragment thereof or the polypeptide comprises the amino acid sequence selected from the group of SEQ ID NO: 2 or the functional fragment thereof, wherein the alanine residue corresponding to position 106 of SEQ ID NO: 4 is replaced by an hydrophilic amino acid. The mutated Semaphorin 3, the functional fragment thereof or the polypeptide as defined in WO/2016135130 is herein incorporated by reference.

Accordingly, the skilled person understands that in case the herein described mutation is defined by a specific position, e.g. the alanine at the position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced, it is clear that also a corresponding amino acid (position) can be meant in other Semaphorin 3 proteins, such as other Semaphorin 3A polypeptides, or Semaphorin 3B, C or D polypeptides, which for instance can be found by comparison of homology. Hence, it is understood herein that for the identification of further wild-type sequences and/or for the detection of the relevant specific amino acid residue corresponding to the alanine on position 106 of wild-type Semaphorin 3A that is mutated according to the invention standard homology screenings (e.g. sequence alignments) or PCR-mediated screening techniques can be employed.

In most preferred embodiments, the inventive combination, the pharmaceutical composition, or the kit of parts comprises the mutated Semaphorin 3A or the functional fragment thereof, wherein the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by a hydrophilic amino acid.

SEQ ID NO: 58, 60, 62, 64, 66, 68, 70 or 72 relates to the full length human or mouse mutated Semaphorin 3A, B, C or D, wherein lysine is in place at the position that by comparison of homology corresponds to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2. Therefore, comprising a hydrophilic amino acid in place of means that the specific alanine, e.g., corresponding to position 106 shown in SEQ ID NO: 2, that is present in the naturally occurring Semaphorin 3A, B, C or D is mutated or changed to the hydrophilic amino acid, preferably lysine, in the mutated Semaphorin 3A, B, C or D.

An exemplary polypeptide comprising a human mutated Semaphorin 3A has an amino acid sequence as given in SEQ ID NO: 58, wherein a lysine is in place at the position 106.

An exemplary polypeptide comprising a mouse mutated Semaphorin 3A has an amino acid sequence as given in SEQ ID NO: 60, wherein a lysine is in place at the position 106.

An exemplary polypeptide comprising a human mutated Semaphorin 3B has an amino acid sequence as given in SEQ ID NO: 62, wherein a lysine is in place at the position 105.

An exemplary polypeptide comprising a mouse mutated Semaphorin 3B has an amino acid sequence as given in SEQ ID NO: 64, wherein a lysine is in place at the position 105.

An exemplary polypeptide comprising a human mutated Semaphorin 3C has an amino acid sequence as given in SEQ ID NO: 66, wherein a lysine is in place at the position 104.

An exemplary polypeptide comprising a mouse mutated Semaphorin 3C has an amino acid sequence as given in SEQ ID NO: 68, wherein a lysine is in place at the position 104.

An exemplary polypeptide comprising a human mutated Semaphorin 3D has an amino acid sequence as given in SEQ ID NO: 70, wherein a lysine is in place at the position 120.

An exemplary polypeptide comprising a mouse mutated Semaphorin 3D has an amino acid sequence as given in SEQ ID NO: 72, wherein a lysine is in place at the position 120.

Therefore, the inventive combination may comprise the mutated Semaphorin 3 or the functional fragment thereof wherein the mutated Semaphorin 3 comprises an amino acid sequence that is selected from the group consisting of SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70 and SEQ ID NO: 72 or a functional fragment thereof. In preferred aspects of the invention, the invention relates to the mutated Semaphorin 3A or the functional fragment thereof wherein the mutated Semaphorin 3A comprises an amino acid sequence that is SEQ ID NO: 58 or SEQ ID NO: 60. In preferred aspects, the functional fragment is the sema domain as detailed herein below.

Further, the inventive combination may comprise a nucleic acid molecule encoding the mutated Semaphorin 3 or the functional fragment thereof that are disclosed in WO/2016135130 and are herein incorporated by reference.

SEQ ID NO: 57, 59, 61, 63, 65, 67, 69 or 71 relates to a nucleic acid sequence encoding the full length human or mouse mutated Semaphorin 3A, B, C or D, wherein lysine is in place at the position that by comparison of homology corresponds to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2.

An exemplary nucleic acid molecule encoding the mutated human Semaphorin 3A comprises a nucleic acid sequence as defined in SEQ ID NO: 57, wherein the nucleotides at position 631 to 633 of SEQ ID NO: 57 encode for the amino acid lysine.

An exemplary nucleic acid molecule encoding the mutated mouse Semaphorin 3A comprises a nucleic acid sequence as defined in SEQ ID NO: 59, wherein the nucleotides at position 965 to 967 of SEQ ID NO: 59 encode for the amino acid lysine.

An exemplary nucleic acid molecule encoding the mutated human Semaphorin 3B comprises a nucleic acid sequence as defined in SEQ ID NO: 61, wherein the nucleotides at position 559 to 561 of SEQ ID NO: 61 encode for the amino acid lysine.

An exemplary nucleic acid molecule encoding the mutated mouse Semaphorin 3B comprises a nucleic acid sequence as defined in SEQ ID NO: 63, wherein the nucleotides at position 712 to 714 of SEQ ID NO: 63 encode for the amino acid lysine.

An exemplary nucleic acid molecule encoding the mutated human Semaphorin 3C comprises a nucleic acid sequence as defined in SEQ ID NO: 65, wherein the nucleotides at position 872 to 874 of SEQ ID NO: 65 encode for the amino acid lysine.

An exemplary nucleic acid molecule encoding the mutated mouse Semaphorin 3C comprises a nucleic acid sequence as defined in SEQ ID NO: 67, wherein the nucleotides at position 498 to 500 of SEQ ID NO: 67 encode for the amino acid lysine.

An exemplary nucleic acid molecule encoding the mutated human Semaphorin 3D comprises a nucleic acid sequence as defined in SEQ ID NO: 69, wherein the nucleotides at position 398 to 400 of SEQ ID NO: 69 encode for the amino acid lysine.

An exemplary nucleic acid molecule encoding the mutated mouse Semaphorin 3D comprises a nucleic acid sequence as defined in SEQ ID NO: 71, wherein the nucleotides at position 904 to 906 of SEQ ID NO: 71 encode for the amino acid lysine.

The nucleic acid given in SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69 or SEQ ID NO: 71 encodes full length mutated Semaphorin 3 proteins. Therefore, the inventive combination may comprise the mutated Semaphorin 3 or the functional fragment thereof wherein the mutated Semaphorin 3 is encoded by a nucleic acid molecule comprising the nucleic acid selected from the group consisting of SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69 and SEQ ID NO: 71. In preferred aspects of the invention, the mutated Semaphorin 3 or the functional fragment thereof is mutated Semaphorin 3A or the functional fragment thereof, wherein the mutated Semaphorin 3A is encoded by a nucleic acid molecule comprising the nucleic acid SEQ ID NO: 57 or SEQ ID NO: 59. In preferred aspects, the functional fragment is the sema domain as detailed herein below.

In certain aspects, the encoded mutated Semaphorin 3 is disclosed in WO/2016135130 incorporated herein by reference.

A codon encoding a hydrophilic amino acid means in accordance with the present invention, a codon, which according to the standard genetic code (as illustrated, inter alia, in Stryer

(1995), "Biochemistry", Freeman and Company, ISBN 0-7167-2009-4) codes for a "hydrophilic amino acid". In certain aspects, K is encoded by a codon coding for K. In particular preferred aspects, K is encoded by the codon AAG or AAA. The degeneracy of the genetic code permits the same amino acid sequence to be encoded and translated in many different ways. For example, leucine, serine and arginine are each encoded by six different codons, while valine, proline, threonine, alanine and glycine are each encoded by four different codons. However, the frequency of use of such synonymous codons varies from genome to genome among eukaryotes and prokaryotes. For example, synonymous codon-choice patterns among mammals are very similar, while evolutionarily distant organisms such as yeast (*S. cerevisiae*), bacteria (such as *E. coli*) and insects (such as *D. melanogaster*) reveal a clearly different pattern of genomic codon use frequencies. Therefore, codon optimized genes can be used in the present invention. The design of codon optimized genes should take into account a variety of factors, including the frequency of codon usage in an organism, nearest neighbor frequencies, RNA stability, the potential for secondary structure formation, the route of synthesis and the intended future DNA manipulations of that gene. It is contemplated herein that codon optimized nucleic acid sequences can be employed. Such codon optimized genes (SEQ ID NOs: 17, 19, 43 and 44) were used in the appended examples.

As disclosed in WO/2016135130 that is incorporated herein by its entirety, the replacement of alanine by a hydrophilic amino acid in the consensus motif $CX_1X_2A_3GKD$ results in beneficial pharmacologic effects.

Therefore, the polypeptides comprised in the inventive combination are mutated Semaphorin 3 or the functional fragment thereof, wherein said mutated Semaphorin 3 or said functional fragment thereof comprises an amino acid sequence $CX_1X_2A_3GKD$, wherein

X_1 is an amino acid, which is K or N,

X_2 is an amino acid selected from the group of W, M and L

and wherein the alanine (A_3) is replaced by said hydrophilic amino acid.

In particular, the mutated Semaphorin 3 is selected from the group consisting of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C, and Semaphorin 3D.

It is herein understood that A_3 refers to the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2; to the alanine corresponding to position 105 of the wild type Semaphorin 3B as shown in SEQ ID NO: 6, to the alanine corresponding to position 104 of the wild type Semaphorin 3C as shown in SEQ ID NO: 10, or to the alanine corresponding to position 120 of the wild type Semaphorin 3D as shown in SEQ ID NO: 14. " A_3 " usually refers to the specific alanine; however, " A_3 " can also refer to an amino acid residue that is homologous to alanine, such as valine, isoleucine, leucine, methionine, phenylalanine, tyrosine or tryptophan. Most preferably, " A_3 " is alanine. Furthermore, the

amino acid residues defined by “C”, “X₁”, “X₂”, “G”, “K” and “D” can also refer to amino acid residues that are homologous to said respective defined amino acid residues as long as the mutated Semaphorin 3 is selected from the group consisting of Semaphorin 3 A, B, C and D. According to the invention, the mutated Semaphorin 3 is not Semaphorin 3E, F or G. In preferred aspects of the invention, “X₁” is not isoleucine or valine.

Said hydrophilic amino acid is selected from the group consisting of lysine, arginine, asparagine, glutamine, serine, threonine, glutamic acid, aspartic acid and histidine. More preferably, said hydrophilic amino acid is lysine or arginine and most preferably, said hydrophilic amino acid is lysine.

The term “hydrophilic amino acid” preferably means an amino acid selected from the group consisting of N, Q, S, T, E, D, K, R and H. According to the standard three letter amino acid code and single letter code arginine can be abbreviated (Arg) or (R). Lysine can be abbreviated (Lys) or (K). Aspartic acid can be abbreviated (Asp) or (D). Glutamic acid can be abbreviated (Glu) or (E). Glutamine can be abbreviated (Gln) or (Q). Asparagine can be abbreviated (Asn) or (N). Histidine can be abbreviated (His) or (H). Serine can be abbreviated (Ser) or (S). Threonine can be abbreviated (Thr) or (T). N, Q, S and T are hydrophilic uncharged amino acids and E, D, K, R and H are hydrophilic charged amino acids. It is also envisaged herein that said hydrophilic amino acid can be a non-proteinogenic and/or non-standard α -amino acid (such as, e.g., ornithine and citrulline).

The inventive combination comprises the amino acid sequences comprising the mutated Semaphorin 3 or the functional fragment thereof, wherein said hydrophilic amino acid is selected from the group of K, R, N, Q, S, T, E, D, and H. In preferred aspects, the hydrophilic amino acid is selected from the group of K, R, E, D, and H. In even more preferred aspects, the hydrophilic amino acid is K or R. In most preferred aspects, the hydrophilic amino acid is K.

In certain aspects, the inventive combination comprises the amino acid sequences comprising the mutated Semaphorin 3 or the functional fragment thereof, wherein the alanine (A₃) in the amino acid sequence motif CX₁X₂A₃GKD is replaced by said hydrophilic amino acid selected from the group of K, R, N, Q, S, T, E, D, and H. In certain aspects, the inventive combination comprises the mutated Semaphorin 3 or the functional fragment thereof, wherein the alanine (A₃) in the amino acid sequence motif CX₁X₂A₃GKD is replaced by said hydrophilic amino acid selected from the group of K, R, E, D, and H. In preferred aspects, the inventive combination comprises the mutated Semaphorin 3 or the functional fragment thereof, wherein the alanine (A₃) in the amino acid sequence motif CX₁X₂A₃GKD is replaced

by said hydrophilic amino acid is K or R. In particularly preferred aspects, the inventive combination comprises the mutated Semaphorin 3 or the functional fragment thereof, wherein the alanine (A₃) in the amino acid sequence motif CX₁X₂A₃GKD is replaced by said hydrophilic amino acid is K.

Further, the inventive combination comprises the mutated Semaphorin 3 or the functional fragment thereof, wherein the mutated Semaphorin 3 or the functional fragment thereof comprises said hydrophilic amino acid at position 106 of SEQ ID NO: 2, at position 105 of SEQ ID NO: 6, at position 104 of SEQ ID NO: 10 or at position 120 of SEQ ID NO: 14 (or a corresponding position) and comprises at least one additional mutation selected from the group consisting of amino acid substitution(s), addition(s), deletions(s), inversion(s) and duplication(s).

The following relates to mutated Semaphorin 3 or the functional fragments thereof that are encompassed in the fusion proteins/polypeptides. Preferably, the inventive combination comprises the fusion protein/polypeptide as defined herein and disclosed in WO/2016135130 incorporated herein by reference. An exemplary fusion protein that is herein preferred is disclosed in Figure 2B that is herein incorporated by reference. The mutated Semaphorin 3 protein encompassed in the fusion protein/polypeptide may preferably be a functional fragment of the mutated Semaphorin 3. In other words, the following relates to the herein provided functional fragments of the non-naturally occurring/artificial/mutated Semaphorin 3 proteins or the herein provided functional fragments of the non-naturally occurring/artificial/mutated Semaphorin 3 proteins that are comprised in the fusion protein/polypeptide:

In most preferred embodiments, the herein provided functional fragment of the mutated Semaphorin 3 comprises a functional sema domain, wherein the sema domain comprises the hydrophilic amino acid at the position that by comparison of homology corresponds to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 and wherein the sema domain has the properties of mutated Semaphorin 3A, B, C or D, as described above, e.g. inhibiting angiogenesis and/or vascular normalizing agent. The term "sema domain" refers to a structural domain of Semaphorin 3 proteins that is defined and explained in WO/2016135130. Therefore, all the definitions, explanations and corresponding embodiments provided in WO/2016135130 in relation to the sema domain or the functional sema domain are herein incorporated by reference unless explicitly disclosed herein. The same holds true for the definitions and explanations of the functional fragment. Thus, the definitions and explanations in relation to the functional fragment disclosed in WO/2016135130 are herein incorporated by reference.

In summary, the inventive combination comprises the functional fragment of the non-naturally occurring/artificial/mutated Semaphorin 3 comprising:

- the amino acid sequence $CX_1X_2A_3GKD$, wherein X_1 is an amino acid, which is K or N, X_2 is an amino acid selected from the group of W, M and L and wherein the alanine (A_3) is replaced by the hydrophilic amino acid;
- the amino acid sequence selected from the group consisting of SEQ ID NO: 25, 28, 31 and 34, wherein the alanine at the position that by comparison of homology corresponds to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 is replaced by the hydrophilic amino acid; or
- the amino acid sequence SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55 or SEQ ID NO: 56,

and wherein the functional fragment has the properties/characteristics of mutated Semaphorin 3A, B, C or D, e.g. inhibiting angiogenesis, and has not the properties/characteristics of Semaphorin 3E, F or G.

The PSI domain that may be comprised in the fusion protein stabilizes the structural conformation and/or the structural integrity of the functional sema domain, the functional fragment of the mutated Semaphorin 3 or the fusion protein/polypeptide. The functional fragment of the mutated Semaphorin 3 can comprise fragments of the PSI domain so long as the fragment of the PSI domain has the function to stabilize the mutated Semaphorin 3 or the functional fragment thereof or the fusion protein/ polypeptide, more preferably, the functional sema domain. The PSI domain of Semaphorins 3A, B, C or D shares conserved amino acid sequences illustrated in the consensus motifs/sequences/motifs SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47 or SEQ ID NO: 48, respectively. Accordingly, the PSI domain of the invention comprises one or more of the following sequences SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47 or SEQ ID NO: 48. An exemplary amino acid sequence of the PSI domain of human Semaphorin 3A spans from amino acid residues 517 to 567 of SEQ ID NO: 2. As shown in the appended examples and thus more preferably, an exemplary amino acid sequence of a shorter PSI domain, which lacks furin protease cleavage sites, spans from amino acid residues 517 to 548 of SEQ ID NO: 2. Exemplary PSI domains are given in the following: an exemplary amino acid sequence of the PSI domain of human Semaphorin 3A spans from amino acid residues 517 to 548 of SEQ ID NO: 2, an exemplary amino acid sequence of the PSI domain of human Semaphorin 3B spans from amino acid residues 516 to 547 of SEQ ID NO: 6, an exemplary amino acid sequence of the PSI domain of human Semaphorin 3C spans from amino acid residues 514 to 545 of SEQ ID NO: 10, and an exemplary amino acid sequence of the PSI domain of human Semaphorin 3D spans from amino acid residues 534 to 565) of SEQ ID NO: 14.

Further, the mutated Semaphorin 3 or the functional fragment of the mutated Semaphorin 3 are disclosed in WO/2016135130 incorporated herein by reference. It is also herein envisaged that these amino acid sequences can be linked together with artificial amino acid linkers as disclosed in WO/2016135130 incorporated herein by reference., e.g., serine-glycine linkers.

The length of the functional fragments of the mutated Semaphorin 3 or the fusion protein is not limited as long as the functional fragments shows one or more of the herein documented functions. The functional fragments disclosed herein may have a length of e.g. about 10, 20, 30, 40, 50, 60, 80, 100, 200, 250, 300, 400, 500 or 600 amino acids. Preferably, such fragments have a length of about 400 to 500 amino acids. Preferably, such fragments have a length of about 300 to 400 amino acids. Preferably, such fragments have a length of about 100 to 300 amino acids. It is herein envisaged that that the amino acid sequence of the herein provided functional fragments of the non-naturally occurring/artificial/mutated Semaphorin 3 proteins can be truncated at the N-terminus, the C-terminus and/or in the body of the amino acid sequence. It is herein envisaged that, e.g., 10, 20, 30, 40, or 50 amino acids can be deleted. These deletions/modifications do not depart from the scope of the invention as long as the functional fragment has the characteristics of mutated Semaphorin 3A, B, C or D. Further, these deletions/modifications are not limited as long as the herein provided functional fragment or the fusion protein/polypeptide comprising the herein provided functional fragment, which has the hydrophilic amino acid in place of the alanine at the position which corresponds by comparison of homology to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2, has the function/activity as defined herein above, e.g., as an angiogenesis inhibitor and/or as a vascular normalizing agent, of the mutated Semaphorin 3, the functional fragment thereof, the fusion protein comprising the functional fragment or the functional sema domain.

In most preferred embodiments, the functional fragment of the mutated Semaphorin 3 comprises the functional sema domain, wherein said sema domain comprises the hydrophilic amino acid at the position that by comparison of homology corresponds to the position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2.

In other words, the mutated Semaphorin 3 may comprise the functional sema domain, wherein said sema domain comprises the hydrophilic amino acid at the position that by comparison of homology corresponds to the position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2. The functional sema domain is disclosed in WO/2016135130 incorporated herein by reference. The functional sema domain and corresponding embodiments as disclosed in WO/2016135130 are herein incorporated by reference.

Furthermore, the functional sema domain can comprise at least one additional mutation selected from the group consisting of amino acid substitution(s), addition(s), deletions(s), inversion(s) and duplication(s). Further, the functional sema domain can comprise additional amino acid deletion(s). It is envisaged herein that the amino acid sequence of the functional sema domain can be truncated at the N-terminus, the C-terminus and/or in the body of the amino acid sequence. It is envisaged herein that, e.g., about 10, 20, 30, 40, 50 or 100 amino acids can be deleted. These deletions/modifications do not depart from the scope of the invention as long as the functional sema domain has the characteristics of the mutated Semaphorin 3A, B, C or D as defined herein above. Further, the deletions/modifications as disclosed in WO/2016135130 are herein incorporated by reference

An exemplary nucleic acid molecule encoding the functional sema domain or the functional fragment of the mutated Semaphorin 3 are disclosed in WO/2016135130 incorporated herein by reference.

In preferred embodiments, the nucleic acid molecule encoding the functional sema domain or the functional fragment of the mutated Semaphorin 3A comprises the nucleotides from 601 to 1206 of SEQ ID NO: 1, wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding the hydrophilic amino acid.

In further preferred embodiments, the nucleic acid molecule encoding the functional sema domain or the functional fragment of the mutated Semaphorin 3A comprises the nucleotides from 601 to 1206 of SEQ ID NO: 57.

Further, an exemplary polypeptide comprises the functional fragment of the mutated Semaphorin 3, wherein the functional fragment comprises or is the functional sema domain of the mutated Semaphorin 3 is disclosed in WO/2016135130 incorporated herein by reference.

SEQ ID NO: 49, 50, 51 or 52 comprises an amino acid sequence of an exemplary functional sema domain or an exemplary functional fragment of the mutated Semaphorin 3A, B, C or D, respectively, wherein the alanine is replaced by a lysine at the position that by comparison of homology corresponds to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2. Thus, an exemplary amino acid sequence of the functional sema domain or the functional fragment of the mutated Semaphorin 3 can comprise the amino acid sequence that is selected from the group consisting of SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51 and SEQ ID NO: 52. In preferred embodiments, the amino acid sequence of the functional sema domain or the functional fragment of the mutated Semaphorin 3A comprises the amino acid sequence as shown in SEQ ID NO: 49. Fragments of the functional sema domain are

also envisaged herein. For example, the sema domain can also comprise shortened versions of the herein defined exemplary sema domains.

The following relates to the most preferred embodiment comprised in the inventive combination, the fusion protein/polypeptide. In most preferred embodiments, the polypeptide is the fusion protein. The fusion protein comprises the non-naturally occurring/artificial/mutated Semaphorin 3 protein, the non-naturally occurring/artificial/mutated functional fragment of the Semaphorin 3, and/or the non-naturally occurring/artificial/mutated functional sema domain of the Semaphorin 3. The fusion protein comprises preferably the stabilizer domain and/or the dimerization domain. Any one of the herein above defined functional fragments of the mutated Semaphorin 3 can be comprised in the fusion protein, wherein the functional fragments have the characteristics/properties of mutated Semaphorin 3A, B, C or D, e.g. inhibiting angiogenesis, and not of Semaphorin 3E, F or G. The fusion protein and its function fragments are disclosed in WO/2016135130 that are incorporated herein by reference. In most preferred embodiments, the inventive combination comprises the fusion protein comprising the functional fragment of the mutated Semaphorin 3, wherein said functional fragment comprises the functional sema domain, wherein the sema domain comprises the hydrophilic amino acid in place of the alanine (A₃) in the amino acid sequence CX₁X₂A₃GKD. In other words, the fusion protein comprises the functional sema domain, wherein the sema domain comprises the hydrophilic amino acid at the position that by comparison of homology corresponds to the position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2. In other words, the fusion protein comprises the functional sema domain, wherein the sema domain comprises the hydrophilic amino acid at the position that by comparison of homology corresponds to the position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2. The functional sema domain comprised in the fusion protein is disclosed in WO/2016135130 incorporated herein by reference.

An exemplary nucleic acid molecule encoding the functional sema domain or the functional fragment of the mutated Semaphorin 3 that can be comprised in the fusion protein is disclosed in WO/2016135130 that is herein incorporated by reference.

In preferred embodiments, the fusion protein comprises the functional sema domain of, wherein the sema domain is encoded by the nucleic acid molecule that comprises the nucleotides from 601 to 1206 of SEQ ID NO: 1, wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding the hydrophilic amino acid.

Further, an exemplary fusion protein/polypeptide can comprise the functional fragment of the mutated Semaphorin 3, wherein the functional fragment comprises or is the functional sema domain of mutated Semaphorin 3 as defined in:

SEQ ID NO: 21, wherein the alanine residue corresponding to position 106 of SEQ ID NO: 2 is replaced by a hydrophilic amino acid;

SEQ ID NO: 22, wherein the alanine residue corresponding to position 105 of SEQ ID NO: 6 is replaced by a hydrophilic amino acid;

SEQ ID NO: 23 wherein the alanine residue corresponding to position 104 of SEQ ID NO: 10 is replaced by a hydrophilic amino acid; or

SEQ ID NO: 24, wherein the alanine residue corresponding to position 120 of SEQ ID NO: 14 is replaced by a hydrophilic amino acid.

In preferred embodiments, the amino acid sequence of the functional sema domain of the mutated Semaphorin 3A comprised in the fusion protein comprises an amino acid sequence as shown in SEQ ID NO: 21, wherein the alanine residue corresponding to position 106 of SEQ ID NO: 2 is replaced by a hydrophilic amino acid.

In further preferred embodiments, the amino acid sequence of the functional sema domain of the mutated Semaphorin 3A comprised in the fusion protein comprises the amino acid sequence as shown in SEQ ID NO: 49.

In preferred embodiments, the fusion protein comprises further to the mutated Semaphorin 3 or the functional fragment thereof a stabilizer domain. Said stabilizer domain stabilizes the structural conformation and/or the structural integrity of the non-naturally occurring/artificial/mutated Semaphorin 3 protein, the herein provided functional fragment of the non-naturally occurring/artificial/mutated Semaphorin 3 protein, or the herein provided functional sema domain. The stabilizer domain disclosed in WO/2016135130 is herein incorporated by reference.. The stabilizer domain can for example be the PSI domain or a fragment thereof, wherein said PSI domain can comprise one of the following consensus sequence motifs SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47 or SEQ ID NO: 48.

In preferred embodiments, the fusion protein can comprise the mutated Semaphorin 3 or the functional fragment thereof, wherein the functional fragment comprises the sema domain and the PSI domain. Such exemplary fusion protein is disclosed in WO/2016135130 that is herein incorporated by reference. In preferred embodiments, the functional fragment of the mutated Semaphorin 3A comprised in the fusion protein has a polypeptide spanning from amino acid residues 1 to 548 of SEQ ID NO: 2, wherein the alanine residue at position 106 of SEQ ID NO: 2 is replaced by the hydrophilic amino acid.

As indicated in the appended examples and as explained above, dimerization of the mutated Semaphorin 3 proteins increases the inhibiting effect of EC migration. Therefore, the mutated Semaphorin 3 proteins are preferably in the form of the dimer. A functional sema domain of the mutated Semaphorin 3 proteins may be responsible for the dimerization and the binding to the Plexin receptors. The binding of the Semaphorin 3 to its Plexin receptor leads to an activation of the cytoplasmic region of the Plexin receptor, which results in active downstream signaling. Without being bound by theory, the Plexin receptors are activated by the Semaphorin induced dimerization. Therefore, in most preferred embodiments, the herein provided mutated Semaphorin 3 or the functional sema domain or functional fragment of the mutated Semaphorin 3 is in the form of a dimer with another herein provided mutated Semaphorin 3 or the functional sema domain or functional fragment of the mutated Semaphorin 3. The dimerization of the non-naturally occurring/artificial/mutated Semaphorin 3 proteins or the herein provided functional fragments of the non-naturally occurring/artificial/mutated Semaphorin 3 proteins that are comprised in the fusion protein can be induced by said functional fragments, e.g. by the functional sema domain itself and/or can be induced/promoted by a dimerization domain.

In most preferred embodiments, the fusion protein comprises a dimerization domain further to the mutated Semaphorin 3, the functional fragment of the mutated Semaphorin 3 and/or the stabilizer domain. In other words, the fusion protein comprises further to the mutated Semaphorin 3 or to the functional fragment thereof a stabilizer domain stabilizing the structural integrity of the molecule and/or a dimerization domain inducing homo- or hetero-dimers. In other words, the fusion protein can comprise the stabilizer domain and/or the dimerization domain. The “dimerization domain” refers to a domain that induce/promote spatial proximity of the non-naturally occurring/artificial/mutated Semaphorin 3 proteins, the herein provided functional fragments of the non-naturally occurring/artificial/mutated Semaphorin 3 proteins, or the herein provided functional sema domains. The dimerization domain, the definitions and the corresponding embodiments as disclosed in WO/2016135130 are herein incorporated by reference. The dimerization domain can for example be selected from the group of a C-terminal IgG constant domain, DARPin and leucine zipper.

The non-naturally occurring/artificial/mutated Semaphorin 3 proteins or the functional fragments thereof or the fusion proteins comprising said Semaphorin 3 protein(s) or said functional fragment(s) thereof form homo- or hetero-dimers with each other. The term “homo-dimer” means that two identical monomers are in the form of a dimer. The term “hetero-dimer” means that two different monomers are in the form of a dimer. In certain aspects, the two monomers of the dimer can be comprised in one fusion protein. Such embodiments disclosed in WO/2016135130 are herein incorporated by reference.

In certain aspects, the fusion protein comprises two polypeptides. Such embodiments disclosed in WO/2016135130 are herein incorporated by reference.

It is also envisaged herein that the fusion protein of the mutated Semaphorin 3 comprises the following domains:

- (i) a sema domain;
- (ii) a PSI domain; and
- (iii) a C-terminal IgG constant domain fused to the C-terminus of the PSI domain, and is further characterized in that the alanine (A₃) residue comprised in the motif CX₁X₂A₃GKD of the Semaphorin 3 is replaced by lysine, wherein the Semaphorin 3 is selected from the group consisting of Semaphorin 3A, B, C and D.

The fusion protein comprises the functional sema domain, wherein said sema domain comprises a hydrophilic amino acid in place of the alanine at the position that by comparison of homology corresponds to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2, and a dimerization domain and/or a stabilizer domain.

In other words, the fusion protein comprises the functional sema domain, wherein said sema domain is selected from the group consisting of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C and Semaphorin 3D, wherein said sema domain comprises

a hydrophilic amino acid in place of the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2;

a hydrophilic amino acid in place of the alanine corresponding to position 105 of the wild type Semaphorin 3B as shown in SEQ ID NO: 6;

a hydrophilic amino acid in place of the alanine corresponding to position 104 of the wild type Semaphorin 3C as shown in SEQ ID NO: 10; or

a hydrophilic amino acid in place of the alanine corresponding to position 120 of the wild type Semaphorin 3D as shown in SEQ ID NO: 14;

and wherein said fusion protein further comprises a dimerization domain and/or a stabilizer domain.

Most preferably, the fusion protein comprises a functional sema domain, wherein within said functional sema domain the alanine corresponding to position 106 of the wild type Semaphorin 3A of SEQ ID NO: 2 is replaced by a hydrophilic amino acid or wherein the alanine corresponding to said alanine 106 in Semaphorin 3B, 3C or 3D is replaced by a hydrophilic amino acid and a dimerization domain, wherein the dimerization domain is IgG1 and/or a stabilizer domain, wherein the stabilizer domain is the PSI domain. In most preferred aspects, the fusion protein comprises the functional sema domain of Semaphorin 3A, wherein said sema domain comprises a hydrophilic amino acid in place of the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2 and wherein said

fusion protein further comprises a dimerization domain and/or a stabilizer domain.

In most preferred embodiments, the fusion proteins/polypeptides comprise the functional sema domain that is fused to the PSI domain (as shown in the appended examples). The resulting sema-PSI domain is fused to the dimerization domain, e.g., the constant fragment of the IgG1 domain. Furthermore, such fusion proteins lack the Nrp1 binding and/or the furin cleavable Ig-like (amino acids spanning from 580-670 of SEQ ID NO: 2)/basic region (amino acids spanning from 715-771 of SEQ ID NO: 2). Such fusion proteins are herein most preferred embodiments and exemplary nucleic acid molecules of such a encoded fusion protein can comprise a nucleic acid sequence having:

a nucleic acid sequence spanning from nucleotides 316 to 1959 of SEQ ID NO: 1 and a nucleic acid sequence spanning from nucleotides 295 to 990 of SEQ ID NO: 37, wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding the hydrophilic amino acid;

a nucleic acid sequence spanning from nucleotides 247 to 1887 of SEQ ID NO: 5 and a nucleic acid sequence spanning from nucleotides 295 to 990 of SEQ ID NO: 37, wherein the nucleotides GCA at position 559 to 561 of SEQ ID NO: 5 are replaced by nucleotides encoding the hydrophilic amino acid;

a nucleic acid sequence spanning from nucleotides 563 to 2197 of SEQ ID NO: 9 and a nucleic acid sequence spanning from nucleotides 295 to 990 of SEQ ID NO: 37, wherein the nucleotides GCT at position 872 to 874 of SEQ ID NO: 9 are replaced by nucleotides encoding the hydrophilic amino acid; or

a nucleic acid sequence spanning from nucleotides 41 to 1735 of SEQ ID NO: 13 and a nucleic acid sequence spanning from nucleotides 295 to 990 of SEQ ID NO: 37, wherein the nucleotides GCC at position 398 to 400 of SEQ ID NO: 13 are replaced by nucleotides encoding the hydrophilic amino acid.

In most preferred embodiments, the fusion protein of the mutated Semaphorin 3A is encoded by a nucleic acid sequence spanning from nucleotides 316 to 1959 of SEQ ID NO: 1 and a nucleic acid sequence spanning from nucleotides 295 to 990 of SEQ ID NO: 37, wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding the hydrophilic amino acid.

It is contemplated herein that codon optimized nucleic acid sequences can be employed as shown in the appended examples (SEQ ID NOs: 17, 19, 43 and 44).

In most preferred embodiments, the fusion protein comprises a sema domain, a stabilizer domain and a dimerization domain. The functional sema domain is fused to the stabilizer domain, e.g., the PSI domain. The resulting sema-PSI domain is fused to the dimerization domain, e.g., the constant fragment of the IgG1 domain as shown in SEQ ID NO: 38 or 41.

Such an exemplary fusion protein comprises a sema domain, a PSI and a dimerization domain, wherein the fusion protein comprises an amino acid sequence:

spanning from amino acid residues 1 to 548 of SEQ ID NO: 2 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 106 of SEQ ID NO: 2 is replaced by the hydrophilic amino acid;

spanning from amino acid residues 1 to 547 of SEQ ID NO: 6 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 105 of SEQ ID NO: 6 is replaced by the hydrophilic amino acid;

spanning from amino acid residues 1 to 565 of SEQ ID NO: 10 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 104 of SEQ ID NO: 10 is replaced by the hydrophilic amino acid; or

spanning from amino acid residues 1 to 545 of SEQ ID NO: 14 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 120 of SEQ ID NO: 14 is replaced by the hydrophilic amino acid.

In most preferred embodiments, the fusion protein of the mutated Semaphorin 3A comprises a polypeptide: spanning from amino acid residues 1 to 548 of SEQ ID NO: 2 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 106 of SEQ ID NO: 2 is replaced by the hydrophilic amino acid

An exemplary fusion protein comprising the functional sema domain of mutated Semaphorin 3A, the PSI domain, the IgG1 domain is shown in SEQ ID NO: 18 or 20. An exemplary fusion protein comprising the functional sema domain of mutated Semaphorin 3B, mutated Semaphorin 3C, or mutated Semaphorin 3D and the PSI domain, the IgG1 domain is shown in SEQ ID NO: 76, 78 or 79, respectively. SEQ ID NO: 18 shows a fusion protein comprising the functional sema domain of the human mutated Semaphorin 3A, wherein the alanine at the position that by comparison of homology corresponds to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO:2 is replaced by lysine, the PSI domain and the human constant fragment of IgG1. The corresponding nucleic acid sequence encoding the fusion protein comprising the functional sema domain of the human mutated Semaphorin 3A, wherein the alanine at the position that by comparison of homology corresponds to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO:2 is replaced by lysine, the PSI domain and the human constant fragment of IgG1 is given in SEQ ID NO: 17. SEQ ID NOS: 74 and 75 show an amino acid sequence and the encoding nucleic acid sequence of an exemplary fusion protein of Semaphorin 3B without the inventive mutation.

SEQ ID NO: 20 shows a fusion protein comprising the functional sema domain of mouse mutated Semaphorin 3A, wherein the alanine at the position that by comparison of homology corresponds to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO:2 is replaced by lysine, the PSI domain and the human constant fragment of IgG1. The

corresponding nucleic acid sequence encoding the fusion protein comprising the functional sema domain of the mouse mutated Semaphorin 3A, wherein the alanine at the position that by comparison of homology corresponds to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO:2 is replaced by lysine, the PSI domain and the mouse constant fragment of IgG1 is given in SEQ ID NO: 19.

The fusion protein as described herein can be a heterologous protein, wherein the non-naturally occurring/artificial/mutated Semaphorin 3 protein, the non-naturally occurring/artificial/mutated functional fragment of the Semaphorin 3 or the non-naturally occurring/artificial/mutated functional sema domain of the Semaphorin 3, the dimerization domain and/or the stabilization domain are from different sources, e.g. from different species. It is understood herein that the non-naturally occurring/artificial/mutated Semaphorin 3 protein, the non-naturally occurring/artificial/mutated functional fragment of the Semaphorin 3, the non-naturally occurring/artificial/mutated functional sema domain of the Semaphorin 3, the dimerization domain and/or the stabilization domain can be linked/fused together as found in natural occurring Semaphorin 3 proteins, wherein the character of the mutated Semaphorin 3A, B, C or D is maintained. Further, the non-naturally occurring/artificial/mutated Semaphorin 3 protein, the non-naturally occurring/artificial/mutated functional fragment of the Semaphorin 3, the non-naturally occurring/artificial/mutated functional sema domain of the Semaphorin 3, the dimerization domain and/or the stabilization domain can be linked/fused together as is not found in nature. The non-naturally occurring/artificial/mutated Semaphorin 3 protein, the non-naturally occurring/artificial/mutated functional fragment of the Semaphorin 3, the non-naturally occurring/artificial/mutated functional sema domain of the Semaphorin 3, the dimerization domain and/or the stabilization domain can be conjugated/linked together via amino acid linkers as disclosed in WO/2016135130 incorporated herein by reference.

It is also envisaged herein that the stability of the mutated Semaphorin 3 or the functional fragment thereof can be optimized by adding immunoglobulin-like domains and to simultaneously enhance pharmacokinetic properties like prolonged half-life in serum and protection from proteolytic digestion by proteases. Moreover, stability of the formats can be enhanced by optimizing the production. Since linker sequences which are utilized to covalently join domains often leads to aggregates, production lines have been established that first produce two or three polypeptides that can be easily reassembled in order to generate a functional drug. Such techniques utilize directed disulphide-bridges or crosslinking reagents to covalently join two different polypeptides. Other techniques make use of hetero- or homo-dimerization domains like leucine-zipper domains, Fc-domains and others like knob into hole technologies (see, for example, WO 2007/062466).

The nucleic acid sequence(s) or the nucleic acid molecule encoding the herein described polypeptides may be a vector. The vector may be comprised in the inventive combination, the kit of parts, or the pharmaceutical composition.

Many suitable vectors are known to those skilled in molecular biology, the choice of which would depend on the function desired and include plasmids, cosmids, viruses, bacteriophages and other vectors used conventionally in genetic engineering. Methods which are well known to those skilled in the art can be used to construct various plasmids and vectors; see, for example, the techniques described in Sambrook et al. (*loc cit.*) and Ausubel, *Current Protocols in Molecular Biology*, Green Publishing Associates and Wiley Interscience, N.Y. (1989), (1994). Alternatively, the polynucleotides and vectors comprised in the inventive combination can be reconstituted into liposomes for delivery to target cells. Relevant sequences can be transferred into expression vectors where expression of a particular polypeptide is required.

Preferably, the vector is a gene targeting vector and/or a gene transfer vector. Gene therapy, which is based on introducing therapeutic genes (for example for vaccination) into cells by *ex vivo* or *in vivo* techniques, is one of the most important applications of gene transfer. Suitable vectors, vector systems and methods for *in vitro* or *in vivo* gene therapy are described in the literature and are known to the person skilled in the art; see, e.g., Giordano, *Nature Medicine* 2 (1996), 534-539; Schaper, *Circ. Res.* 79 (1996), 911-919; Anderson, *Science* 256 (1992), 808-813, Isner, *Lancet* 348 (1996), 370-374; Muhlhauser, *Circ. Res.* 77 (1995), 1077-1086; Wang, *Nature Medicine* 2 (1996), 714-716; WO 94/29469; WO 97/00957, Schaper, *Current Opinion in Biotechnology* 7 (1996), 635-640 or Verma, *Nature* 389 (1997), 239-242 and references cited therein. In certain aspects, the vector is an adeno-associated-virus (AAV) vector. In particular aspects, the AAV virus is an AAV8 and thus the vector is an AAV8 vector. AAV vectors are attractive for gene therapy. The AAV system has several advantages including long-term gene expression, the inability to autonomously replicate without a helper virus, transduction of dividing and nondividing cells, and the lack of pathogenicity from wild-type infections. It is envisaged herein that AAV serotypes display different organ tropism. Accordingly, different AAV serotypes can be employed to target the proteins/polypeptides of the invention to cancers of different organs. It is envisaged herein that different AAV vectors can be employed in gene therapy according to standard protocols (Grieger et al., 2012 and Asokan et al., 2012).

The nucleic acid molecules and vectors as described herein above may be designed for direct introduction or for introduction via liposomes, or viral vectors (e.g. adenoviral, retroviral)

into the cell. Methods for obtaining the herein disclosed polypeptides are disclosed in WO/2016135130 incorporated herein by reference.

In certain aspects, the vector comprises a nucleic acid sequence which is a regulatory sequence operably linked to said nucleic acid sequence defined in WO/2016135130 incorporated herein by reference.

The recited vector can also be an expression vector. An "expression vector" is a construct that can be used to transform a selected host and provides for expression of a coding sequence in the selected host. Expression vectors can for instance be cloning vectors, binary vectors or integrating vectors. Expression comprises transcription of the nucleic acid molecule preferably into a translatable mRNA. Regulatory elements ensuring expression in prokaryotes and/or eukaryotic cells are well known to those skilled in the art. In the case of eukaryotic cells they comprise normal promoters ensuring initiation of transcription and optionally poly-A signals ensuring termination of transcription and stabilization of the transcript. Possible regulatory elements permitting expression in prokaryotic host cells comprise, e.g., the P_L , *lac*, *trp* or *tac* promoter in *E. coli*, and examples of regulatory elements permitting expression in eukaryotic host cells are the *AOXI* or *GALI* promoter in yeast or the CMV-, SV40-, RSV-promoter (Rous sarcoma virus), CMV-enhancer, SV40-enhancer or a globin intron in mammalian and other animal cells.

Beside elements, which are responsible for the initiation of transcription such regulatory elements may also comprise transcription termination signals, such as the SV40-poly-A site or the tk-poly-A site, downstream of the polynucleotide. Furthermore, depending on the expression system used leader sequences capable of directing the polypeptide to a cellular compartment or secreting it into the medium may be added to the coding sequence of the recited nucleic acid sequence and are well known in the art. The leader sequence(s) is (are) assembled in appropriate phase with translation, initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein, or a portion thereof, into the periplasmic space or extracellular medium. Accordingly, such a leader sequence can also be a signal peptide. Thus, the mutated Semaphorin 3 polypeptides can comprise a signal peptide. The signal peptide is a short stretch of amino acids usually present at the N-terminus of proteins that are destined towards the secretory pathway. Such proteins include those that reside either inside certain organelles, like the endoplasmic reticulum, golgi or endosomes, or are secreted from the cell. The signal peptide is cleaved off and active polypeptides usually do not comprise signal peptides. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product; see supra. In this context, suitable expression vectors are known in the art such as Okayama-

Berg cDNA expression vector pcDV1 (Pharmacia), pCDM8, pRc/CMV, pcDNA1, pcDNA3 (Invitrogen), pEF-DHFR, pEF-ADA or pEF-neo (Mack et al. PNAS (1995) 92, 7021-7025 and Raum et al. Cancer Immunol Immunother (2001) 50(3), 141-150) or pSPORT1 (GIBCO BRL).

Preferably, the expression control sequences may be eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells, but control sequences for prokaryotic hosts may also be used. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and as desired, the collection and purification of the polypeptide of the invention may follow; see, e.g., the appended examples.

The present invention also relates to a host cell transformed with a vector described herein above or the nucleic acid molecule described herein above and further comprises the antimetabolite as described herein. Said host may be eukaryotic cell. Said eukaryotic host may be a mammalian cell.

The mammalian cell may be a cell of a subject, e.g. a neuronal cell and/or a cultured cell like.

The term "cell" or "mammalian cell" as used in this context may also comprise a plurality of cells as well as cells comprised in a tissue. The cell/host may be obtained from samples from a (transgenic) non-human animal or human suffering from a disease, e.g. angiogenic disease, cancer or a disease associated with Semaphorin dependent Plexin receptor activation. The cell (e.g. a tumor cell and the like) may also be obtained or derived from patient samples (e.g. biopsies), in particular a biopsy/biopsies from a patient/subject suffering from a disease as defined herein above or below. Accordingly, the cell may be a human cell. The invention also provides for a host transformed or transfected with a vector described herein. Said host may be produced by introducing the above described vector of the invention or the above described nucleic acid molecule into the host. The presence of at least one vector or at least one nucleic acid molecule in the host may mediate the expression of a gene encoding the above described mutated Semaphorin 3 or the fragment thereof. The described nucleic acid molecule or vector of the invention, which is introduced in the host, may either integrate into the genome of the host or it may be maintained extrachromosomally. The host can be any prokaryote or eukaryotic cell.

Methods for obtaining the polypeptides or the nucleic acids as described herein are disclosed in WO/2016135130 that is incorporated herein by its entirety. For example, the polypeptides as described herein may be obtained by expression in HEK cells, HEK293E cells, HEK293

cell line stably expressing the Epstein-Barr virus nuclear antigen-1 (HEK293-EBNA1, or 293E) or insect cells.

As described above, the recited nucleic acid molecule can be used alone or as part of a vector to express the polypeptide of the invention in cells, for, e.g., for gene therapy purposes. The nucleic acid molecules or vectors containing the DNA sequence(s) encoding any one of the above described polypeptide of the invention is introduced into the cells which in turn produce the polypeptide of interest. Gene therapy, which is based on introducing therapeutic genes into cells by *ex vivo* or *in vivo* techniques, is one of the most important applications of gene transfer. Suitable vectors, methods or gene-delivery systems for *in-vitro* or *in-vivo* gene therapy are described in the literature and are known to the person skilled in the art; see, e.g., Giordano, *Nature Medicine* 2 (1996), 534-539; Schaper, *Circ. Res.* 79 (1996), 911-919; Anderson, *Science* 256 (1992), 808-813; Verma, *Nature* 389 (1994), 239; Isner, *Lancet* 348 (1996), 370-374; Muhlhauser, *Circ. Res.* 77 (1995), 1077-1086; Onodera, *Blood* 91 (1998), 30-36; Verma, *Gene Ther.* 5 (1998), 692-699; Nabel, *Ann. N.Y. Acad. Sci.* 811 (1997), 289-292; Verzeletti, *Hum. Gene Ther.* 9 (1998), 2243-51; Wang, *Nature Medicine* 2 (1996), 714-716; WO 94/29469; WO 97/00957, US 5,580,859; US 5,589,466; or Schaper, *Current Opinion in Biotechnology* 7 (1996), 635-640. The recited nucleic acid molecules and vectors may be designed for direct introduction or for introduction via liposomes, or viral vectors (e.g., adenoviral, retroviral) into the cell. Preferably, said cell is a germ line cell, embryonic cell, or egg cell or derived there from, most preferably said cell is a stem cell. An example for an embryonic stem cell can be, *inter alia*, a stem cell as described in Nagy, *Proc. Natl. Acad. Sci. USA* 90 (1993), 8424-8428.

Herein envisaged is also a process for the production of a polypeptide to be used in accordance with the present invention, said process comprising culturing/raising the host under conditions allowing the expression of the polypeptide of the invention and optionally recovering/isolating the produced polypeptide from the culture.

The transformed hosts can be grown in fermenters and cultured according to techniques known in the art to achieve optimal cell growth. The polypeptide described herein can then be isolated from the growth medium, cellular lysates, or cellular membrane fractions. The isolation and purification of the, e.g., microbially expressed polypeptides of the invention may be by any conventional means such as, for example, preparative chromatographic separations and immunological separations such as those involving the use of monoclonal or polyclonal antibodies directed.

The invention relates to use in medicine of the combination, the kit of parts and/or the

pharmaceutical composition as provided herein. The terms “medicament” and “pharmaceutical composition” are used interchangeably herein. Accordingly, definitions and explanations provided herein in relation to the term “combination”, apply, *mutatis mutandis*, to the term “pharmaceutical composition” and “kit of parts”.

The combination, the kit of parts and/or the pharmaceutical composition may be used in the treatment of a disorder or disease, such as a tumor. The term “treatment of a disorder or disease” implies that a disorder or disease is suspected or has been diagnosed in a patient/subject. A patient/subject suspected of suffering from a disorder or disease typically shows specific clinical and/or pathological symptoms which a skilled person can attribute to a specific pathological condition (i.e., diagnose a disorder or disease). The “treatment” of a disorder or disease may, for example, lead to a halt in the progression of the disorder or disease (e.g., no deterioration of symptoms) or a delay in the progression of the disorder or disease (in case the halt in progression is of a transient nature only). The “treatment” of a disorder or disease may also lead to a partial response (e.g., amelioration of symptoms) or complete response (e.g., disappearance of symptoms) of the subject/patient suffering from the disorder or disease. Accordingly, the “treatment” of a disorder or disease may also refer to an amelioration of the disorder or disease, which may, e.g., lead to a halt in the progression of the disorder or disease or a delay in the progression of the disorder or disease. Such a partial or complete response may be followed by a relapse. It is to be understood that a subject/patient may experience a broad range of responses to a treatment (e.g., the exemplary responses as described herein above). The treatment of a disorder or disease may, *inter alia*, comprise curative treatment (preferably leading to a complete response and eventually to healing of the disorder or disease) and palliative treatment (including symptomatic relief). Thus, the term “treatment” means obtaining a desired pharmacological and/or physiological effect. The effect may also be prophylactic in terms of completely or partially preventing a disease/medical condition/disorder or symptom thereof and/or may be therapeutic in terms of partially or completely curing a disease/medical condition/disorder and/or adverse effect attributed to the disease/medical condition/disorder.

The term “prevention of a disorder or disease” as used herein, such as “prevention of cancer”, is also well known in the art. For example, a patient/subject suspected of being prone to suffer from a disorder or disease as defined herein may, in particular, benefit from a prevention of the disorder or disease. The subject/patient may have a susceptibility or predisposition for a disorder or disease, including but not limited to hereditary predisposition. Such a predisposition can be determined by standard assays, using, for example, genetic markers or phenotypic indicators. It is to be understood that a disorder or disease to be prevented in accordance with the present invention has not been diagnosed or cannot be diagnosed in the patient/subject (for example, the patient/subject does not show any clinical

or pathological symptoms). Thus, the term “prevention” comprises the use of compounds of the present invention before any clinical and/or pathological symptoms are diagnosed or determined or can be diagnosed or determined by the attending physician.

The combination, the kit of parts or the pharmaceutical composition may comprise one or more excipients. The excipient may particularly be a pharmaceutical excipient. The pharmaceutical excipient can comprise or is a pharmaceutical carrier, vehicle and/or diluent.

In one specific embodiment, said pharmaceutical carrier can be a virus. In a preferred embodiment said virus is an adeno-associated-virus (AAV), wherein the adeno-associated-virus is AAV8. AAV can be for instance employed in gene therapy. Thus, in one aspect, the invention provides a nucleotide sequence which contains elements of an adenovirus genome as well as a mutated Semaphorin 3 or fragments thereof that is under the control of a eukaryotic transcriptional promoter. This nucleic acid sequence can function as a vector allowing expression of the aforementioned heterologous gene when the vector is introduced in a cell of an individual.

Further, the invention provides the combination, the pharmaceutical composition and/or the kit of parts for use in the treatment of an angiogenic disorder, cancer, tumor, tumorous disease, vascular retinopathy, blood-brain barrier permeability alterations, neuroinflammatory disorders, inflammatory disorders, osteoporosis, psoriasis, obesity Mycobacterial infections, and/or granulomas.

In particular aspects of the invention, the combination, the pharmaceutical composition and/or the kit of parts is used for the treatment of tumor and/or cancer. The appended examples document the surprising strong medical effects in the treatment of a cancer, in particular of pancreatic cancer.

Further, the invention relates to the combination, the pharmaceutical composition and/or the kit of parts for use in the treatment of a solid tumor. The term “tumor” refers to an abnormal mass of tissue that lacks the ability to invade neighboring tissue or metastasize (benign tumor). The term “cancer” refers to an abnormal mass of tissue that has the potential to invade neighboring tissue or metastasize. Accordingly, the term “tumor or cancer” may refer to cancer, a tumorous disease, or a solid tumor.

Further, the invention relates to the combination, the pharmaceutical composition and/or the kit of parts for use in the treatment of a tumor or cancer selected from the group consisting of

pancreatic cancer, cervical cancer, breast cancer, colon cancer, melanoma, prostate cancer, bladder cancer and tongue cancer.

In preferred aspects of the invention, the inventive combination, the pharmaceutical composition and/or the kit of parts may be used for the treatment of a pancreatic tumor or pancreatic cancer. In most preferred aspects of the invention, the inventive combination, the pharmaceutical composition and/or the kit of parts may be used for the treatment of pancreatic cancer. In particular aspects of the invention, the inventive combination, the pharmaceutical composition and/or the kit of parts may be used in the treatment of metastatic pancreatic cancer.

“Cancer”, in accordance with the present invention, refers to a class of diseases or disorders characterized by uncontrolled division of cells and the ability of these to spread, either by direct growth into adjacent tissue through invasion, or by implantation into distant sites by metastasis, where cancer cells are transported through the bloodstream or lymphatic system. The “tumorous disease” may refer to a cancer, a pre-tumor, pancreas cancer, myeloma, leukemia, breast cancer, epithelial cancer, hepatocellular carcinoma, cholangiocellular cancer, stomach cancer, colon cancer, prostate cancer, bladder cancer, tongue cancer, head and neck cancer, skin cancer (melanoma), a cancer of the urogenital tract, e.g., ovarian cancer, endometrial cancer, cervix cancer, kidney cancer; lung cancer, gastric cancer, a cancer of the small intestine, liver cancer, gall bladder cancer, a cancer of the bile duct, esophagus cancer, a cancer of the salivary glands or a cancer of the thyroid gland.

Further, the inventive combination, the pharmaceutical composition and/or the kit of parts may be administered in combination with a further medicament, e.g. an anti-proliferative drug, an anticancer drug, a cytostatic drug, a cytotoxic drug and/or radiotherapy.

Furthermore, the inventive combination, the pharmaceutical composition, and/or the kit of parts may be used in the treatment of a tumor or cancer for inhibiting cancer growth, reducing liver metastasis or metastasis volume, reducing vessel area and/or promoting cancer vessel normalization by enhancing pericyte coverage, and/or increasing blood vessel perfusion and inhibiting cancer hypoxia.

The combination, the pharmaceutical composition, and/or the kit of parts of the invention may be administered parenterally, orally, rectally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch) or buccally.

In particular preferred embodiments, the combination, the pharmaceutical composition, and/or the kit of parts of the invention is administered parenterally.

The at least two components of the inventive combination, the pharmaceutical composition, or the kit of parts (e.g. the antimetabolite and the mutated Semaphorin 3, the functional fragment thereof, the fusion protein, or the nucleic acid molecule) are to be administered to a subject in need thereof via different administration routes or via the same routes, e.g. parenterally.

The individual components of such combinations may be administered either sequentially or simultaneously/concomitantly in separate or combined pharmaceutical formulations by any convenient route. When administration is sequential, either the mutated Semaphorin 3 (the functional fragment thereof), the fusion protein (the functional fragment thereof), or the nucleic acid molecule may be administered first or the antimetabolite may be administered first. When administration is simultaneous, the combination may be administered either in the same or different pharmaceutical compositions. When combined in the same formulation it will be appreciated that the two components must be stable and compatible with each other and the other components of the formulation. When formulated separately, they may be provided in any convenient formulation and may be comprised in the kit of parts.

In particular aspects of the invention, the antimetabolite and the mutated Semaphorin 3, a functional fragment thereof, the fusion protein (or the functional fragment thereof), or the nucleic acid molecule are to be administered to a subject in need thereof sequentially.

The invention also comprises that the antimetabolite and the mutated Semaphorin 3, a functional fragment thereof, the fusion protein (or the functional fragment thereof), or the nucleic acid molecule are to be administered to a subject in need thereof concurrently.

The at least two components may be comprised in such an amount in the combination, the pharmaceutical composition, or the kit of parts that the components (in combination) may lead to some therapeutic response or desired effect in some fraction of the subjects. Such a dose may be referred as pharmaceutical effective amount. For example, the antimetabolite may be administered in a dose of about 100 to 10000 mg/m² body surface, and wherein the mutated Semaphorin 3, the functional fragment of the mutated Semaphorin 3, or the fusion protein or the functional fragment thereof may to be administered in a dose of about 1 µg protein /kg/day to 15 mg protein /kg/day of patient body weight, although, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg protein/kg/day, and most preferably for humans between about 0.01 and 1 mg protein /kg/day. The dose of the combination of the two components will be subject to therapeutic discretion. For example,

the combination of the two components may further reduce the therapeutic effective dose of the antimetabolite and/or the mutated Semaphorin 3 protein/polypeptide.

It is envisaged herein that the amount of the components can be different depending on the disease, the administration site, the number of doses, the desired duration of therapy, the age or body weight of a patient or the like. The pharmaceutical effective amount of the nucleic acid molecule can be usually about 0.01 - 2000 mg and preferably 0.1 - 100 mg of DNA encoding a protein of this invention for a patient (the body weight is 60 kg).

If given continuously, the at least two components may typically be administered at a dose rate of about 1 µg/kg/hour to about 50 µg/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed.

The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect. The particular amounts may be determined by conventional tests which are well known to the person skilled in the art.

The inventive combination, the pharmaceutical composition, and/or the kit of parts will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient, the site of delivery of the pharmaceutical composition, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" of the pharmaceutical composition for purposes herein is thus determined by such considerations.

The skilled person knows that the effective amount of the at least two components of the inventive combination, the pharmaceutical composition, and/or the kit of parts administered to an individual will, inter alia, depend on the nature of the compound.

The administration of the herein provided combination, the pharmaceutical composition, and/or the kit of parts may, inter alia, comprise an administration twice daily, every day, every other day, every third day, every fourth day, every fifth day, once a week, once every second week, once every third week, once every month, etc.

The inventive combination, the pharmaceutical composition, and/or the kit of parts can be used in combination with further therapeutic agents as mentioned above. When the at least two components comprised in the inventive combination, the pharmaceutical composition, and/or the kit of parts are used with a further therapeutic agent against the same disease, the dose of each compound may differ from that when the compound is used alone. The combination with the further therapeutic agent may comprise the administration of the second

therapeutic agent with the inventive combination, the pharmaceutical composition, and/or the kit of parts. Such an administration may comprise simultaneous/concomitant administration. However, also sequential/separate administration is envisaged.

Preferably, the further therapeutic agent can be: a tumor angiogenesis inhibitor (for example, a protease inhibitor, an epidermal growth factor receptor kinase inhibitor, or a vascular endothelial growth factor receptor kinase inhibitor); a cytotoxic drug (for example, a further antimetabolite); an antimetabolic agent (for example, a microtubule stabilizing drug or an antimetabolic alkaloid); a platinum coordination complex; an anti-tumor antibiotic; an alkylating agent (for example, a nitrogen mustard or a nitrosourea); an endocrine agent (for example, an adrenocorticosteroid, an androgen, an anti-androgen, an estrogen, an anti-estrogen, an aromatase inhibitor, a gonadotropin-releasing hormone agonist, or a somatostatin analogue); or a compound that targets an enzyme or receptor that is overexpressed and/or otherwise involved in a specific metabolic pathway that is misregulated in the tumor cell (for example, ATP and GTP phosphodiesterase inhibitors, histone deacetylase inhibitors, protein kinase inhibitors (such as serine, threonine and tyrosine kinase inhibitors (for example, Abelson protein tyrosine kinase)) and the various growth factors, their receptors and corresponding kinase inhibitors (such as epidermal growth factor receptor kinase inhibitors, vascular endothelial growth factor receptor kinase inhibitors, fibroblast growth factor inhibitors, insulin-like growth factor receptor inhibitors and platelet-derived growth factor receptor kinase inhibitors)); methionine, aminopeptidase inhibitors, proteasome inhibitors, cyclooxygenase inhibitors (for example, cyclooxygenase-1 or cyclooxygenase-2 inhibitors) and topoisomerase inhibitors (for example, topoisomerase I inhibitors or topoisomerase II inhibitors).

An alkylating agent which can be used as an anticancer drug in combination with the pharmaceutical composition, the nucleic acid, the vector, the mutated Semaphorin 3, the functional fragment thereof and/or the fusion protein/polypeptide of the present invention may be, for example, a nitrogen mustard (such as cyclophosphamide, mechlorethamine (chlormethine), uramustine, melphalan, chlorambucil, ifosfamide, bendamustine, or trofosfamide), a nitrosourea (such as carmustine, streptozocin, fotemustine, lomustine, nimustine, prednimustine, ranimustine, or semustine), an alkyl sulfonate (such as busulfan, mannosulfan, or treosulfan), an aziridine (such as hexamethylmelamine (altretamine), triethylenemelamine, ThioTEPA (N,N,N'-triethylenethiophosphoramidate), carboquone, or triaziquone), a hydrazine (such as procarbazine), a triazene (such as dacarbazine), or an imidazotetrazines (such as temozolomide).

A platinum coordination complex which can be used as an anticancer drug in combination with the pharmaceutical composition, the nucleic acid molecule, the vector, the mutated Semaphorin 3, the functional fragment thereof and/or the fusion protein/polypeptide of the present invention may be, for example, cisplatin, carboplatin, nedaplatin, oxaliplatin, satraplatin, or triplatin tetranitrate.

A cytotoxic drug which can be used as an anticancer drug in combination with the inventive combination, the pharmaceutical composition, and/or the kit of parts may be, for example, a further antimetabolite, including folic acid analogue antimetabolites (such as aminopterin, methotrexate, pemetrexed, or raltitrexed), purine analogue antimetabolites (such as cladribine, , fludarabine, 6-mercaptopurine (including its prodrug form azathioprine), pentostatin, or 6-thioguanine), and pyrimidine analogue antimetabolites (such as decitabine, 5-fluorouracil (including its prodrug forms and tegafur), enocitabine, or sapacitabine).

An antimitotic agent which can be used as an anticancer drug in combination with the inventive combination, the pharmaceutical composition, and/or the kit of parts may be, for example, a taxane (such as docetaxel, larotaxel, ortataxel, paclitaxel/taxol, or tesetaxel), a Vinca alkaloid (such as vinblastine, vincristine, vinflunine, vindesine, or vinorelbine), an epothilone (such as epothilone A, epothilone B, epothilone C, epothilone D, epothilone E, or epothilone F) or an epothilone B analogue (such as ixabepilone/azaepothilone B).

An anti-tumor antibiotic which can be used as an anticancer drug in combination with the inventive combination, the pharmaceutical composition, and/or the kit of parts may be, for example, an anthracycline (such as aclarubicin, daunorubicin, doxorubicin, epirubicin, idarubicin, amrubicin, pirarubicin, valrubicin, or zorubicin), an anthracenedione (such as mitoxantrone, or pixantrone) or an anti-tumor antibiotic isolated from *Streptomyces* (such as actinomycin (including actinomycin D), bleomycin, mitomycin (including mitomycin C), or plicamycin).

A tyrosine kinase inhibitor which can be used as an anticancer drug in combination with the inventive combination, the pharmaceutical composition, and/or the kit of parts may be, for example, axitinib, bosutinib, cediranib, dasatinib, erlotinib, gefitinib, imatinib, lapatinib, lestaurtinib, nilotinib, semaxanib, sorafenib, sunitinib, or vandetanib.

A topoisomerase-inhibitor which can be used as an anticancer drug in combination with the inventive combination, the pharmaceutical composition, and/or the kit of parts may be, for example, a topoisomerase I inhibitor (such as irinotecan, topotecan, camptothecin, belotecan,

rubitecan, or lamellarin D) or a topoisomerase II inhibitor (such as amsacrine, etoposide, etoposide phosphate, teniposide, or doxorubicin).

Further anticancer drugs may be used in combination with inventive combination, the pharmaceutical composition, and/or the kit of parts. The anticancer drugs may comprise biological or chemical molecules, like TNF-related apoptosis-inducing ligand (TRAIL), tamoxifen, amsacrine, bexarotene, estramustine, iriffulven, trabectedin, cetuximab, panitumumab, tositumomab, alemtuzumab, bevacizumab, edrecolomab, gemtuzumab, trastuzumab, pertuzumab, alvocidib, seliciclib, aminolevulinic acid, methyl aminolevulinate, efaproxiral, porfimer sodium, talaporfin, temoporfin, verteporfin, alitretinoin, tretinoin, anagrelide, arsenic trioxide, atrasentan, bortezomib, carmofur, celecoxib, demecolcine, elesclomol, elsamitucin, etoglucid, lonidamine, lucanthone, masoprocol, mitobronitol, mitoguazone, mitotane, oblimersen, omacetaxine, sitimagene, ceradenovec, tegafur, testolactone, tiazofurine, tipifarnib, and vorinostat.

Also biological drugs, like antibodies, antibody fragments, antibody constructs (for example, single-chain constructs), and/or modified antibodies (like CDR-grafted antibodies, humanized antibodies, “full humanized” antibodies, etc.) directed against cancer or tumor markers/factors/cytokines involved in proliferative diseases can be employed in co-therapy approaches with the inventive combination, the pharmaceutical composition, and/or the kit of parts. Examples of such biological molecules are anti-HER2 antibodies (e.g. trastuzumab, Herceptin®), anti-CD20 antibodies (e.g. Rituximab, Rituxan®, MabThera®, Reditux®), anti-CD19/CD3 constructs (see, e.g., EP-B1 1071752) and anti-TNF antibodies (see, e.g., Taylor PC. Antibody therapy for rheumatoid arthritis. *Curr Opin Pharmacol.* 2003. 3(3):323-328). Further antibodies, antibody fragments, antibody constructs and/or modified antibodies to be used in co-therapy approaches with the inventive combination, the pharmaceutical composition, and/or the kit of parts can be found in Taylor PC. *Curr Opin Pharmacol.* 2003. 3(3):323-328; Roxana A. *Maedica.* 2006. 1(1):63-65.

The further therapeutic agent(s) may be comprised in the same pharmaceutical composition, the combination or the kit of parts or in separate pharmaceutical composition.

The inventive combination, the pharmaceutical composition, and/or the kit of parts may also be administered in combination with physical therapy, such as radiotherapy. Radiotherapy may commence before, after, or simultaneously with administration of the compounds of the invention. For example, radiotherapy may commence 1-10 minutes, 1-10 hours or 24-72 hours after administration of the compounds. Yet, these time frames are not to be construed as limiting. The subject is exposed to radiation, preferably gamma radiation, whereby the

radiation may be provided in a single dose or in multiple doses that are administered over several hours, days and/or weeks. Gamma radiation may be delivered according to standard radiotherapeutic protocols using standard dosages and regimens.

The term "Angiogenesis" means that a vascular EC germinates from a pre-existing vessel and a capillary vessel is formed in a way that goes into a tissue. A formative process is the digestion of the vascular basement membrane by a protease, the migration/growth of a vascular EC, and the lumen formation. "Angiogenic disorder" is a vascular disease such as arterial sclerosis, hypertonia, angina pectoris, obstructive arteriosclerosis, myocardial infarction, cerebral infarction, diabetic angiopathy or vascular malformation; inflammatory disease such as hepatitis, pneumonitis, glomerular nephritis, thyroiditis, osteitis, arthromeningitis, osteoclasia, chondrolysis, rheumatism, bronchial asthma, sarcoidosis, Crow-Fukase syndrome, pannus, allergic oedema, ulcers, hydroperitoneum, peritoneal sclerosis or tissular conglutination; entoptic neovascular disease such as diabetic retinopathy, occlusion of retinal vein or aging macular degeneration; reproductive system disease such as uterus dysfunction, placental dysfunction, ovarian hyperergasia or follicle cyst; central nervous system disease such as retinosis, cerebral apoplexy, vascular dementia or Alzheimer disease; cancer such as solid cancer, angiomatous, hemangioendothelioma, sarcomas, Kaposi's sarcoma or hematopoietic organic ulcer.

The angiogenesis inhibitor can be used as a preventive or therapeutic agent for a disease whose condition can become serious by angiogenesis in the above diseases. The disease on which the angiogenesis inhibitor has effect is vascular disease, inflammatory disease, entoptic neovascular disease, reproductive system disease, central nervous system disease, cancer or the like. A form of formulation of a vector of this invention (a gene therapy agent) can be one of different forms according to the above each form of administration. For example, when it is an injection comprising DNA of this invention which is an active ingredient, the injection can be prepared by a usual method. A base ingredient for a gene therapy agent is not especially restricted as long as it is a base ingredient usually used for an injection. It is, for example, distilled water, sodium chloride, a salt solution such as mixture of sodium chloride and mineral salts, a solution such as mannitol, lactose, dextran or glucose, an amino acid solution such as glycine or arginine, or mixture of an organic acid solution or a salt solution and a glucose solution. An injection can be prepared with an auxiliary such as an osmotic adjustment agent, pH adjustment agent, plant oil such as sesame oil or soybean oil, surfactant such as lecithin or nonionic surfactant or the like according to a usual method as a solution, suspension or dispersion. The injection as above can be a preparation dissolved in use by manipulation such as disintegration or lyophilization.

Further, the invention provides a method of treatment for angiogenic disorder and/or tumorous disease and/or cancer comprising the step of administering to a subject in need of such treatment a pharmaceutical effective amount of the combination or the pharmaceutical composition.

In particular, the invention provides a method of treatment for a tumor comprising the step of administering to a subject in need of such treatment a pharmaceutical effective amount of the combination or the pharmaceutical composition.

The subject or patient to be treated in accordance with the invention may be an animal (e.g., a non-human animal), a vertebrate animal, a mammal, a rodent (e.g., a guinea pig, a hamster, a rat, a mouse), a murine (e.g., a mouse), a canine (e.g., a dog), a feline (e.g., a cat), a porcine (e.g., a pig), an equine (e.g., a horse), a primate, a simian (e.g., a monkey or ape), a monkey (e.g., a marmoset, a baboon), an ape (e.g., a gorilla, chimpanzee, orang-utan, gibbon), or a human. In the context of this invention, it is particularly envisaged that animals are to be treated which are economically, agronomically or scientifically important. Scientifically important organisms include, but are not limited to, mice, rats, and rabbits. Lower organisms such as, e.g., fruit flies like *Drosophila melanogaster* and nematodes like *Caenorhabditis elegans* may also be used in scientific approaches. Non-limiting examples of agronomically important animals are sheep, cattle and pigs, while, for example, cats and dogs may be considered as economically important animals. Preferably, the subject/patient is a mammal; more preferably, the subject/patient is a human or a non-human mammal (such as, e.g., a guinea pig, a hamster, a rat, a mouse, a rabbit, a dog, a cat, a horse, a monkey, an ape, a marmoset, a baboon, a gorilla, a chimpanzee, an orang-utan, a gibbon, a sheep, cattle, or a pig); most preferably, the subject/patient is a human.

Pharmaceutical compositions, the combination or the kit of parts or the components comprised in the kit of parts of the invention preferably comprise a pharmaceutically acceptable carrier. By "pharmaceutically acceptable carrier" / "excipient" / "pharmaceutical excipient" is meant a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intratracheal, intranasal, intrasternal, subcutaneous and intraarticular injection and infusion.

The pharmaceutical composition, the combination or the components of the kit of parts may be administered by sustained release systems. Suitable examples of sustained-release compositions include semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules. Sustained-release matrices include polylactides (U.S. Pat. No.

3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman, U. et al., *Biopolymers* 22:547-556 (1983)), poly (2-hydroxyethyl methacrylate) (R. Langer et al., *J. Biomed. Mater. Res.* 15:167-277 (1981), and R. Langer, *Chem. Tech.* 12:98-105 (1982)), ethylene vinyl acetate (R. Langer et al., *Id.*) or poly-D-(-)-3-hydroxybutyric acid (EP 133,988). Sustained release pharmaceutical composition also include liposomally entrapped compound. Liposomes containing the pharmaceutical composition are prepared by methods known per se: DE 3,218,121; Epstein et al., *Proc. Natl. Acad. Sci. (USA)* 82:3688-3692 (1985); Hwang et al., *Proc. Natl. Acad. Sci. (USA)* 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal therapy.

For parenteral administration, the pharmaceutical composition is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation.

The carrier/excipient may be a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes. The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) (poly)peptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The pharmaceutical composition, the combination or the components comprised in the kit of parts to be used for therapeutic administration must be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes).

The components of the pharmaceutical composition, the combination or the components of the kit of parts ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized compound(s) using bacteriostatic Water-for-Injection.

The term "nucleic acid molecule" in accordance with the present invention comprises coding and, wherever applicable, non-coding sequences (like promoters, enhancers etc.).

The terms "polypeptide", "(poly)peptide", "peptide" and "protein" are used herein interchangeably and refer to a polymer of two or more amino acids linked via amide bonds that are formed between an amino group of one amino acid and a carboxyl group of another amino acid. The amino acids comprised in the peptide or protein, which are also referred to as amino acid residues, may be selected from the 20 standard proteinogenic α -amino acids (i.e., Ala, Arg, Asn, Asp, Cys, Glu, Gln, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val) but also from non-proteinogenic and/or non-standard α -amino acids (such as, e.g., ornithine, citrulline, homolysine, pyrrolysine, or 4-hydroxyproline) as well as β -amino acids (e.g., β -alanine), γ -amino acids and δ -amino acids. Preferably, the amino acid residues comprised in the peptide or protein are selected from α -amino acids, more preferably from the 20 standard proteinogenic α -amino acids (which can be present as the L-isomer or the D-isomer, and are preferably all present as the L-isomer). The peptide or protein may be unmodified or may be modified, e.g., at its N-terminus, at its C-terminus and/or at a functional group in the side chain of any of its amino acid residues (particularly at the side chain functional group of one or more Lys, His, Ser, Thr, Tyr, Cys, Asp, Glu, and/or Arg residues). Such modifications may include, e.g., the attachment of any of the protecting groups described for the corresponding functional groups in: Wuts PG & Greene TW, Greene's protective groups in organic synthesis, John Wiley & Sons, 2006. Such modifications may also include the covalent attachment of one or more polyethylene glycol (PEG) chains (forming a PEGylated peptide or protein), the glycosylation and/or the acylation with one or more fatty acids (e.g., one or more C₈₋₃₀ alkanolic or alkenolic acids; forming a fatty acid acylated peptide or protein). The amino acid residues comprised in the peptide or protein may, e.g., be present as a linear molecular chain (forming a linear peptide or protein) or may form one or more rings (corresponding to a cyclic peptide or protein). The peptide or protein may also form oligomers consisting of two or more identical or different molecules. As used herein, the term "domain" relates to any region/part of an amino acid sequence that is capable of autonomously adopting a specific structure and/or function. In the

context of the present invention, accordingly, a "domain" may represent a functional domain or a structural domain.

The term "consensus sequence" or "consensus sequence motif" is the calculated order of most frequent residues, either nucleotide or amino acid, found at each position in a sequence alignment. It represents the results of a multiple sequence alignments in which related sequences are compared to each other and similar sequence motifs are calculated.

As used herein, the terms "comprising" and "including" or grammatical variants thereof are to be taken as specifying the stated features, integers, steps or components but do not preclude the addition of one or more additional features, integers, steps, components or groups thereof. This term encompasses the terms "consisting of" and "consisting essentially of."

Thus, the terms "comprising"/"including"/"having" mean that any further component (or likewise features, integers, steps and the like) can/may be present.

The term "consisting of" means that no further component (or likewise features, integers, steps and the like) is present.

The term "consisting essentially of" or grammatical variants thereof when used herein are to be taken as specifying the stated features, integers, steps or components but do not preclude the addition of one or more additional features, integers, steps, components or groups thereof but only if the additional features, integers, steps, components or groups thereof do not materially alter the basic and novel characteristics of the claimed composition, device or method.

Thus, the term "consisting essentially of" means those specific further components (or likewise features, integers, steps and the like) can be present, namely those not materially affecting the essential characteristics of the composition, device or method. In other words, the term "consisting essentially of" (which can be interchangeably used herein with the term "comprising substantially"), allows the presence of other components in the composition, device or method in addition to the mandatory components (or likewise features, integers, steps and the like), provided that the essential characteristics of the device or method are not materially affected by the presence of other components.

The term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures

either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, biological and biophysical arts.

The term "about" preferably refers to $\pm 10\%$ of the indicated numerical value, more preferably to $\pm 5\%$ of the indicated numerical value, and in particular to the exact numerical value indicated.

As used herein, the term "about" refers to $\pm 10\%$ of the indicated numerical value, and in particular to $\pm 5\%$ of the indicated numerical value. Whenever the term "about" is used, a specific reference to the exact numerical value indicated is also included. If the term "about" is used in connection with a parameter that is quantified in integers, such as the number of nucleotides in a given nucleic acid, the numbers corresponding to $\pm 10\%$ or $\pm 5\%$ of the indicated numerical value are to be rounded to the nearest integer. For example, the expression "about 25 nucleotides" refers to the range of 23 to 28 nucleotides, in particular the range of 24 to 26 nucleotides, and preferably refers to the specific value of 25 nucleotides.

The present invention is further described by reference to the following non-limiting figures and examples. Unless otherwise indicated, established methods of recombinant gene technology were used as described, for example, in Sambrook, Russell "Molecular Cloning, A Laboratory Manual", Cold Spring Harbor Laboratory, N.Y. (2001)) which is incorporated herein by reference in its entirety.

As used herein, the term "isolated" refers to a composition that has been removed from its in-vivo location. Preferably the isolated compositions or compounds of the present invention are substantially free from other substances (e.g., other proteins or other compounds) that are present in their in-vivo location (i.e. purified or semi-purified compositions or compounds.)

The present invention is further described by reference to the following non-limiting figures and examples.

Unless otherwise indicated, established methods of recombinant gene technology were used as described, for example, in Sambrook, Russell "Molecular Cloning, A Laboratory Manual", Cold Spring Harbor Laboratory, N.Y. (2001)) which is incorporated herein by reference in its entirety.

The Figures show:

Fig. 1. Sema3A_A106K_ΔIg-b synergizes with Gemcitabine to block metastases in mPDAC model (A,B) mPDAC mice were treated for 3 weeks with: i) Sema3A_A106K_ΔIg-b protein (3mg/kg, 3 times/week, i.p); ii) Gemcitabine (GEM) 50 mg/kg (twice/week, i.v.); iii) Sema3A_A106K_ΔIg-b protein + GEM; iv) vehicle (saline solution) (n=11/group). **(A)** Sema3A_A106K_ΔIg-b protein alone or GEM alone inhibited tumor growth by 50% and 40% respectively; the combination of the two drugs (Sema3A_A106K_ΔIg-b + GEM) more efficiently reduced tumor burden (by 66%) compared with the vehicle-treatment group. **(B)** Analysis of the total metastatic area of liver metastasis. Sema3A_A106K_ΔIg-b + GEM more efficiently impaired the liver metastasis fraction compared to single drugs (i.e., by 90%, compared to controls; by 87% compared to GEM and 67% compared with Sema3A_A106K_ΔIg-b protein alone respectively). A) Analysis of tumor volume; § treated sample vs. control. B) Analysis of the total area of liver metastasis; § treated sample vs. control. Results are the mean ± SD and were analyzed by non-parametric 2-tailed, unpaired Mann-Whitney U test; * or § p<0.05, ** or §§ p<0.01, *** or §§§ p<0.001.

The following non-binding Examples illustrate the invention.

Results and methods of the combination of Sema3A_A106K_ΔIg-b + Gemcitabine

The fusion protein comprising the mutated Sema 3A (Sema3A_A106K_ΔIg-b) is an effective vascular normalizing agent that enhances the anti-cancer potential of chemotherapeutic drugs

To evaluate whether Sema3A_A106K_ΔIg-b-elicited tumor blood vessel normalization may improve the delivery and the anti-cancer activity of chemotherapeutic drugs, we treated cohorts of PDAC mice with either Sema3A_A106K_ΔIg-b protein alone, or Gemcitabine (GEM) alone, or the combination of both molecules (Sema3A_A106K_ΔIg-b + GEM). We discovered that Sema3A_A106K_ΔIg-b protein, as single agent, was more efficient in reducing liver metastases compared to GEM alone (**Figure 1B**). Remarkably, the combination of Sema3A_A106K_ΔIg-b protein with GEM was significantly more efficient to hamper tumor growth and metastatic spread to the liver compared to the corresponding drugs employed as single agents (**Figure 1A, B**). In fact, Sema3A_A106K_ΔIg-b protein or GEM monotherapy reduced tumor volume by 50% and 40%, respectively, whereas the combination of the two drugs efficiently inhibited by 66% cancer growth, compared to controls (**Fig. 1A**). Remarkably, the combination Sema3A_A106K_ΔIg-b + GEM displayed an unexpected synergistic effect in impairing metastasis formation compared to single drugs. Of note, while Sema3A_A106K_ΔIg-b efficiently reduces liver metastases area, GEM did

not exert any statistical significant anti-metastatic effect in mPDAC. Sema3A_A106K_ΔIg-b + GEM decreased metastases by 67% compared to Sema3A_A106K_ΔIg-b (alone) and by 87% compared to GEM (alone). (**Fig. 1B**). Without being bound by theory, it can be hypothesized that the mutated Semaphorin 3 constructs as used in the present invention, in particular the Sema3A_A106K_ΔIg-b protein, functions as a vessel normalizing drug that efficiently enhances the delivery and the anti-cancer properties of GEM in PDAC tumors.

A major clinical benefit of tumor blood vessel normalization consists in the possibility to enhance delivery drugs to cancer and thus their efficacy (1). In the case of PDAC patients, the combination of targeted agents with chemotherapy did not significantly extend survival and available anti-angiogenic agents failed to improve the reduced supply of drugs that is typical of PDAC (2). Herein, we showed that Sema3A_A106K_ΔIg-b protein significantly prolongs the survival of mPDAC mice. Moreover, by enhancing the anti-cancer properties of the standard of care Gemcitabine, Sema3A_A106K_ΔIg-b protein efficiently hampers tumor growth and hinders PDAC metastatic dissemination to the liver. Interestingly, Sema3A_A106K_ΔIg-b protein was therapeutically more effective than recombinant wildtype Sema3A, further demonstrating that A106K mutation endows this protein with enhanced anti-cancer properties.

Methods

Mouse tumor models

Orthotopic PDAC mouse models have been previously described (Gilles *et al.*, *Cancer Res* 2016 Gilles *et al.*, Nucleolin Targeting Impairs the Progression of Pancreatic Cancer and Promotes the Normalization of Tumor Vasculature. *Cancer Res* **76**, 7181-7193 (2016)).

For the mPDAC model, cohorts of eight-week-old female FVB/n (strain code 207, weight average of 20 g, Charles River) were anesthetized by 1.5% isoflurane anesthesia and mice were injected orthotopically with syngeneic PDAC cells (1×10^3 cells/mouse) isolated from transgenic mice ($p48^{cre}$, $Kras^{LSL-G12D}$, $p53^{R172H/+}$, $Ink4a/Arf^{fllox/+}$, kindly provided by Doug Hanahan and Ksenya Shchors, ISREC, EPFL, Lausanne, Switzerland). For the orthotopic model, we previously performed time-course analyses of mPDAC growth to define the amount of time required for tumor establishment. Based on these observations, next we decided to start the regression trial one week after cancer cells inoculation. This model develops tumors with an average latency of 3-4 weeks (Gilles *et al.*, *Cancer Res* 2016). Total tumor burden was quantified by measuring with a caliper and calculating the volume of individually excised macroscopic tumors ($>1 \text{ mm}^3$) with the formula $V = a \cdot b^2 \cdot 0.52$ (where a and b represent the longer and shorter diameters of the tumor, respectively). All animal

procedures were approved by the Ethics Committee of the University of Torino and by the Italian Ministry of Health, in compliance with international laws and policies.

Therapeutic interventions

The therapeutic treatment in mPDAC model started one week after PDAC cells injection, as previously described (Gilles *et al.*, *Cancer Res* 2016, *supra*). The animals were treated for three weeks, 3 times a week with saline solution as control, or with 3 mg/kg, *i.p.*, of SEMA3A_A106K_ΔIg-b. Mice were regularly monitored for changes in weight and health status. For combinatorial treatment experiments, gemcitabine (GEM) alone or combined with SEMA3A_A106K_ΔIg-b was administered intravenously (50 mg/kg, 2 days a week) to mPDAC mice for 3 weeks.

Metastasis analysis

In the PDAC model, liver metastases were evaluated as previously described (Gilles *et al.*, *Cancer Res* 2016, *supra*). Liver tissues from all the treatment groups were serially cut and stained by H&E. Sections were scored under the optical DM2000 microscope (Leica), equipped with a color Qicam Fast 1394-digital CCD camera (12 bits; QImaging Corp.), and metastatic surface was calculated as the total surface occupied by metastasis divided by the total area of liver sections (metastatic liver fraction), using ImageJ software.

All references cited herein are fully incorporated by reference. Having now fully described the invention, it will be understood by a person skilled in the art that the invention may be practiced within a wide and equivalent range of conditions, parameters and the like, without affecting the spirit or scope of the invention or any embodiment thereof.

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Claims

1. A combination for use in the treatment of a tumor and/or cancer, wherein the combination comprises:
 - a) an antimetabolite; and
 - bi) a mutated Semaphorin 3 or a functional fragment thereof, wherein the mutated Semaphorin 3 is selected from the group consisting of Semaphorin 3A, Semaphorin 3B, Semaphorin 3C, and Semaphorin 3D, and wherein the mutated Semaphorin 3 or the functional fragment thereof comprises an amino acid sequence $CX_1X_2A_3GKD$, wherein
 X_1 is an amino acid, which is K or N,
 X_2 is an amino acid selected from the group of W, M and L,
and wherein the alanine (A_3) is replaced by a hydrophilic amino acid; or
 - bii) a fusion protein comprising the mutated Semaphorin 3 or the functional fragment thereof; or
 - biii) a nucleic acid molecule encoding the mutated Semaphorin 3 or the functional fragment thereof, or a nucleic acid molecule encoding the fusion protein.
2. The combination of claim 1, wherein the mutated Semaphorin 3, the functional fragment thereof, or the fusion protein inhibits angiogenesis and/or functions as vascular normalizing agent.
3. The combination of claim 1 or 2, wherein the antimetabolite is selected from gemcitabine, azacytidine, capecitabine, clofarabine, cytarabine, doxifluridine, floxuridine, 5-fluorouracil and pharmaceutically acceptable salts and solvates thereof.
4. The combination according to any one of claims 1 to 3, wherein the antimetabolite is selected from gemcitabine and pharmaceutically acceptable salts and solvates thereof.
5. The combination according to any one of claims 1 to 4, wherein the tumor or cancer is

selected from the group consisting of a tumor, preferably a solid tumor, and a tumorous disease.

6. The combination according to any one of claims 1 to 5, wherein the tumor is a solid tumor.
7. The combination according to any one of claims 1 to 6, wherein the tumor or cancer is selected from the group consisting of pancreatic cancer, cervical cancer, breast cancer, colon cancer, melanoma, prostate cancer, bladder cancer and tongue cancer.
8. The combination according to any one of claims 1 to 7, wherein the tumor or cancer is a pancreatic tumor or pancreatic cancer, in particular wherein the cancer is a metastatic pancreatic cancer.
9. The combination according to any one of claims 1 to 8, wherein the combination prolongs the survival of a subject suffering from a tumor or cancer compared to a pharmaceutical composition comprising the mutated Semaphorin 3, the functional fragment thereof, the fusion protein, or the antimetabolite alone.
10. The combination according to any one of claims 1 to 9, wherein the combination reduces the tumor growth compared to a pharmaceutical composition comprising the mutated Semaphorin 3, the functional fragment thereof, the fusion protein, or the antimetabolite alone.
11. The combination according to any one of claims 1 to 10, wherein the combination reduces metastasis, in particular liver metastasis, compared to a pharmaceutical composition comprising the mutated Semaphorin 3, the functional fragment thereof, the fusion protein, or the antimetabolite alone.
12. The combination according to any one of claims 1 to 11, wherein the mutated Semaphorin 3 comprises Semaphorin 3A or a functional fragment thereof and comprises a hydrophilic amino acid at position 106 of SEQ ID NO: 2, and wherein the antimetabolite is gemcitabine.

13. The combination according to any one of claims 1 to 12, wherein the mutated Semaphorin 3 comprises SEQ ID NO: 18 or functional fragments thereof, and wherein the antimetabolite is gemcitabine.
14. The combination of any one of claims 1 to 13, wherein the mutated Semaphorin 3 or the functional fragment thereof comprises
the hydrophilic amino acid in place of the alanine corresponding to position 106 of the wild type Semaphorin 3A as shown in SEQ ID NO: 2;
the hydrophilic amino acid in place of the alanine corresponding to position 105 of the wild type Semaphorin 3B as shown in SEQ ID NO: 6;
the hydrophilic amino acid in place of the alanine corresponding to position 104 of the wild type Semaphorin 3C as shown in SEQ ID NO: 10; or
the hydrophilic amino acid in place of the alanine corresponding to position 120 of the wild type Semaphorin 3D as shown in SEQ ID NO: 14.
15. The combination of any one of claims 1 to 14, wherein the mutated Semaphorin 3 is selected from the group of:
 - (a) a polypeptide that is encoded by a nucleic acid sequence selected from the group of SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 9 and SEQ ID NO: 13, wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding the hydrophilic amino acid, wherein the nucleotides GCA at position 559 to 561 of SEQ ID NO: 5 are replaced by nucleotides encoding the hydrophilic amino acid, wherein the nucleotides GCT at position 872 to 874 of SEQ ID NO: 9 are replaced by nucleotides encoding the hydrophilic amino acid, and wherein the nucleotides GCC at position 398 to 400 of SEQ ID NO: 13 are replaced by nucleotides encoding the hydrophilic amino acid;
 - (b) a polypeptide having the amino acid sequence selected from the group of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 10 and SEQ ID NO: 14, wherein the alanine residue at position 106 of SEQ ID NO: 2, at position 105 of SEQ ID NO: 6, at position 104 of SEQ ID NO: 10 or at position 120 of SEQ ID NO: 14 is replaced by the hydrophilic amino acid;

- (c) a polypeptide that is encoded by a nucleic acid sequence that hybridizes under stringent conditions to the complementary strand of a nucleic acid molecule encoding a polypeptide as defined in (a) or (b);
 - (d) a polypeptide that inhibits of angiogenesis and/or functions as a vascular normalizing agent and has at least 55% identity to any one of the polypeptides referred to in (b).
16. The combination of any one of claims 1 to 15, wherein the mutated Semaphorin 3 or the functional fragment thereof comprises a hydrophilic amino acid
- (a) at position 106 of SEQ ID NO: 2;
 - (b) at position 105 of SEQ ID NO: 6;
 - (c) at position 104 of SEQ ID NO: 10; or
 - (d) at position 120 of SEQ ID NO: 14.
17. The combination of any one of claims 1 to 16, wherein the mutated Semaphorin 3 or the functional fragment thereof comprises a functional sema domain and comprises at least one additional mutation selected from the group consisting of amino acid substitution(s), addition(s), deletions(s) and duplication(s).
18. The combination of any one of claims 1 to 17, wherein the hydrophilic amino acid is selected from the group of K, R, N, Q, S,T, E, D, and H.
19. The combination of any one of claims 1 to 18, wherein the hydrophilic amino acid is K or R.
20. The combination of claim 19, wherein the hydrophilic amino acid replacing the alanine is K.
21. The combination of any one of claims 1 to 20, wherein the mutated Semaphorin 3 or the functional fragment thereof comprises one or more of the following sequence(s) as defined in any one of SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47 or SEQ ID NO: 48.

22. The combination of any one of claims 1 to 21, wherein the nucleic acid molecule encoding the mutated Semaphorin 3 or the functional fragment thereof comprises:
- (a) the nucleotides from 601 to 1206 of SEQ ID NO: 1 and wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding a hydrophilic amino acid;
 - (b) the nucleotides from 529 to 1137 of SEQ ID NO: 5 and wherein the nucleotides GCA at position 559 to 561 of SEQ ID NO: 5 are replaced by nucleotides encoding a hydrophilic amino acid;
 - (c) the nucleotides from 842 to 1444 of SEQ ID NO: 9 and wherein the nucleotides GCT at position 872 to 874 of SEQ ID NO: 9 are replaced by nucleotides encoding a hydrophilic amino acid; or
 - (d) the nucleotides from 368 to 982 of SEQ ID NO: 13 and wherein the nucleotides GCC at position 398 to 400 of SEQ ID NO: 13 are replaced by nucleotides encoding a hydrophilic amino acid.
23. The combination of any one of claims 1 to 22, wherein the mutated Semaphorin 3 or the functional fragment thereof comprises the functional sema domain, wherein the sema domain is selected from an amino acid sequence as shown in:
- (a) SEQ ID NO: 21, wherein the alanine residue corresponding to position 106 of SEQ ID NO: 2 is replaced by a hydrophilic amino acid;
 - (b) SEQ ID NO: 22, wherein the alanine residue corresponding to position 105 of SEQ ID NO: 6 is replaced by a hydrophilic amino acid;
 - (c) SEQ ID NO: 23, wherein the alanine residue corresponding to position 104 of SEQ ID NO: 10 is replaced by a hydrophilic amino acid; or
 - (d) SEQ ID NO: 24, wherein the alanine residue corresponding to position 120 of SEQ ID NO: 14 is replaced by a hydrophilic amino acid.
24. The combination of any one of claims 1 to 23, wherein the mutated Semaphorin 3 comprises an amino acid sequence that is selected from the group consisting of SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70 and SEQ ID NO: 72.

25. The combination of any one of claims 1 to 24, wherein the fusion protein comprises a polypeptide as defined in:
- (a) SEQ ID NO: 21, wherein the alanine residue corresponding to position 106 of SEQ ID NO: 2 is replaced by a hydrophilic amino acid;
 - (b) SEQ ID NO: 22, wherein the alanine residue corresponding to position 105 of SEQ ID NO: 6 is replaced by a hydrophilic amino acid;
 - (c) SEQ ID NO: 23, wherein the alanine residue corresponding to position 104 of SEQ ID NO: 10 is replaced by a hydrophilic amino acid; or
 - (d) SEQ ID NO: 24, wherein the alanine residue corresponding to position 120 of SEQ ID NO: 14 is replaced by a hydrophilic amino acid.
26. The combination of any one of claims 1 to 25, wherein the fusion protein comprises:
- (a) a stabilizer domain; and/or
 - (b) a dimerization domain.
27. The combination of claim 26, wherein
- (a) the stabilizer domain is a Plexin Semaphorin Integrin (PSI) domain, wherein the PSI domain comprises one or more of the following sequences SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47 or SEQ ID NO: 48; and/or
 - (b) the dimerization domain has a dissociation constant K_D in the range of 10^{-5} M to 10^{-6} M with another such dimerization domain and/or wherein the dimerization domain is selected from the group of a C-terminal IgG constant domain, DARPin and leucine zipper.
28. The combination of claim 27, wherein the IgG constant domain is IgG1 or IgG3.
29. The combination of any one of claims 1 to 28, wherein the nucleic acid molecule encoding the fusion protein comprises a nucleic acid sequence having:
- a nucleic acid sequence spanning from nucleotides 316 to 1959 of SEQ ID NO: 1 and a nucleic acid sequence spanning from nucleotides 295 to 990 of SEQ ID NO: 37, wherein the nucleotides GCT at position 631 to 633 of SEQ ID NO: 1 are replaced by nucleotides encoding a hydrophilic amino acid;

a nucleic acid sequence spanning from nucleotides 247 to 1887 of SEQ ID NO: 5 and a nucleic acid sequence spanning from nucleotides 295 to 990 of SEQ ID NO: 37, wherein the nucleotides GCA at position 559 to 561 of SEQ ID NO: 5 are replaced by nucleotides encoding a hydrophilic amino acid;

a nucleic acid sequence spanning from nucleotides 563 to 2197 of SEQ ID NO: 9 and a nucleic acid sequence spanning from nucleotides 295 to 990 of SEQ ID NO: 37, wherein the nucleotides GCT at position 872 to 874 of SEQ ID NO: 9 are replaced by nucleotides encoding a hydrophilic amino acid; or

a nucleic acid sequence spanning from nucleotides 41 to 1735 of SEQ ID NO: 13 and a nucleic acid sequence spanning from nucleotides 295 to 990 of SEQ ID NO: 37, wherein the nucleotides GCC at position 398 to 400 of SEQ ID NO: 13 are replaced by nucleotides encoding a hydrophilic amino acid.

30. The combination of any one of claims 1 to 29, wherein the fusion protein comprises an amino acid sequence:
- spanning from amino acid residues 1 to 548 of SEQ ID NO: 2 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 106 of SEQ ID NO: 2 is replaced by a hydrophilic amino acid;
 - spanning from amino acid residues 1 to 547 of SEQ ID NO: 6 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 105 of SEQ ID NO: 6 is replaced by a hydrophilic amino acid;
 - spanning from amino acid residues 1 to 565 of SEQ ID NO: 10 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 104 of SEQ ID NO: 10 is replaced by a hydrophilic amino acid; or
 - spanning from amino acid residues 1 to 545 of SEQ ID NO: 14 and an amino acid sequence as shown in SEQ ID NO: 41, wherein the alanine residue at position 120 of SEQ ID NO: 14 is replaced by the hydrophilic amino acid.
31. The combination of any one of claims 1 to 30, wherein the fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 18, SEQ ID NO: 76, SEQ ID NO: 78 and SEQ ID NO: 79.

32. The combination of any one of claims 1 to 31, wherein the antimetabolite and the mutated Semaphorin 3, a functional fragment thereof, the fusion protein, or the nucleic acid molecule are to be administered to a subject in need thereof sequentially.
33. The combination of any one of claims 1 to 32, wherein the antimetabolite and the mutated Semaphorin 3, a functional fragment thereof, the fusion protein, or the nucleic acid molecule are to be administered to a subject in need thereof concurrently.
34. The combination of any one of claims 1 to 32, wherein the antimetabolite and the mutated Semaphorin 3, a functional fragment thereof, the fusion protein, or the nucleic acid molecule are to be administered to a subject in need thereof via different administration routes.
35. The combination of any one of claims 1 to 34, wherein the combination is to be administered parenterally.
36. The combination of any one of claims 1 to 35, wherein the antimetabolite is to be administered in a dose of about 100 to 10000 mg/m² body surface, and wherein the mutated Semaphorin 3, the functional fragment thereof, or the fusion protein is to be administered in a dose of about 1 µg protein /kg/day to 15 mg protein /kg/day mg/kg of body weight
37. The combination according to any one of claim 1 to 36, wherein the nucleic acid molecule is a vector.
38. The combination according to claim 37, wherein the vector is a gene targeting vector or a gene transfer vector.
39. The combination according to claim 36 or 37, wherein the vector is an adeno-associated-virus (AAV) vector.
40. The combination according to claim 39, wherein the adeno-associated-virus is an AAV8 vector.

41. A kit comprising:
 - a) the antimetabolite as defined in any one of the preceding claims; and
 - b) the mutated Semaphorin 3 or the functional fragment thereof, the fusion protein comprising the mutated Semaphorin 3 or the functional fragment thereof, or the nucleic acid molecule encoding the mutated Semaphorin 3 or the functional fragment thereof, or the nucleic acid molecule encoding the fusion protein as defined in any one of the preceding claims.

42. A pharmaceutical composition comprising:
 - a) the antimetabolite as defined in any one of the preceding claims; and
 - b) the mutated Semaphorin 3 or the functional fragment thereof, the fusion protein comprising the mutated Semaphorin 3 or the functional fragment thereof, or the nucleic acid molecule encoding the mutated Semaphorin 3 or the functional fragment thereof, or the nucleic acid molecule encoding the fusion protein as defined in any one of the preceding claims; andoptionally comprising one or more pharmaceutical excipients.

43. The kit or the pharmaceutical composition of any one of the preceding claims for use in medicine.

44. The kit or the pharmaceutical composition of any one of the preceding claims for use in the treatment of a tumor, tumorous disease and/or a cancer.

45. The kit or the pharmaceutical composition of any one of the preceding claims for use in the treatment of a pancreatic tumor or pancreatic cancer.

46. A method of treatment for a tumor comprising the step of administering to a subject in need of such treatment a pharmaceutical effective amount of the combination or the pharmaceutical composition of any one of the preceding claims.

Figure 1A

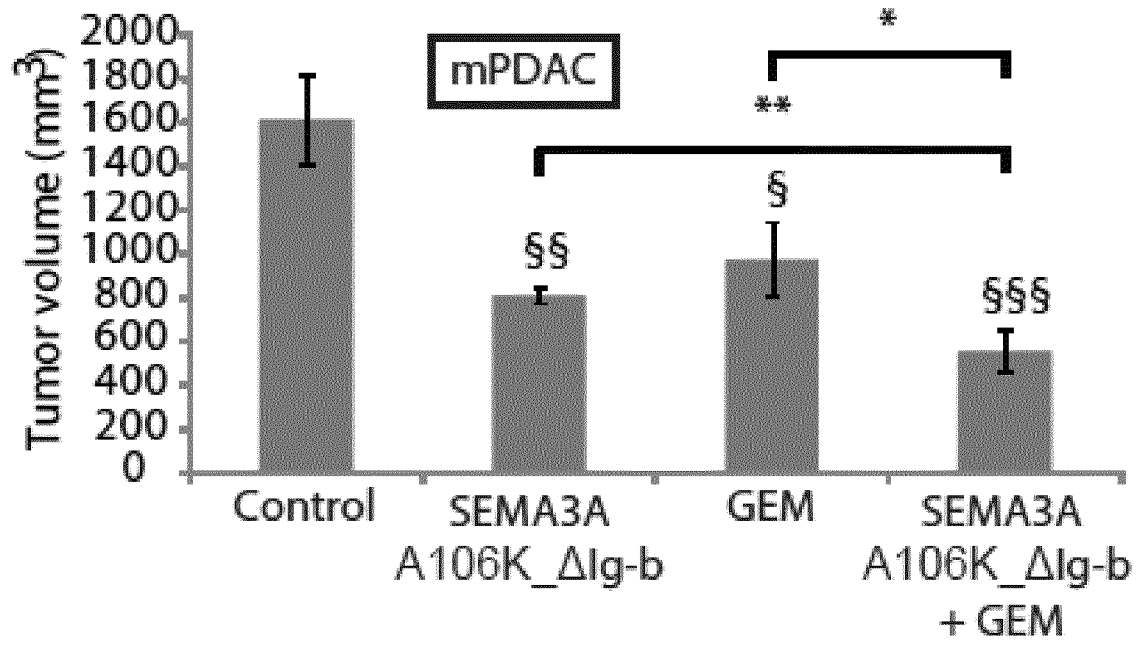
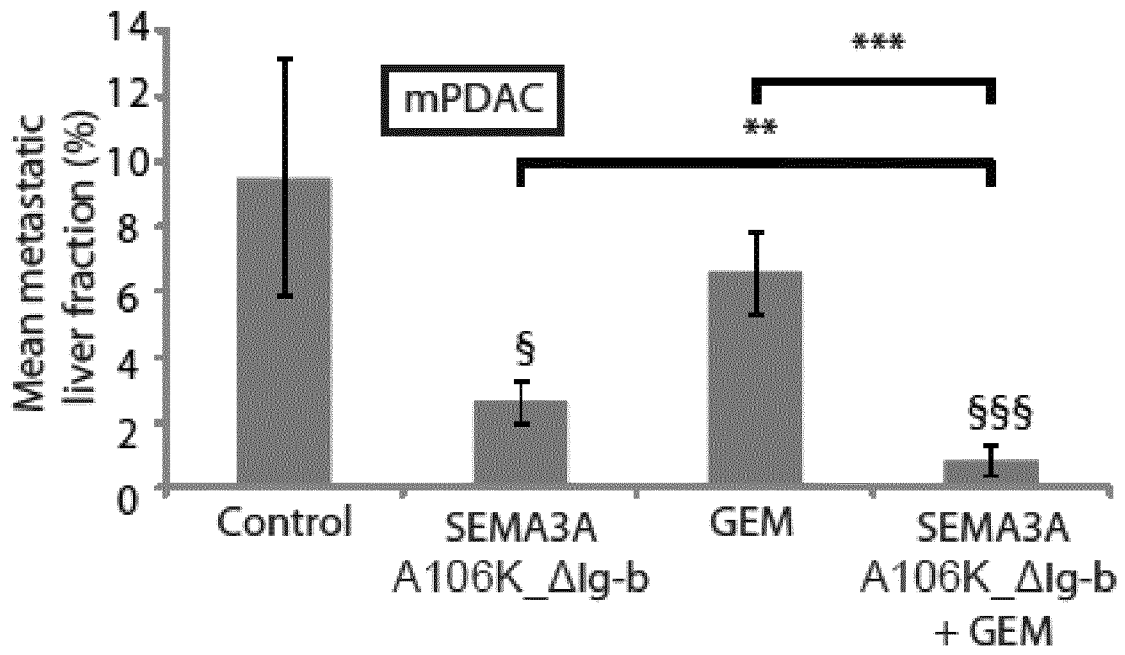


Figure 1B



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/060626

A. CLASSIFICATION OF SUBJECT MATTER
 INV. A61K38/17 A61K31/7068 A61P35/00
 ADD.
 According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
 Minimum documentation searched (classification system followed by classification symbols)
 A61K A61P
 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 EPO-Internal, WPI Data, BIOSIS, CHEM ABS Data, Sequence Search, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2016/135130 A1 (SERINI GUIDO [IT]; GIRAUDO ENRICO [IT]; TAMAGNONE LUCA [IT]) 1 September 2016 (2016-09-01) cited in the application claims; example 2 page 96, last paragraph	1-46
A	AKASHI: "Anticancer effects of gemcitabine are enhanced by co-administered iRGD peptide in murine pancreatic cancer models that overexpressed neuropilin-1", BRITISH JOURNAL OF CANCER, vol. 110, 1 January 2014 (2014-01-01), pages 1481-1487, XP055509796, abstract	1-46

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 13 June 2019	Date of mailing of the international search report 27/06/2019
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Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Vandenbogaerde, Ann
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2019/060626

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