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(54) **Title:** PDGFRα POLYPEPTIDES AS VIRAL DECOY RECEPTORS

(57) **Abstract:** The present invention relates to the field of pharmacology and specifically to PDGFRα polypeptides which can be used as viral decoy receptors. It includes fusion proteins comprising these polypeptides as well as nucleic acids, vectors, cells and pharmaceutical compositions. The use in medicine is envisioned specifically for the prevention and treatment of CMV infections.

5 PDGFRα POLYPEPTIDES AS VIRAL DECOY RECEPTORS

FIELD OF THE INVENTION

The present invention relates to the field of pharmacology and specifically to PDGFRα
10 polypeptides which can be used as viral decoy receptors. It includes fusion proteins comprising these polypeptides as well as nucleic acids, vectors, cells and pharmaceutical compositions. The use in medicine is envisioned specifically for the prevention and treatment of CMV infections.

BACKGROUND OF THE INVENTION

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Human cytomegalovirus (HCMV) is a ubiquitous pathogen that is found worldwide in 45-100% of the population. Although the vast majority of infections is asymptomatic or mild, HCMV is the leading infectious cause of congenital birth defects in the western world and a continuous risk factor for transplant recipients. The currently available drugs inhibit replication
20 and packaging of the viral genome and they are essential for successful transplantations. Unfortunately, their use is limited by severe side effects and/or resistance, also none of them has been approved for prevention or treatment of intrauterine HCMV infection. Therefore, there is a continuing need for alternative treatment options.

One alternative strategy is to block virus entry, which has been successfully applied in
25 anti-retroviral therapy. In recent years, several receptors for CMV glycoprotein complexes which mediate cell-entry have been identified, including PDGFRα, NRP2 and OR14I1. It was demonstrated previously that a soluble PDGFRα-Fc fusion protein efficiently inhibits cell-free HCMV infection (WO 2018/002081 A1). This decoy receptor binds to virus particles and prevents them from binding to and penetrating into host cells in a highly efficient manner as
30 compared to neutralization by monoclonal antibodies. Importantly, PDGFRα-Fc inhibits the infection of various cell types, while many of the highly potent monoclonal antibodies that are currently in development are directed against the viral pentamer complex and inhibit only infection of endothelial and epithelial cells efficiently. Using a PDGFRα-Fc as a decoy receptor against HCMV infection may impair cellular signaling by sequestration of the natural ligands
35 of PDGFRα. The interplay between platelet-derived growth factors (PDGFs) and their receptors

PDGFRα and PDGFRβ plays an important role in development as well as in the regulation of various physiological processes like cell migration and proliferation in adults. Hence, administering soluble PDGFRα-Fc could cause side effects by sequestration of PDGFs and in consequence reduction of PDGF-dependent signaling. For further development of the decoy receptor as an entry inhibitor, circumvention of PDGF sequestration is desirable. While substitutions or deletions could be an option, it has been shown that PDGFs can outcompete HCMV, indicating that the binding site of PDGFRα for the HCMV glycoprotein complex gH/gL/gO and the PDGFs are located in the same domains and might overlap.

The finding of the inventors that all of the single amino acid exchange mutants tested affected either both binding activities or none, confirms that the binding sites are actually overlapping. Importantly, their analysis provides proof of principle that a dissection of the two functions is nonetheless possible and identifies mutations on individual amino acid positions that increase the selectivity index. Specifically, the inventors identified amino acid positions within PDGFRα that are important for interaction with growth factors but dispensable for neutralization of the virus. These amino acid positions can be mutated, i.e. deleted or substituted, to reduce growth factor binding while maintaining virus binding, thereby reducing possible side effects of a PDGFRα-derived decoy as an HCMV entry inhibitor.

SUMMARY OF THE INVENTION

In a first aspect, the present invention relates to a PDGFRα polypeptide comprising (i) at least a PDGFRα D2 domain and a PDGFRα D3 domain, and (ii) mutations at positions 139 and 206 corresponding to the PDGFRα sequence according to SEQ ID NO: 1.

In a second aspect, the invention relates to a fusion protein comprising the PDGFRα polypeptide of the first aspect.

In a third aspect, the invention relates to a multimer comprising at least two fusion proteins of the second aspect.

In a fourth aspect, the present invention relates to a nucleic acid encoding the PDGFRα polypeptide of the first aspect, the fusion protein according to the second aspect, or one or more fusion proteins forming the multimer of the third aspect.

In a fifth aspect, the present invention relates to a vector comprising the nucleic acid of the fourth aspect.

In a sixth aspect, the present invention relates to a cell comprising the nucleic acid of the fourth aspect or the vector of the fifth aspect.

In a seventh aspect, the present invention relates to a pharmaceutical composition comprising the PDGFRα polypeptide of the first aspect, the fusion protein of the second aspect, the multimer of the third aspect, the nucleic acid of the fourth aspect, the vector of the fifth aspect or the cell of the sixth aspect, and a pharmaceutically acceptable carrier.

5 In an eighth aspect, the invention relates to an in *vitro* method of treating a device or a composition, comprising the steps of:

(i) providing a device or a composition, and

(ii) contacting the device or the composition with the PDGFRα polypeptide of the first aspect, the fusion protein of the second aspect, the multimer of the third aspect, or the pharmaceutical

10 composition of the seventh aspect.

In a ninth aspect, the present invention relates to the PDGFRα polypeptide of the first aspect, the fusion protein of the second aspect, the multimer of the third aspect, the nucleic acid of the fourth aspect, the vector of the fifth aspect, the cell of the sixth aspect or the pharmaceutical composition of the seventh aspect for use in medicine.

15 In a tenth aspect, the present invention relates to a polypeptide comprising a fragment of PDGFRα, wherein the fragment consists of a PDGFRα D2 domain and a PDGFRα D3 domain, and to the use of this polypeptide for binding to a virus.

LEGENDS TO THE FIGURES

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Figure 1: Screening of deletions in the predicted ligand binding sites of PDGFR-alpha-Fc for their ability to inhibit HCMV infection. A: Overview of the relative locations of the predicted ligand binding sites in domains 2 and 3 of PDGFR-alpha-Fc. Position numbers are based on the amino acid sequence of cellular PDGFRα. B to C The effect of PDGFRα-Fc

25 wild type (in the context of a PDGFRα-Fc protein, "wild type" herein refers to the PDGFRα part of the fusion) and deletion mutants on HCMV infection of fibroblasts was tested. Different concentrations of the soluble receptor variants were preincubated with a MOI of 1 of HCMV TB40-BAC4-IE-GLuc reporter virus. B: Expression of GLuc in samples treated with the different PDGFRα-Fc variants were compared to cells incubated with untreated virus. Dose

30 response curves were generated as an average of 3 independent experiments using 2 different preparations of the luciferase expressing TB40-BAC4-IE-GLuc reporter virus. C: Three mutants showed near complete inhibition at high doses (shown in black). For those mutants and the wild type PDGFRα-Fc the half-maximal effective concentration (EC50, shown in grey) was

calculated from the neutralization curves shown in B. Error bars indicate the standard error of the mean (SEM) calculated from 3 experiments.

Figure 2: Mutation of isoleucine 139 and tyrosine 206 reduces sequestration of PDGF-BB. A + B: Quantification of HCMV inhibition by PDGFRα-Fc alanine exchange mutants was assessed by preincubation of the HCMV TB40 GLuc reporter virus with PDGFRα-Fc derivatives before infection of fibroblasts. The degree of infection was measured relative to a virus only condition. A depicts the average dose-response curves as calculated from at least 3 independent experiments. B: The individual dose-response curves from each experiment were used as a basis to calculate the half-maximal effective concentrations (EC50s) needed for HCMV inhibition C + D: Biological interference of PDGFRα-Fc alanine exchange mutants with PDGF-dependent signaling was tested by preincubation of 50 ng/ml PDGF-BB with various concentrations of PDGFRα-Fc derivatives before stimulation of fibroblasts. Immunoblot staining of Phospho-Akt served as a measure for PDGF induced signaling. C shows immunoblot examples and D summarizes the interference of 4000 ng/ml of the different PDGFRα-Fc variants with Akt phosphorylation. All signals were normalized to actin to control for consistent loading. 100% interference equals complete inhibition of Akt phosphorylation to the level of cells that not receive PDGF-BB. 0% interference is full signal intensity to the level of cells stimulated with untreated PDGF-BB. Summarized are experiments using two independent preparations of the soluble receptors. Error bars indicate SEM.

Figure 3: Replacement of isoleucine 139 with glutamic acid improves the inhibition profile of PDGFRα-Fc. A + B: Quantification of HCMV inhibition by the PDGFRα-Fc I139 mutants. HCMV TB40 at a MOI $\leq$ 1 was preincubated with different concentrations of PDGFRα-Fc derivatives before infection of fibroblasts. After 1 day the cells were fixed and stained for the viral immediate early antigens and the percentage of infected cells was determined. Dose-response curves showing the percent of infection relative to a virus only control (A) were used as a basis to calculate the (B). Shown are the means of 5 independent experiments. C to F: Biological interference of PDGFRα-Fc I139 mutants with PDGF-dependent signaling was tested by preincubation of 6 ng/ml PDGF-BB with different concentrations of PDGFRα-Fc before stimulation of fibroblasts. C + D: PDGF-dependent signaling was assessed by staining for Phospho-Akt. C shows immunoblot examples and D summarizes the change in phospho-Akt signals after pretreatment of PDGF-BB with 4000g/ml PDGFRα-Fc. 100% interference equals complete inhibition of Akt phosphorylation to the level

of cells that not receive PDGF-BB. E + F PDGF-dependent signaling was quantified with a commercial phospho-Akt ELISA. The dose-response curves shown in E depict the mean of 3 independent experiments and F shows the EC50s calculated thereof in grey. The black bars depict the change in Akt phosphorylation when PDGF-BB was pretreated with 562 ng/ml of PDGFRα-Fc wild type or I139A. 100% interference equals complete inhibition of Akt phosphorylation to the level of cells that not receive PDGF-BB. All error bars indicate SEM and all experiments were performed with at least 2 independent protein preparations. Asterisks indicate statistically significant differences as compared to PDGFRα-Fc wild type determined by unpaired t-tests.

Figure 4: Dose-response relationship between PDGF-BB and Akt phosphorylation. Serum-starved fibroblasts were incubated for 15 min with various concentrations of PDGF-BB before the cells were lysed. The PDGF-dependent signaling was assessed by immunoblot and staining for phospho-Akt. Actin was included as a loading control. A shows a representative example of such an immunostaining. B depicts the average dose-response in 4 independent experiments. Error bars indicate SEM.

Figure 5: Replacement of tyrosine 206 with serine abolishes PDGF sequestration while maintaining HCMV inhibition. A + B: HCMV at a MOI ≤ 1 was pretreated with various concentrations of PDGFRα-Fc fusion proteins before infection of fibroblasts. Shown is the number of cells expressing the viral immediate early antigens relative to a virus only condition. Dose-response curves, as shown averaged in A, were used as a basis to calculate the half-maximal effective concentrations (B) in 3 independent experiments. C to F: Biological interference with PDGF-dependent signaling was tested by preincubation of 6 ng/ml PDGF-BB with various concentrations of PDGFRα-Fc mutants or wild type before stimulation of fibroblasts. C to D: Staining of Phospho-Akt served as a measure for PDGF induced signaling. C shows immunoblot examples and D summarizes the interference of different PDGFRα-Fc variants with Akt phosphorylation. 100% interference equals complete inhibition of Akt phosphorylation and negative values indicate enhanced Akt phosphorylation as compared to cells that were stimulated with untreated PDGF-BB. E +F: PDGF-dependent signaling was quantified using a phospho-Akt ELISA. The dose-response curves shown in E depict the mean of 3 independent experiments and F shows the EC50s calculated thereof (grey bars) as well as the interference of 562 ng/ml of PDGFRα-Fc variants with PDGF-dependent Akt

phosphorylation (black bars). N.d. stands for not determined and indicates that in this case no EC50 could be calculated due to lack of response.

All error bars indicate SEM and all experiments were performed with at least 2 independent protein preparations. Asterisks indicate statistical significant differences as compared to
5 PDGFRα-Fc wild type determined by unpaired t-tests.

Figure 6: Combination of multiple amino acid exchanges in PDGFRα further reduce inhibition of PDGF dependent signaling. A + B: Quantification of HCMV inhibition by PDGFRα-Fc double amino acid exchange mutants. To measure the inhibition of infection,
10 the rate of immediate early positive cells after infection with HCMV untreated or pretreated with PDGFRα-Fc was measured relative to a virus only condition. An average of 3 independent dose-response experiments is shown in A. The corresponding EC50 values are shown in B. C + D: PDGF-dependent signaling after preincubation of 6 ng/ml PDGF-BB with various concentrations of PDGFRα-Fc mutants was quantified with a commercial phospho-Akt ELISA.
15 The dose-response curves shown in C depict the mean of 3 independent experiments and D compares the interference of 562 ng/ml of the different PDGFRα-Fcs with PDGF-dependent cellular signaling. 100% interference equals complete inhibition of Akt phosphorylation to level of unstimulated cells whereas negative values indicate enhanced Akt phosphorylation as compared to cells that were stimulated with untreated PDGF-BB.

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Figure 7: Mutations in PDGFRα-Fc reduce binding affinity for PDGF-BB. Quantification of biochemical binding affinity of PDGFRα-Fc variants for PDGF-BB was assessed by microscale thermophoresis. All experiments were performed with 0.1 nmol/l (2.5 ng/ml) fluorescently labelled (NT-647) PDGF-BB. A to C: Various concentrations of PDGFRα-
25 Fc wildtype and I139E (A), Y206S (B) or I139A + Y206A (C) were mixed with PDGF-BB. This resulted in a dose dependent fluorescence quenching which was used to compare wild type and mutants using the fluorescence analysis mode. The concentration dependent change in fluorescence over time from 3 measurements was used to generate binding curves which were fitted to a one site binding model to determine K_d values which are graphed in D. Error bars in
30 indicate K_d confidence. E: No changes in the initial fluorescence were observed when PDGF-BB was mixed with the PDGFRα-Fc I139E + Y206S mutant, therefore, the binding affinity for PDGF-BB was determined by standard microscale thermophoresis analysis. Shown is the ratio of (F1) fluorescence 5 seconds after excitation over (F0) the steady-state fluorescence (ΔF_{norm}) for each PDGFRα-Fc wild type or I139E + Y206S concentration. Error bars indicate standard

deviation from 3 replicate measurements. F depicts the corresponding K_d values with confidence intervals.
For comparison with other graphs in this application: 0.14 nmol/l of PDGFRα-Fc correspond to about 24 ng/ml, while 200 nmol/l equal 33,000 ng/ml.

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Figure 8: MST traces for PDGF-BB bound to PDGFRα-Fc variants. Microscale thermophoresis (MST) was performed with 0.1 nM (2.5 ng/ml) fluorescently labelled (NT-647) PDGF-BB which was mixed with different concentrations of PDGFRα-Fc wild type and mutants (2fold dilution series starting from A + E: 5 nM, B: 500 nM, C: 650 nM, D: 550 nM or
10 F: 500 nM). In all graphs, the darkest line represents the highest concentration of soluble receptor and lightest grey depicts the lowest concentration. Each concentration was tested three times, shown is one example. All experiments were performed with medium MST power and 60% excitation power. A to D: Direct fluorescence analysis measuring binding-induced fluorescence quenching. After 5 sec of initial photobleaching the change in fluorescence was
15 monitored over the indicated time (shaded in grey). The rate of change in fluorescence per second was used to generate dose-response curves shown in Figure 6 A-C. E + F: After 5 sec of initial photobleaching the change in fluorescence was monitored for 6 seconds. For the dose response curves shown in Figure 7, the fluorescence intensity after infrared laser activation (F1, second grey bar) over the initial fluorescence (F0, first grey bar) was calculated from the data
20 shown here.

Figure 9: Serum induced cellular signaling is unaffected by treatment with the PDGFRα-Fc I139E Y206S mutant. To test for cellular signaling under more physiological conditions, the effect of PDGFRα-Fc wild type and I139E+Y206S double mutant on serum-
25 induced phosphorylation of Akt was measured. A pool of sera from 3 donors was preincubated with 562 ng/ml of soluble receptors before stimulation of fibroblasts. The absolute amount of Akt phosphorylation was measured using a commercial ELISA. Shown are the mean values for 3 experiments testing 2 independent protein preparations. The error bars indicate SEM. The asterisk indicates that only PDGFRα-Fc wild type but not the I139E + Y206S mutant
30 significantly interfered with serum induced signaling as determined by unpaired t-tests comparing the response of untreated serum to the treated sera.

Figure 10: Mutation of V242K abolishes PDGF sequestration without impact on HCMV inhibition. A + B: Quantification of HCMV inhibition by PDGFRα-Fc V242K

mutants. To measure the inhibition of infection, the rate of immediate early antigen-positive cells after infection with HCMV untreated or pretreated with PDGFRα-Fc was measured. A shows the average of 3 independent dose-response experiments and B shows the corresponding EC50 values. C + D: PDGF-dependent signaling after preincubation of 6 ng/ml PDGF-BB with various concentrations of PDGFRα-Fc mutants was quantified with a phospho-Akt ELISA. The dose-response curves shown in C depict the mean of 3 independent experiments and D compares the interference of 500 ng/ml of the different PDGFRα-Fcs with PDGF-dependent cellular signaling. 100% interference equals complete inhibition of Akt phosphorylation to the level of unstimulated cells whereas negative values indicate enhanced Akt phosphorylation as compared to cells that were stimulated with untreated PDGF-BB. All error bars indicate standard error of the mean.

Figure 11: Combination of multiple mutations affecting PDGF binding reduces the affinity of PDGFRα-Fc for PDGF beyond detection. Quantification of biochemical binding affinity of PDGFRα-Fc variants for PDGF-BB was assessed by microscale thermophoresis. Various concentrations of PDGFRα-Fc wildtype, PDGFRα-Fc V242K (A), PDGFRα-Fc I139E + V242K (B), PDGFRα-Fc Y206S + V242K (C) or PDGFRα-Fc I139E + Y206S + V242K (D) were mixed with 0.1 nmol/l (A) or 1 nmol/l (B to D) fluorescently labeled PDGF-BB. Binding curves were generated by analysis of the ratio (ΔF_{norm}) of fluorescence at MST-on time (1.5–2.5) seconds over the steady-state fluorescence (F0) for each concentration. Measurements were performed at 60% excitation power (A) or 20% excitation power (B). Error bars indicate standard deviation from 3 replicate measurements.

DETAILED DESCRIPTION OF THE INVENTION

Before the present invention is described in detail below, it is to be understood that this invention is not limited to the particular methodology, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention, which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art. Preferably, the terms used herein are defined as described in "A multilingual glossary of biotechnological terms: (IUPAC Recommendations)", Leuenberger,

H.G.W, Nagel, B. and Kölbl, H. eds. (1995), Helvetica Chimica Acta, CH-4010 Basel, Switzerland).

Several documents are cited throughout the text of this specification. Each of the documents cited herein (including all patents, patent applications, scientific publications, manufacturers' specifications, instructions etc.), whether supra or infra, is hereby incorporated by reference in its entirety.

In the following, the elements of the present invention will be described. These elements are listed with specific embodiments, however, it should be understood that they may be combined in any manner and in any number to create additional embodiments. The variously described examples and preferred embodiments should not be construed to limit the present invention to only the explicitly described embodiments. This description should be understood to support and encompass embodiments, which combine the explicitly described embodiments with any number of the disclosed and/or preferred elements. Furthermore, any permutations and combinations of all described elements in this application should be considered disclosed by the description of the present application unless the context indicates otherwise.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word “comprise”, and variations such as “comprises” and “comprising”, are to be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integer or step. In preferred embodiments, “comprise” can mean “consist of”. As used in this specification and the appended claims, the singular forms “a”, “an”, and “the” include plural referents, unless the content clearly dictates otherwise.

In a first aspect, the present invention relates to a PDGFRα polypeptide comprising (i) at least a PDGFRα D2 domain and a PDGFRα D3 domain, and (ii) mutations at positions 139 and 206 corresponding to the PDGFRα sequence according to SEQ ID NO: 1 (also referred to herein as “139 mutation” and “206 mutation”).

The term “PDGFRα polypeptide” herein refers to a polypeptide derived from PDGFRα, in particular according to SEQ ID NO: 1. PDGFRα comprises several domains, an extracellular domain (ECD), a transmembrane domain (TM) and an intracellular domain (ICD). The ECD comprises domains 1, 2, 3, 4 and 5 (D1, D2, D3, D4 and D5) as well as a signal peptide (SP) prior to maturation. In SEQ ID NO: 1, the SP extends from amino acid position 1 to 23, the ECD extends from amino acid position 24 to 528, the TM extends from amino acid position 529 to 549 and the ICD extends from amino acid position 550 to 1089. D1, D2, D3, D4 and D5

extend from amino acid position 24 to 113, 117 to 201, 202 to 306, 319-410 and 414 to 517, respectively. PDGFRα also comprises a PDGF binding domain and a virus binding domain within the ECD. While both may involve some residues outside of D2 and D3, the inventors found that D2 and D3 are sufficient for PDGF and virus binding. Therefore, when referring to "the PDGF binding domain" and "the virus binding domain" herein, in particular the PDGF binding domain and the virus binding domain herein as comprised in D2 and D3 are meant. The virus is a virus binding to (usually mature) PDGFRα, preferably a virus binding to mature PDGFRα according to amino acids 24-1089 of SEQ ID NO: 1, in particular to the ECD or only D2 and D3 thereof. More preferably, the virus is CMV, with respect to all references to "a virus" or "viral" herein. The CMV is preferably HCMV, with respect to all references to CMV herein.

The PDGFRα from which the PDGFRα polypeptide of the first aspect is derived can be any PDGFRα, specifically from any species (although preferably it is from a mammal, more preferably human), or a variant thereof. The positions and domains boundaries of homologous PDGFRα or of variants can be determined by the skilled person without undue burden by sequence alignment. In a preferred embodiment it is PDGFRα according to SEQ ID NO: 1 or a variant thereof. Generally, it is preferred that the PDGFRα polypeptide is derived from mature PDGFRα or a variant thereof, i.e. PDGFRα or a variant thereof lacking the SP (or any other signal peptide). With regard to SEQ ID NO: 1, mature PDGFRα corresponds to amino acids 24-1089 of SEQ ID NO: 1. Nevertheless, it is preferred that a nucleic acid of the invention encodes for the PDGFRα polypeptide comprising a (any) signal peptide (preferably one which directs the PDGFRα polypeptide into the endoplasmic reticulum of a cell such that it is secreted, such as a signal peptide comprising amino acids 1-20 of SEQ ID NO: 2 or a variant thereof), preferably the PDGFRα SP (e.g. comprising amino acids 1-23 of SEQ ID NO: 1 or a variant thereof).

The above also applies to any PDGFRα domain recited herein (SP, ECD, TD, ICD, D1-D5), i.e. these domains can be from any PDGFRα (any species, preferably mammalian, more preferably of SEQ ID NO: 1), and it can be a variant of domains of that PDGFRα. Accordingly, "SP, ECD, TD, ICD, or D1-D5" as referred to herein can be any PDGFRα SP, ECD, TD, ICD, or D1-D5, respectively, and in particular can be a variant of the PDGFRα SP, ECD, TD, ICD, or D1-D5, respectively according to SEQ ID NO: 1.

The PDGFRα polypeptide of the first aspect differs from the PDGFRα from which it is derived by at least the 139 and 206 mutations. The PDGFRα polypeptide may further differ from the PDGFRα from which it is derived by deletion of one or more domains or parts thereof,

wherein the deleted domains (or domains with deletion of at least a part) are preferably the TM and/or the ICD, and optionally further D4 and/or D5, and optionally also D1. While the PDGFRα polypeptide of the first aspect comprises (i) D2 and (ii) D3, in a preferred embodiment it further comprises (iii) D1, and optionally (iv) D4 and/or D5. Alternatively or preferably in
5 addition, it lacks (a) at least a part (preferably all) of the TM, preferably (b) the SP, and optionally (c) at least a part (preferably all) of the ICD. In a particular embodiment, the PDGFRα polypeptide of the first aspect consists of the ECD and lacks the SP, TM and ICD. As indicated above, any of these domains can be from any PDGFRα (any species, preferably mammalian, more preferably of SEQ ID NO: 1), and it can be a variant of corresponding
10 domains of that PDGFRα. A PDGFRα polypeptide lacking SP may comprise a different signal peptide, but preferably does not comprise any signal peptide.

The above variants (of PDGFRα or of any of its domains) independently have at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity (higher identities
15 being preferred over lower ones) to the sequence of polypeptide they vary (e.g. SEQ ID NO: 1 or domains thereof). For example, if the PDGFRα polypeptide comprises only the ECD of PDGFRα, the ECD has at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the ECD of SEQ ID NO: 1 (rather than the ECD of SEQ ID NO: 1 or of a
20 variant of SEQ ID NO: 1).

The results of the examples provide evidence that mutations of distinct sites within the PDGF binding domain can act synergistically against binding of PDGFs (the 139 and 206 mutations render the PDGF binding domain largely non-functional), so it is reasonable to assume that also mutations without a strong individual impact on PDGF sequestration by
25 themselves may lead to a synergistic reduction when replaced e.g. with amino acids other than alanine and in combination with the 139 and 206 mutations. Accordingly, the PDGFRα polypeptide may further differ from the PDGFRα (or variant) from which it is derived by one or more further mutations (e.g. up to 10, 9, 8, 7, 6, 5, 4, 3 or 2, lower numbers preferred over higher ones), including deletions and preferably substitutions, within the PDGF binding
30 domain, preferably within the D2 and/or the D3 domains. Preferred amino acids to be mutated are those that are predicted by *in silico* models to be relevant for PDGF binding. Particular examples are M133, V184, G185, N204, N240, E241, V242, V243, D244, L245, T259, M260, L261, E262, K270, T273, Q294, A295, T296 and/or E298, preferably M133, N204, V242, Q294, T296 and/or E298, more preferably M133, N204, Q294, T296 and/or E298,

corresponding to the PDGFRα sequence according to SEQ ID NO: 1. Substitutions are preferably non-conservative and change one or more properties of the substituted amino acid selected from the group consisting of from polarity, charge and size. Exemplary substitutions of e.g. V242 are V242H, V242K and V242R, specifically V242K. While the V242 mutation may be a deletion or substitution, a substitution is preferred. Most preferred is a point mutation.

Preferably, the PDGFRα polypeptide comprises a functional virus binding domain. Functional in this respect means that the PDGFRα polypeptide has a substantial affinity for the virus. Substantial in this respect preferably means at least 5%, at least 10%, at least 15%, or at least 20% of the virus affinity of a corresponding PDGFRα according to SEQ ID NO: 1, e.g. at least at least 5%, 10%, at least 15%, or at least 20% of the virus affinity of the ECD of SEQ ID NO: 1. Alternatively or additionally, functional may mean that the PDGFRα polypeptide has a substantial inhibitory effect on virus cell-entry. Substantial in this respect preferably means at least 5%, at least 10%, at least 15%, or at least 20% of the inhibitory effect of a corresponding PDGFRα according to SEQ ID NO: 1, e.g. at least at least 5%, 10%, at least 15%, or at least 20% of the virus affinity of the ECD of SEQ ID NO: 1. Higher percentages are preferred over lower ones. A "corresponding PDGFRα according to SEQ ID NO: 1" above and below has the same PDGFRα domains (or parts thereof) as the PDGFRα polypeptide, but of exactly SEQ ID NO: 1.

The 139 and 206 mutations alter the PDGF binding domain, i.e. the PDGFRα polypeptide comprises an altered PDGF binding domain. The altered PDGF binding domain is characterized functionally as a result of these mutations by a reduced affinity for PDGF (at least PDGF-BB) compared to the corresponding PDGFRα according to SEQ ID NO: 1. Reduced affinity in this respect preferably means that the altered PDGF binding domain has 5% or less, 4% or less, 3% or less, 2% or less, 1% or less, 0.5% or less, 0.2% or less, 0.1% or less, 0.05% or less, 0.01% or less, 0.005% or less, or 0.001% or less of the PDGF affinity of the corresponding PDGFRα according to SEQ ID NO: 1. Lower percentages are preferred over higher ones. The preferred minimal affinity is, if zero cannot be achieved, 0.001% or 0.0001% of the corresponding PDGFRα according to SEQ ID NO: 1.

Mutations referred to herein, specifically the 139 and 206 mutations, may independently be selected from the group consisting of a deletion and a substitution, preferably a non-conservative substitution. Preferably the mutation is a point mutation. This means that at least the one adjacent position upstream and the one adjacent position downstream are not mutated. This applies in particular to the mutation being a deletion, i.e. at least the one adjacent position upstream and the one adjacent position downstream are not deleted.

In embodiments where a mutation is a substitution, the 139 substitution preferably is to a polar, more preferably polar and charged, most preferably polar and negatively charged amino acid and/or the 206 substitution is to a non-polar amino acid or preferably to a smaller amino acid. The smaller amino acid is preferably polar, and more preferably also uncharged.

5 Polar amino acids have either an OH or NH_2 group (when in an aqueous environment) and can therefore form hydrogen bonds with other suitable groups. Due to the hydrophilic properties of their side chain, they have a tendency to be on the outside of a protein and to affect the overall protein structure. Polar amino acids are D and E (negatively charged); R, K and H (positively charged); and N, Q, S, T and Y (uncharged). Non-polar amino acids have side chains 10 consisting of hydrocarbons and optionally other side chain atoms such as sulphur which do not confer polar properties. Non-polar amino acids are G, A, P, V, L, I, M, W and F.

The expression “smaller” relates to the Tyrosine at position 206 in SEQ ID NO: 1 and preferably means that the amino acid has a lower molecular weight than Tyrosine. Alternatively or in addition, it means that is has a shorter side chain than Tyrosine, e.g. a side chain 15 comprising less than 6, less than 5, less than 4, less than 3 C, N, O, S and Se atoms in total (lower numbers preferred over higher ones). In preferred embodiments, the 139 substitution is selected from the group consisting of I139E and I139A, more preferably it is I139E, and/or the 206 substitution is selected from the group consisting of Y206S, Y206T, Y206N, Y206Q, Y206C, Y206U, Y206G, Y206A and Y206F, preferably from the group consisting of Y206S, 20 Y206T, Y206N, Y206Q, Y206C, Y206U, Y206A and Y206G, more preferably from the group consisting of Y206S, Y206T, Y206N, Y206A and Y206Q, even more preferably from the group consisting of Y206S and Y206T. Most preferably it is Y206S. In the most preferred embodiment, the 139 substitution is I139E and the 206 substitution is Y206S.

In one embodiment, the PDGFRα polypeptide comprises or consists of a fragment 25 consisting of amino acids 60-206, 60-243, 60-262, 60-273, 60-294, 60-528, 30-206, 30-243, 30-262, 30-273, 30-294, 30-528, 24-206, 24-243, 24-262, 24-273, or 24-528 (the latter being preferred) of SEQ ID NO: 1 or a variant of any of the fragments, wherein the fragments and variants have the 139 and 206 mutations.

In a preferred embodiment, the PDGFRα polypeptide is a decoy receptor. The term 30 “decoy receptor” as used herein refers to viral decoy receptor, i.e. to a receptor that binds to a virus, but is not capable of mediating cell entry of the virus. In order to exert its function, the decoy receptor is usually not integrated into or attached to the plasma membrane of a cell. In a preferred embodiment, the decoy receptor is soluble. The term "soluble" as used herein indicates that the PDGFRα polypeptide is not bound to a cellular membrane, and it is usually

characterized by the functional disruption of the TM (preferably by deletion of at least part of it, e.g. of at least 10, at least 14 or at least 18 amino acids of the TM, higher values being preferred), so that the soluble PDGFRα polypeptide is devoid of any membrane anchoring function. It is therefore generally secreted by the cell producing it. At least part of the ICD may
5 also be absent.

Alternatively, it is envisaged that the decoy receptor, e.g. if not soluble, can be linked to a carrier. The carrier can be any suitable biocompatible substance, but is not a host cell. For example, the decoy receptor may be covalently bound to a particle, e.g. a nanobead, or may be integrated in an artificial lipid membrane of e.g. a liposome.

10 Additional definitions and embodiments are described further below.

In an alternative to the first aspect described above, the present invention relates to a PDGFRα polypeptide comprising a mutation at (a) a position selected from the group consisting of M133, V184, G185, N204, N240, E241, V242, V243, D244, L245, T259, M260, L261, E262, K270, T273, Q294, A295, T296 and E298 and (b) position 206 and/or 139 corresponding
15 to the PDGFRα sequence according to SEQ ID NO: 1. Regarding (a), the mutation can be at one position (e.g. V242) or at more than one of the positions. This is based on the finding of the inventors that mutations of distinct sites within the PDGF binding domain can act synergistically against binding of PDGFs, and mutations (even if they do not have a strong individual impact on PDGF sequestration by themselves) may lead to a synergistic reduction
20 when in combination with the 139 and/or the 206 mutation. The mutation of (a) is preferably a point mutation. It may be a deletion or substitution. Preferably it is a substitution, more preferably a non-conservative substitution, changing one or more properties of the substituted amino acid selected from the group consisting of from polarity, charge and size. Exemplary substitutions of e.g. V242 are V242H, V242K and V242R, specifically V242K.

25 Definitions and embodiments described herein for the PDGFRα polypeptide of the first aspect apply also to this PDGFRα polypeptide, and corresponding fusion proteins, multimers, nucleic acids, vectors, cells, pharmaceutical compositions and methods uses as described below (but corresponding to the (a) and (b) mutations instead) are also intended.

30 In a second aspect, the invention relates to a fusion protein comprising the PDGFRα polypeptide of the first aspect.

In some embodiments, the fusion protein further comprises one or more moieties selected from the group consisting of a moiety facilitating the crossing of the blood-brain-barrier (BBB moiety); a moiety targeting the brain; a moiety facilitating the crossing of the

placenta from the mother towards the fetus (placenta moiety); a moiety preventing the crossing of the placenta from the mother towards the fetus; a multimerization moiety, preferably a dimerization moiety; a half-life extending moiety; and an extracellular anti-viral agent (EAA). The moieties are preferably polypeptide moieties.

5 An example for a BBB moiety is an insulin receptor binding moiety, e.g. an antibody or an antigen-binding fragment thereof binding to the insulin receptor. An example for a moiety targeting the brain is a scopine moiety (1R,2R,4S,5S,7S)-9-Methyl-3-oxa-9-azatricyclononan-7-ol). An example for a placenta moiety is an Fc domain of IgG. An example for a moiety preventing the crossing of the placenta from the mother towards the fetus is an Fc domain of 10 IgM. The multimerization moiety, preferably a dimerization moiety, can be a heterodimerization moiety or preferably a homodimerization moiety. In preferred embodiments, the homodimerization domain is selected from the group consisting of an Fc domain, a CH3 domain, a CH2-CH3 domain, and a domain where homodimerization is mediated by an Ig-like fold, a rossmann- or rossmann-like alpha-beta-alpha sandwich fold, an 15 alpha-sandwich fold, a continuous-beta-sheet fold, a beta-sandwich fold, a mixed beta-sheet fold, a 2-helix orientation, an antiparallel alpha-helix-orientation, a parallel alpha-helix orientation, a 4-helix bundle motif, a leucine zipper or a coiled-coil domain. Preferably it is an Fc domain, more preferably of IgG (specifically IgG1) or IgM, most preferably of human.

In some embodiments, the heterodimerization domain is selected from the group 20 consisting of a knob or a hole CH3 (or CH2-CH3) domain of a pair of knob-into-hole CH3 (or CH2-CH3) domains; an Fc-domain with mutations forcing heterodimerization (e.g. charged mutations); a domain of a pair of interchanged domains (such as Fc-one/kappa heterodimerization domain, CL and CH domains); an Ig-like fold with introduced mutations to force heterodimerization; and a domain mediating heterodimerization containing a rossmann- 25 or rossmann-like alpha-beta-alpha sandwich fold, an alpha-sandwich fold, a continuous-beta-sheet fold, a beta-sandwich fold, a mixed beta-sheet fold, a 2-helix orientation, an antiparallel alpha-helix-orientation, a parallel alpha-helix orientation, a 4-helix bundle motif, a leucine zipper or a coiled-coil domain. The half-life extending moiety can be a moiety selected from the group consisting of an Fc domain, albumin, an elastin-like polypeptide (ELP), an XTEN 30 polypeptide, a PAS polypeptide, a polyethylene glycol (PEG) moiety and a lipid moiety. The EAA (e.g. an agent, like an antibody, disrupting the virus capsid) is preferably an EAA active against CMV.

In preferred embodiments, the fusion protein comprises a multimerization moiety or a half-life extending moiety. More preferably, it comprises both. In especially preferred

embodiments, the fusion protein comprises a half-life extending moiety which is a multimerization moiety. Most preferably, the half-life extending moiety which is a multimerization moiety is an Fc domain (e.g. of IgM or preferably IgG, specifically IgG1). The Fc domain increases the plasma half-life of fusion proteins. This is caused by binding of the Fc domain to the receptor FcRn, which enables recycling during circulation. It also is a dimerization moiety. One of the advantages of the use of a dimerization domain considered by the inventors is that it increases the binding efficiency which suggests that the Fc-mediated dimerization of PDGFRα-Fc further enhances virus inhibition by increasing the avidity. Furthermore, it has placenta-crossing properties as described above.

SEQ ID NO: 2 represents the fusion protein of the examples. It comprises (i) a signal peptide (which is not the PDGFRα SP) at positions 1-20, (ii) the PDGFRα ECD at positions 26-530 with the 139 and 206 mutations (numbering of the mutations according to SEQ ID NO: 1 as in the entire disclosure), (iii) the Fc domain of human IgG1 at positions 533-759, and (iv) non-functional (with respect to effects described herein) residues which are remnants of the recombination process at positions 21-25 and 531-532. Thus, it is preferred that the fusion protein comprises or consists of an amino acid sequence according to positions 26-530 of SEQ ID NO: 2 and an amino acid sequence according to positions 533-759 of SEQ ID NO: 2, or variants of these amino acid sequences. Alternatively, it comprises or consists of an amino acid sequence according to positions 26-530 of SEQ ID NO: 2 and an amino acid sequence according to positions 533-759 of SEQ ID NO: 2, or variants of these amino acids sequences, as well as any signal peptide (preferably any that directs the fusion protein into the endoplasmic reticulum of a cell, e.g. the signal peptide of SEQ ID NO: 2 or of SEQ ID NO: 1 including variants; with or without a linking sequence to the ECD) and/or any linking sequence between the ECD and the Fc domain. Linking sequences are up to 20, preferably up to 15 or 10 or more preferably up to 5 amino acids long. In a specific embodiment, the fusion protein comprises or consists of an amino acid sequence according to SEQ ID NO: 2 or a variant thereof. Variants have at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity (higher identities being preferred over lower ones) to the sequence of polypeptide they vary (here SEQ ID NO: 2 or parts thereof). Variants maintain the 139 and 206 mutations and all other mutation that may be intended for the PDGFRα polypeptide of the first aspect, in as far as they include the corresponding positions.

In a third aspect, the invention relates to a multimer comprising at least two fusion proteins of the second aspect. This is preferably afforded by the fusion protein comprising a multimerization domain as described above. In a preferred embodiment, the multimer is a dimer. The dimer preferably is a homodimer, but it may also be a heterodimer.

5

In a fourth aspect, the present invention relates to a nucleic acid encoding the PDGFRα polypeptide of the first aspect, the fusion protein according to the second aspect, or one or more fusion proteins forming the multimer of the third aspect. It is preferred that the nucleic acid encoding the PDGFRα polypeptide of the first aspect encodes also a (any) signal peptide
10 preferably one directing to the endoplasmic reticulum of a cell, more preferably the PDGFRα SP or the signal peptide of SEQ ID NO: 2 or a variant thereof with at least 80%, at least 90% or preferably at least 95% sequence identity.

In a fifth aspect, the present invention relates to a vector comprising the nucleic acid of
15 the fourth aspect. Any suitable vector known in the art can be used, preferably it is a DNA vector e.g. an expression vector like a plasmid. Examples are given further below.

In a sixth aspect, the present invention relates to a cell comprising the nucleic acid of the fourth aspect or the vector of the fifth aspect. The cell may be any prokaryotic or eukaryotic
20 cell. In instances where the decoy receptor is soluble, the cell secretes the PDGFRα polypeptide of the first aspect, the fusion protein of the second aspect or the multimer of the third aspect.

In a seventh aspect, the present invention relates to a pharmaceutical composition comprising the PDGFRα polypeptide of the first aspect, the fusion protein of the second aspect,
25 the multimer of the third aspect, the nucleic acid of the fourth aspect, the vector of the fifth aspect or the cell of the sixth aspect, and a pharmaceutically acceptable carrier.

It is envisaged that the pharmaceutical composition may further comprise an anti-viral agent, e.g. an agent selected from the group consisting of Letermovir, Formiviren, Foscarnet, Cidofovir, Ganciclovir, Valganciclovir, a direct antiviral (DAA), a viral antigen, and an
30 antibody to a viral antigen. Preferred viral antigens of CMV are CMV complexes comprising gH and gL, such as the gH/gL/gO trimer or the gH/gL/pUL128/pUL130/pUL131A pentamer, or one or more of the CMV proteins gH, gL, gO, pUL128, pUL130 and/or pUL131A.

The therapeutically effective amount results in a therapeutically effective concentration in blood of a subject that is suitable for neutralizing a virus, but does not detectably influence

PDGF cellular signaling, i.e. it does not have any unspecific effects on cells. Wide ranges of concentrations are possible since the PDGFRα polypeptide of the first aspect is engineered to maintain virus neutralization but to have a diminished PDGF binding capability. Suitable steady state concentrations in blood are for example from about 0.3 nM to about 12 nM, from about 0.6 nM to about 10 nM, from about 1.5 nM to about 6 nM, or from about 3 nM to about 3.6 nM. Such concentrations can be achieved, for example, by administering from about 0.03 mM to about 0.5 mM, from about 0.1 mM to about 0.3 mM, or from about 0.12 mM to about 0.2 mM per week in total, in one or more doses (e.g. daily or weekly doses).

The pharmaceutically acceptable carrier is not limited, definitions and examples can be found further below.

In an eighth aspect, the invention relates to an in *vitro* method of treating a device or a composition, comprising the steps of:

(i) providing a device or a composition, and

(ii) contacting the device or the composition with a PDGFRα agent.

The term "PDGFRα agent" refers to a PDGFRα polypeptide of the first aspect, a fusion protein of the second aspect, a multimer of the third aspect, or a pharmaceutical composition of the seventh aspect.

The device can be e.g. a medical device including an intravaginal device, and the composition, which is preferably liquid and more preferably aqueous, can be a body liquid such as blood or breast milk. Specifically, the *in vitro* method can be one of the following methods:

An *in vitro* method of coating a medical device, in particular an intravaginal device, comprising the steps of:

(i) providing the medical device,

(ii) contacting the medical device with a PDGFRα agent such that it adheres to the medical device, and

(iii) optionally washing the medical device to remove excess (i.e. non-adhered) PDGFRα agent. Adherence is such that it is maintained during washing steps. More preferably, it is such that it is maintained within an animal, preferably human, body. Methods for conferring adherence are well-known in the art and are not limited herein.

An *in vitro* method of treating a body liquid comprising virus (preferably breast milk, more preferably human breast milk), comprising the steps of

(i) providing the body liquid, and

(ii) contacting the body liquid with a PDGFRα agent such that virus comprised in the body liquid binds to the PDGFRα agent, and

(iii) optionally removing the PDGFRα agent from the body liquid.

An *in vitro* method of treating a body liquid at least potentially comprising virus (preferably blood, more preferably human blood; e.g. from a donor), comprising the steps of

(i) providing the body liquid, and

(ii) contacting the body liquid with a PDGFRα agent such that virus at least potentially comprised in the body liquid can bind to the PDGFRα agent, and

(iii) optionally removing the PDGFRα agent from the body liquid.

"At least potentially comprising virus" means that only the body liquid comprising virus is treated, or that body liquid for which it is unknown whether it comprises virus is treated for the event that it does comprise it. The latter would be a routine treatment applied to any body liquid (specifically blood) without ascertaining that it comprises virus. Any step of optionally removing the PDGFRα agent from the body liquid also removes all virus from the body liquid, which can be afforded by contacting the body liquid with an excess amount of PDGFRα agent (compared to virus comprised or at least potentially comprised, e.g. at least 10x, at least 50x, at least 100x or at least 1000x; larger x preferred over lower ones).

The treated medical device can be used to treat a patient that is infected with virus or to prevent infection of a patient or a child to be born, the treated blood can be used to prevent infection of a patient in need of blood, and the treated breast milk can be used to prevent infection of an infant in need of the breast milk.

In a ninth aspect, the present invention relates to the PDGFRα polypeptide of the first aspect, the fusion protein of the second aspect, the multimer of the third aspect, the nucleic acid of the fourth aspect, the vector of the fifth aspect, the cell of the sixth aspect or the pharmaceutical composition of the seventh aspect for use in medicine.

The use in medicine includes various uses, such as the use of a medical device coated with a PDGFRα in particular an intravaginal device, e.g. produced with the method of the eighth aspect, or the use of a body liquid (preferably human breast milk or blood, e.g. of a donor, i.e. of a subject who will not receive the blood) treated with a PDGFRα agent to capture (decoy) virus, e.g. as described in the eighth aspect. In the case of breast milk (expressed from the adult body), this can be advantageous compared to methods like short term pasteurization, since there are concerns that short term pasteurization may destroy valuable components comprised in the milk.

Preferred, however, is the use according to the ninth aspect as a medicament. The use as a medicament is in an amount or concentration that is therapeutically effective as described above. The subject is preferably a mammal, more preferably human. In some embodiments, it is female and optionally pregnant. In other embodiments, the subject has a malfunctioning organ and is going to have transplant surgery, or the subject had transplant surgery (preferably within 3 months of the start administration of the medicament), or the subject is characterized by having recurring acute transplant rejections.

It is envisaged that the use as a medicament is the use as a virus-decoying medicament. The terms "virus-trapping", "virus-sinking" and "virus-capturing" may be used instead to describe this function. In other words, this use is a use as a viral entry inhibitor, since the decoying prevents cell-entry of decoyed viral particles.

Preferred specific uses may be one or more of the following:

(i) use in preventing or treating a viral infection;

(ii) preventing viral transmission to a fetus;

(iii) use in preventing or treating a viral infectious disease;

(iv) use in preventing or treating a virus-facilitated progression of cancer; and/or

(v) use in preventing or treating a transplant rejection.

The term "infection" as used herein refers to a viral infection, i.e. to the entry of a virus into at least one cell of a host and its replication within the at least one cell. An infection may be acute (i.e. active) or, as e.g. in the case of CMV, also latent (i.e. inactive, hidden, dormant). In an acute infection, the virus is replicating, infects cells and potentially causes symptoms, whereas in a latent infection, the virus does not replicate independent from the host cell genome and infect further cells, it rather "hides" in a cell. A latent infection can be interrupted by acute infections in which the hidden virus starts replicating and infecting further cells. In the case of a pre-existing latent infection, the use in preventing of an infection preferably relates to preventing an acute infection by preventing the hidden virus from infecting further cells, i.e. from spreading. In other words, this can be described as a treatment of a latent infection, wherein the treatment is not curative (but keeps the virus in check).

The term "infectious disease" as used herein refers to a disease resulting from an infection. In the context of a viral infection, the infectious disease is a viral infectious disease, i.e. a disease resulting from a viral infection (primary viral disease) or a "virus-associated diseases", which is a related (secondary) disease that is caused or contributed to by a viral infection. For instance, the virus-associated disease, e.g. a CMV-associated disease, is selected from the group consisting of retinitis, encephalitis, ventriculitis, hepatitis, nephritis, cystitis,

myocarditis, pancreatitis, esophagitis, colitis, pneumonia, atherosclerosis, neonatal infection sequelae, transplant rejection, mucoepidermoid carcinoma, glioblastoma, prostate, breast and colon cancer, a cardiovascular disease, a gastrointestinal disease, an acute or chronic inflammatory disease (including e.g. rheumatoid arthritis, and an age-related disease (including e.g. diabetes, particularly of type 2, immunosenescence and cognitive impairment).

Transmission of a virus to a fetus, i.e. an intrauterine infection, may occur when the mother is infected (or has an acute infection) with the virus during pregnancy and can be prevented. This includes an infection of the membranes surrounding the fetus, of the umbilical cord, of the amniotic fluid, and of the fetus itself. Infections can be through the vagina, the cervix, the fallopian tubes, invasive procedures such as amniocentesis, or through the placenta (transplacental infection).

Prevention or treatment of virus infection can also prevent maternal pregnancy complications including preeclampsia. Adverse pregnancy outcomes that can be due to preeclampsia include stillbirth, neonatal death, intrauterine growth restriction and preterm birth, and can therefore also be prevented.

Children born with a viral infection (congenital infection) may have one or more of the following secondary disorders ("neonatal sequelae"): hepatomegaly, splenomegaly, jaundice, pneumonitis, fetal growth retardation, petechiae, purpura, thrombocytopenia and/or major neurological sequelae including microcephaly, intracranial calcifications, sensorineural hearing loss (SNHL), vision loss, optic atrophy, strabismus, chorioretinitis, intellectual disabilities, motor disabilities, and/or seizure disorders. Thus, preventing transmission of virus to a fetus is highly desirable to prevent these secondary disorders. It is envisioned that the virus-decoying medicament used in the mother prevents virus transmission by inhibiting entry of the virus into the fetus.

The cancer is generally one which is adversely oncomodulated by the viral infection, with respect to CMV infection, e.g. glioblastoma or other cancers including but not limited to colon cancer, prostate cancer and breast cancer. This is based on known oncomodulatory and malignancy increasing effects of viral infection, including CMV infection, of tumour cells.

The transplant rejection can be an acute or chronic rejection. It is known that infection with pathogens including viruses, specifically CMV, increases morbidity and decreases transplant survival, and that control of the infection can control the transplant rejection. An acute rejection usually occurs any time from the first week to 3 months after the infection, and in a preferred embodiment, the medical use according to the invention prevents an acute rejection. A chronic rejection can take place over several years, during which the transplant is

slowly damaged. A chronic rejection usually comprises several episodes of acute rejections, and it is preferred that the medical use according to the invention treats a chronic rejection.

The route of administration is not particularly limited and can for example be oral, intravenous or subcutaneous.

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In a tenth aspect, the present invention relates to a polypeptide comprising a fragment of PDGFRα, wherein the fragment consists of a PDGFRα D2 domain and a PDGFRα D3 domain. The polypeptide comprising the fragment may further comprise other polypeptides which are not domains of. PDGFRα. In a preferred embodiment, the fragment comprises the 139 and 206 mutations. Preferably, the polypeptide is a decoy receptor. Parts of this aspect of the invention are further a fusion protein comprising the polypeptide, a multimer comprising the fusion protein, a nucleic acid encoding for the polypeptide, a vector comprising the nucleic acid, a cell comprising the nucleic acid or the vector, a pharmaceutical composition comprising the polypeptide, fusion protein, multimer, nucleic acid, vector or cell, and their (and of the polypeptide of the tenth aspect) use for binding a virus, specifically for use in medicine. All definitions and embodiments described above with regard to the first to ninth aspect apply here, not necessarily limited to the 206 and 139 mutations, in as far as they are applicable.

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Definitions and further embodiments of the invention

20 The specification uses a variety of terms and phrases, which have certain meanings as defined below. Preferred meanings are to be construed as preferred embodiments of the aspects of the invention described herein. As such, they and also further embodiments described in the following can be combined with any embodiment of the aspects of the invention and in particular any preferred embodiment of the aspects of the invention described above.

25 The terms "platelet-derived growth factor receptor α", "PDGFRα" or "PDGFRA" are used interchangeably herein. PDGFRα is further known as “CD140a”. The human *PDGFRA* gene, which is located on the long arm of chromosome 4 (4q12), encodes a 1089 amino acid protein precursor (platelet-derived growth factor receptor alpha isoform 1 precursor, SEQ ID NO: 1), which is processed into a mature polypeptide consisting of amino acids 24-1089. The

30 extracellular portion of PDGFRα interacts with ligands of the platelet-derived growth factor (PDGF) family, in particular with the homo- or heterodimers PDGF-AA, -AB, -BB, and -CC.

The term “fusion protein” relates to a protein comprising two or more polypeptides, preferably functional domains, derived from different proteins. The two or more polypeptides are linked directly or indirectly by peptide bonds. A fusion protein is generated by joining two

or more nucleic acid sequences. This can be done recombinantly and also via nucleic acid synthesis. Translation of this fusion construct results in a single protein with the functional properties derived from the two or more polypeptides.

The expression “Fc domain” as used herein refers to the crystallisable fragment of the constant region of an antibody. Suitable Fc domains may be derived from IgG, IgA, IgD or IgM antibody isotypes.

Knob-into-hole mutations are amino acid substitutions in order to create a “knob” on one CH3 domain and a “hole” on the other CH3 domain. Various knob-into-hole mutations are known in the art, for instance the knob is represented by a tyrosine (Y), whereas the hole is represented by a threonine (T). In particular, knob-into-hole mutations are T366Y in one CH3 domain and Y407T in the other, wherein the two CH3 domains are IgG1 constant domains, and optionally wherein the Fc region comprising the T366Y mutation ("knob" chain) further comprises the mutations S354 and T166W and the Fc region comprising the Y407T mutation ("hole" chain) further comprises the mutations Y349C, T366S, L368A and Y407V.

The term "variant" is, with respect to polypeptides, to be understood as a polypeptide which differs in comparison to the polypeptide from which it is derived by one or more changes in the amino acid sequence. The polypeptide from which a protein variant is derived is also known as the parent polypeptide. The changes in the amino acid sequence may be amino acid exchanges, insertions, deletions, N-terminal truncations, or C-terminal truncations, or any combination of these changes, which may occur at one or several sites. Amino acid exchanges may be conservative (preferred) or non-conservative. A “variant” herein is characterized by a certain degree of sequence identity (as provided above) to the parent polypeptide from which it is derived.

The term "identity" or "identical" in the context of sequences refers to the number of residues in the two sequences that are identical when aligned for maximum correspondence. Specifically, the percent sequence identity of two sequences is the number of exact matches between two aligned sequences divided by the length of the shorter sequence and multiplied by 100. Alignment tools that can be used to align two sequences are well known to the person skilled in the art and can, for example, be obtained on the World Wide Web, e.g. Needle (EMBOSS) (https://www.ebi.ac.uk/Tools/psa/emboss_needle/), MUSCLE (http://www.ebi.ac.uk/Tools/msa/muscle/), MAFFT (http://www.ebi.ac.uk/Tools/msa/ mafft/) or WATER (http://www.ebi.ac.uk/Tools/psa/ emboss_water/). The alignments between two sequences may be carried out using default parameters settings, e.g. for Needle preferably MATRIX: BLOSUM62, Gap Open: 10.0, Gap Extend: 0.5, for MAFFT preferably: Matrix:

Blosum62, Gap Open 1.53, Gap Extend 0.123, for WATER polynucleotides preferably: MATRIX: DNAFULL, Gap Open: 10.0, Gap Extend 0.5 and for WATER polypeptides preferably MATRIX: BLOSUM62, Gap Open: 10.0, Gap Extend: 0.5. Those skilled in the art understand that it may be necessary to introduce gaps in either sequence to produce a satisfactory alignment. The "best sequence alignment" is defined as the alignment that produces the largest number of aligned identical residues while having a minimal number of gaps. Preferably, it is a global alignment, which includes every residue in every sequence in the alignment.

The term "nucleic acid" includes RNA and DNA, but preferably refers to DNA.

The term “vector” as used herein includes any vectors known to the skilled person including plasmid vectors, cosmid vectors, phage vectors such as lambda phage, viral vectors such as adenovirus (Ad) vectors), adeno-associated virus (AAV) vectors, alphavirus vectors (e.g., Venezuelan equine encephalitis virus (VEE), sindbis virus (SIN), semliki forest virus (SFV), and VEE-SIN chimeras), herpes virus vectors, measles virus vectors, pox virus vectors (e.g., vaccinia virus, modified vaccinia virus Ankara (MVA), NYVAC (derived from the Copenhagen strain of vaccinia), and avipox vectors: canarypox (ALVAC) and fowlpox (FPV) vectors), and vesicular stomatitis virus vectors, or virus like particles. As used herein, the term "virus-like particle" or "VLP" refers to a non-replicating, empty viral shell. VLPs are generally composed of one or more viral proteins, such as, but not limited to those proteins referred to as capsid, coat, shell, surface and/or envelope proteins. They contain functional viral proteins responsible for cell penetration by the virus, which ensures efficient cell entry. Methods for producing particular VLPs are known in the art.

The term "prokaryotic cell" as used herein refers to any kind of bacterial organism suitable for application in recombinant DNA technology such as cloning or protein expression includes both Gram-negative and Gram-positive microorganisms. Preferred is *Escherichia* (in particular *E. coli*).

A eukaryotic cell is in particular a yeast or an animal cell. A yeast cell can be, in the broadest sense, any cell of a yeast organism. Preferably, the yeast cell is a *Saccharomyces* cell, in particular a *Saccharomyces cerevisiae* cell.

An animal cell may be a cell of a primate, mouse, rat, rabbit, dog, cat, hamster, cow, insect (e.g. Sf9 or Sf21) etc., preferably a human. Also, it may be a suspension or an adherent cell. In one embodiment, the adherent cell is a monolayer cell. For example, the cell is selected from the group consisting of a hybridoma cell, a primary epithelial cell, an endothelial cell, a keratinocyte, a monocyte/macrophage, a lymphocyte, a hematopoietic stem cell, a fibroblast, a

chondrocyte and a hepatocyte. More specifically, it may be selected from the group consisting of a CHO-K1 SV cell, a CHO DG44 cell, a CHO DP-12 cell, a CHO DHFR$^-$ cell, a CHO-GS cell, a BHK-21 cell, a HEK-293 embryonic kidney cell, a HeLa cervical epithelial cell, a PER-C6 retinal cell, an MDCK cell, an HDMEC cell, a HepG2 cell, an HL-60 cell, an HMEC-1 cell, a HUVEC cell, an HT1080 cell, a Jurkat cell, an MRC5 cell,a K562 cell, a HeLa cell, an NS0 cell, an Sp20 cell, a COS cell, and a VERO cell.

The term “pharmaceutically acceptable carrier” refers to any substrate which serves to improve the selectivity, effectiveness, and/or safety of drug administration. Such carriers can be used to control the release of a drug into systemic circulation. This can be accomplished either by slow release of the drug over a long period of time (typically diffusion) or by triggered release at the drug's target by some stimulus, such as changes in pH, application of heat, and activation by light. Carriers can also be used to improve the pharmacokinetic properties, specifically the bioavailability, of many drugs with poor water solubility and/or membrane permeability. A wide variety of drug carrier systems have been developed and studied. Examples include liposomes, polymeric micelles, microspheres, nanoparticles, nanofibers, protein-drug conjugates, erythrocytes, virosomes and dendrimers. Different methods of attaching the drug to the carrier can be used, including adsorption, integration into the bulk structure, encapsulation, and covalent bonding.

Various modifications and variations of the invention will be apparent to those skilled in the art without departing from the scope of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in the relevant fields are intended to be covered by the present invention.

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The invention is described by way of the following examples which are to be construed as merely illustrative and not limitative of the scope of the invention.

EXAMPLES

Materials and Methods

Human cells, sera and viruses

Primary human foreskin fibroblasts (HFFs) were obtained anonymized from residuals of routine procedures. Written informed consent was given by the parents of the donors in agreement with the recommendations of the council of Europe (articles 21 and 23, year 2006). Propagation of the cells was performed in MEM supplemented with GlutaMAX (Life Technologies), 5 % fetal bovine serum (FBS, PAN Biotech), 0.5 ng/ml basic fibroblast growth factor (bFGF) and 100 μg/ml gentamicin. For use in experiments the cells were kept in maintenance medium without bFGF. Serum starvation was performed in MEM with GlutaMAX but without serum or other supplements.

HEK 293 F suspension cells were propagated in 293 Freestyle medium (Thermo) supplemented with 100 μg/ml gentamicin under 8% CO2 and agitation at 130 rpm to a maximal density of 3×10^6 cells/ml. Human sera were obtained from adult healthy seronegative donors.

All virological experiments were performed with HCMV TB40-BAC4-IE-GLuc, a BAC-cloned reporter virus based on strain TB40/E that expresses Gaussia luciferase under control of the major immediate early (IE) promotor/enhancer (Falk et al. 2016, Journal of Virological Methods 235 (September): 182–89). Virus stocks were harvested 5 to 7 days after infection of HFFs. The virus was cleared from cells and cell debris by centrifugation for 10 min at 2,700 × g. To additionally remove the luciferase released by the producer cells, the virus particles were ultra-centrifuged for 70min at 100,000×g. The virus pellet was immediately resuspended in medium and stored at -80°C until used.

Cloning of PDGFRα-Fc mutants

The extracellular domain (corresponding to aa 24 to 528) of PDGFRα (CD140a) has been cloned previously into pFuse-hIgG1-Fc2 as described previously (Stegmann et al. 2019, Journal of Virology 93 (11)). Mutagenesis primers were designed using the Agilent primer design tool, because the mutagenesis was performed using the QuikChange Lightning site-directed mutagenesis kit (Agilent). In short, the template plasmid was amplified with primers containing the desired mutation, then the template DNA was digested with Dpn I and transformed into XL10 chemically competent cells. Sanger sequencing of the PDGFR sequence was performed to exclude unwanted secondary changes.

Expression and purification of soluble PDGFRα-Fc

Soluble PDGFR-alpha-Fc fusion proteins were transiently expressed from HEK 293 cells. One day prior to transfection, 293 cells were diluted to 5×10^5 cells per ml in gentamycin-free medium. For transfection, 1 μg of sterile DNA and 3 μg of polyethylenimine (PEI, 25K linear, Polysciences #23966) per 10^6 cells were mixed in serum-free Opti-MEM (Thermo) and incubated for 15min before the mixture was added to the cells. Incubation for 6 hours, then a medium exchange was performed. The PDGFRα-Fc containing medium was harvested at day 5 or 6 after transfection by pelleting the cells at 2,700×g for 10 min. To remove smaller cell debris, the supernatants were filtered through a 0.22 μm filter prior to concentrating the proteins with centrifugal filters pore size 100kDa (Amicon). The PDGFR-Fc fusion proteins were then purified using Protein A beads. The proteins were eluted from the beads with an elution buffer of pH 2.8 (Thermo #21004) and stored in the same buffer supplemented with 0.1 M Tris pH 8.0 at 4°C. The purified proteins were quantified photometrically and for additional quality control, all protein preparations were checked by gel electrophoresis followed by visualization using TCE (2,2,2-trichloroethanol).

HCMV inhibition Assay

One day prior to infection, HFFs were seeded on 96well plates at a density of 15,000 cells per well. The next day, HCMV TB40-BAC4-IE-GLuc virus stocks were diluted to obtain a final infection rate of about 50% in the untreated sample. These virus dilutions were then mixed with either only maintenance medium (untreated control) or with serial dilutions of PDGFRα-Fc proteins. The virus-inhibitor mixtures were incubated for 2 h at 37°C, before they were added to the cells for infection. One day later the percentage of inhibition was determined either by quantification of Gaussia-luciferase activity in the supernatants or by immunofluorescence staining of the cells.

The use of Gaussia luciferase for quantification of infection has been described previously (Falk et al. 2016, Journal of Virological Methods 235 (September): 182–89). Briefly, the Gaussia luciferase-containing cell culture supernatants were either stored at -20°C or mixed immediately with the luciferase substrate coelenterazine (PjK GmbH). Coelenterazine was diluted to 0.2 μg/ml in phosphate buffered saline with 5 mM NaCl. The substrate was added to the cell culture supernatants automatically in a plate reader (Hidex Chameleon) to ensure timely measurement of the luminescence signal. The percentage of infection was calculated from the resulting luminescence signals by normalization to the untreated virus control.

For quantification of infection by immunofluorescence, the cells were fixed with 80% acetone for 5 min at room temperature, before sequential incubation with an antibody against the viral immediate early antigens (E13, Argene) and secondary antibody goat anti-mouse IgG F(ab')^2-Cy3 (Jackson ImmunoResearch). Cell nuclei were stained with 4′,6-Diamidin-2-
5 phenylindol (DAPI). Quantification was performed using Zen software (Zeiss).

Quantification of Akt phosphorylation

Stimulation of cellular signaling: HFFs were seeded at a density of 100,000 cells per well in maintenance medium onto 24well plates. About 4 hours after seeding, when the cells
10 had adhered to the plates, a medium exchange was performed and the FBS-containing medium was replaced by MEM without any additives. The cells were kept in serum- and growth factor-free medium for 1 day. Prior to stimulation of the cells, PDGF-BB (R&D # 220-BB) was diluted in MEM to 12 ng/ml and then mixed 1:1 with different concentrations of PDGFRα-Fc, resulting in a final PDGF concentration of 6 ng/ml. For stimulation with human sera, the sera were diluted
15 to a final concentration of 5%. Ligand/Serum and receptor were preincubated at 37°C for 2h. Then the mixture was added to the serum starved HFFs and incubated for 15min at 37°C to allow signaling, cumulating in Akt phosphorylation. The stimulation was terminated by lysis of the cells for either immunoblot analysis or ELISA. Please note that the protocol described here was optimized for increased sensitivity. The first experiments using alanine exchange
20 mutants (Fig. 2) had been performed under slightly different conditions, using 50 ng/ml of PDGF-BB and stimulating the cells for 2h.

Detection of Akt phosphorylation by immunoblot: The cells were lysed in laemmli lysis buffer with β-mercaptoethanol on ice, then scraped off the plate and boiled for 10 min at 95°C. An equivalent of 28,500 cells was loaded onto 10% polyacrylamide gels and electrophoresis
25 was performed in tris glycine SDS buffer. The proteins were transferred onto PVDF membranes in tris-glycine buffer with 15% methanol. Membranes were blocked with TBS plus 0.1 % Tween and 5 % milk powder before staining with rabbit anti-phospho-Akt (Cell Signaling #4060) and rabbit anti-actin (Sigma # A 5060). Horseradish peroxidase (HRP)-conjugated goat anti-rabbit (Santa Cruz #sc-2054) was used as secondary antibody. Visualization and
30 Quantification of the signals was performed with Super Signal West Dura Extended Duration substrate (Thermo) using FusionCapt Advance Solo (v.7 Vilber Lourmat). Phospho-Akt and actin signals were quantified from the same membranes to allow normalization of the Phospho-Akt signal to the corresponding actin signal in order to account for slight differences in loading.

Detection of Akt phosphorylation by ELISA: The PathScan Phospho-Akt1 (Ser473) Sandwich ELISA Kit (Cell Signaling #7160) was used for detection of Akt phosphorylation according to the manufacturer's instructions. In short, the cells of each well were lysed with 150 µl of ice-cold cell lysis buffer containing 1 mM phenylmethylsulfonyl fluoride for 5 min on ice. Then the lysate was homogenized using a syringe before the insoluble parts were removed by centrifugation for 5 min at 18,800×g. The samples were stored at -80°C until the assay was performed. 100 µl of undiluted sample per well was added to a microwell plate coated with phospho-Akt (Ser473) rabbit antibodies. After washing off unbound lysate, the samples were incubated subsequently with Akt1 mouse antibody and HRP conjugated anti-mouse antibody. Detection was performed at 450 nm in a plate reader (Hidex Chamaeleon) after incubation of the sample with TMB substrate for 10 min.

Quantification of binding affinities

Binding affinities were quantified using Monolith NT.115 (NanoTemper) instrumentation and analysis was performed using inbuild MO.Affinity Analysis Software. To allow for fluorescence detection, PDGF-BB (R&D # 220-BB) was labelled with NT-647 using the amine reactive protein labelling kit RED-NHS (NanoTemper) according to the manufacturer's instructions. For all measurements, PDGF-BB was used at a constant concentration of 0.1 nmol/l. To allow for proper curve fitting, the PDGFRα-Fc variants were added in 16 different concentrations spanning 5log steps. It was observed that with all tested PDGFRα-Fc variants, except I139E + Y206S, the initial fluorescence was reduced in a concentration dependent manner. Therefore, direct fluorescence analysis mode was chosen to compare the change in fluorescence over time between different PDGFRα-Fc concentrations. For best signal to noise ratio, an initial 5 seconds of photobleaching was performed prior to fluorescence measurement over 7 seconds. The resulting change in fluorescence per second for each concentration was analyzed using the fluorescence analysis mode to perform curve fitting for calculation of the respective dissociation constants (K_D). In case when the initial fluorescence of PDGF-BB was not significantly changed upon addition of the receptor (PDGFRα-Fc I139E + Y206S), a standard microscale thermophoresis (MST) measurement was used to calculate the K_D for PDGF binding. For this, after 5 seconds of photobleaching, the sample was heated and the change of fluorescence was recorded for 7 seconds. To generate dose response curves, the normalized difference between the steady state (4 sec after heating (F1)) and the initial fluorescence (F0) was plotted against the respective PDGFRα-Fc

concentration to calculate the K_D. All experiments were performed at 25°C, medium MST power and with 60% excitation power.

Statistics

Data sets were compared to test for significant differences using the t.test function of Excel. All tests were performed under two-sided hypotheses. Statistical significance was assumed for $p \leq 0.05$.

Example 1: Deletions of predicted PDGF binding sites in PDGFRα-Fc differentially affect HCMV inhibition

As a starting point for the identification of sites that are essential for ligand binding but dispensable for HCMV inhibition, a set of seven small deletions was introduced at the respective sites in domains two and three of PDGFRα in the background of the Fc fusion vector pFuse-PDGFRα-ECD-hIgG1-Fc2 (Fig. 1A, Stegmann et al. 2019, Journal of Virology 93 (11)) based on a computational prediction of amino acids in the extracellular domain of PDGFRα that are likely to be important for binding to PDGF-AA and PDGF-BB. The soluble receptor mutants were expressed in 293F cells and affinity purified using Protein A. To identify promising sites for further optimization, the capacity of the PDGFRα-Fc mutants to inhibit HCMV infection was assessed with a standard neutralization assay utilizing a Gaussia luciferase reporter virus (TB40-BAC4-IE-GLuc, Falk et al. 2016, Journal of Virological Methods 235 (September): 182–89). Cell- and luciferase-cleared preparations of the virus were diluted to a MOI of ≤ 1 (resulting in less than 68% infection) and preincubated with various concentrations (4,000 to 1 ng/ml) of purified PDGFRα-Fc. After an incubation period of 2 hours at 37°C, the virus was incubated with the fibroblasts for 2 hours. One day later, the luciferase-containing medium was collected and the Gaussia activity therein was measured. The extent of infection initiated by the pretreated virus was quantified relative to untreated control samples.

Wild type PDGFRα-Fc inhibited HCMV at a halfmaximal effective concentration (EC50) of <20 ng/ml and fully blocked HCMV inhibition already at 100 ng/ml. All deletions reduced the inhibitory effect of PDGFRα-Fc to some extent, as indicated by a shift of the dose-response towards higher concentrations (Fig. 1B). With four out of seven mutations a concentration of 4,000 ng/ml was insufficient to achieve full inhibition. Yet, PDGFRα-Fc deletion mutants delM133-I139, delN204-Y206 and delQ294-E298 completely blocked HCMV entry at 2000 ng/ml (Fig. 1C). When compared with wild type PDGFRα-Fc, the EC50s of these mutants were about 10fold increased to 100-300 ng/ml. These results indicated that the

predicted PDGF binding sites M133-I139, N204-Y206 and Q294-E298 contribute also to some extent to HCMV binding but are in principle dispensable for neutralization of the virus.

With regard to the binding site for HCMV in PDGFRα, the data provide evidence that domains 2 and 3 of PDGFRα are involved. This is partially discrepant from a previous study that has investigated the effect of large deletions within PDGFRα on its interaction with HCMV. In this study the ability of different PDGFRα mutants to support HCMV infection was tested and domain 2 of PDGFRα was found to be dispensable for the virus to use PDGFRα as a receptor for entry (Wu et al. 2018, PNAS 115 (42): E9889–98). In contrast, in our screening of small deletion mutants, certain sites within domain 2 (M133-I139 and V184-G185) impaired the interaction between PDGFRα and gH/gL/gO. These slightly different results regarding the importance of domain 2 for interaction with HCMV can be explained by the different experimental systems. Wu et al. tested the impact of mutations on the ability of cellular PDGFRα to support HCMV entry. In this case additional interactions between virus and cell surface could help to stabilize the gH/gL/gO-PDGFRα interaction, e. g. by binding of other glycoproteins to heparan sulfate proteoglycans. In contrast, in a neutralization assay the virus-receptor interaction can dissociate more freely, which makes it a more stringent assay for detecting mutations that destabilize virus binding.

Example 2: Mutations of isoleucine 139 and tyrosine 206 in PDGFRα-Fc reduce sequestration of PDGF-BB

Based on the identification of PDGF binding sites that are dispensable for HCMV inhibition, a further scanning of the amino acid positions within these sites was performed. For this second screening step, we considered only amino acids that were a) included in deletions that were compatible with full inhibition of HCMV infection and b) predicted to be involved in binding of PDGF-A and PDGF-B. These amino acids were M133, I139, N204, Y206, Q294, T296 and E298. An alanine exchange approach was chosen in order to avoid gross structural changes in the protein. The seven alanine exchange mutations were introduced into PDGFRα-Fc and the purified soluble receptor mutants were tested regarding their ability to inhibit HCMV infection. The shift in dose-dependent inhibition of HCMV by these more subtle mutations were less pronounced as compared to those of the deletions (Fig. 2A and 2B). Notably, both slightly enhanced and slightly reduced HCMV neutralization was observed. All three mutations in the peptide site 294-298 slightly increased the inhibitory effect of PDGFRα-Fc about 3fold. On the other side, mutation of isoleucine 139 and tyrosine 206 slightly decreased the efficiency of HCMV inhibition by about 4fold. Mutation of asparagine 204 and methionine 133 did not

change the EC50. Overall, the impact on the HCMV neutralization capacity of PDGFRα-Fc was mild for all alanine exchange mutants.

The next step was to test whether any of those mutations would reduce interference with cellular signaling. To assess the risk of such a potential side effect, a biological assay was preferred over biochemical measurements of protein-protein interaction. The effect of soluble PDGFRα-Fcs on PDGF-dependent signaling in cells was measured directly. PDGFRα can bind to a variety of different growth factors including PDGF-A, PDGF-B and PDGF-C, which will start signaling cascades that include phosphorylation of Akt. Therefore, the effect of soluble PDGFRα-Fc on Akt phosphorylation was chosen as a downstream measure of changes in binding to growth factors. The experiments were performed with PDGF-BB as the cellular response to PDGF-BB has been shown to be more pronounced than to PDGF-A or -C. Dimeric PDGF-B at a final concentration of 50 ng/ml was preincubated with different concentrations of PDGFRα-Fc wild type and mutants for 2 hours at 37°C before stimulation of fibroblasts. To reduce background phosphorylation, the cells were serum-starved prior to addition of the PDGF-B/PDGFRα-Fc mixtures. After stimulation for 90 min, the cells were lysed and Akt phosphorylation was determined in immunoblots (Fig. 2C). It was observed that those mutations that reduced inhibition of HCMV also reduced sequestration of PDGF-B. At the highest concentration tested in these experiments (4000 ng/ml), wild type as well as five of the seven alanine-exchange mutants caused full interference. PDGF-dependent phosphorylation of Akt was reduced close to background level (Fig. 2D). In contrast, preincubation of PDGF-BB with 4,000 ng/ml of PDGFRα-Fc I139A and Y206A reduced Akt phosphorylation by only 20 % and 60 %, respectively. These results demonstrated that mutation of isoleucine (I) 139 and tyrosine (Y) 206 strongly mitigate the negative effect of PDGFRα-Fc on Akt phosphorylation which indicates that these mutations can reduce the sequestration of PDGFs by PDGFRα-Fc.

Example 3: Selected permutations at positions 139 and 206 of PDGFRα improve the interaction profile of the soluble receptor

To identify amino acid exchanges that would have the reduced PDGF sequestration the most and HCMV inhibition the least, a hypothesis driven permutation at the positions 139 and 206 was conducted. It was assumed that binding to PDGFs may be disrupted by changing either size or charge of the side chains of I139 and Y206 and that change of these characteristics might differentially affect the ability of PDGFRα-Fc to sequester PDGFs and to neutralize HCMV. Isoleucines are often found at internal positions within the protein with their non-polar side chains involved in formation of beta sheets. As it was not clear whether the interaction with the

HCMV glycoprotein complex gH/gL/gO requires structural integrity at this position or not, three amino acid exchanges with varying theoretical outcomes were tested. Isoleucine to Valine exchange should maintain the non-polar character of the site, yet due to the smaller side chain might preclude hydrophobic interactions that are regarded important for PDGFRα-PDGF binding. Isoleucine to glutamic acid exchange in contrast does not change the size much but alters it´s biochemical characteristic to polar and preferentially surface exposed, potentially disrupting the structural integrity. We also exchanged isoleucine with leucine which is a very mild change, only affecting the stereoisomerism of the side chain. To evaluate the effects that these single amino acid exchanges have on the interactions of soluble PDGFRα-Fc, the recombinant proteins were expressed and tested regarding their ability to inhibit HCMV infection and their impact on PDGF-dependent signaling (Fig. 3).

For measurement of HCMV inhibition the virus was preincubated with the soluble receptors before incubation with the cells for 1 day. To obtain even more precise data, infection was measured by immunofluorescence staining for the viral immediate early antigens instead of by luciferase activity. Replacement of isoleucine 139 with leucine or valine had no or only a marginal effect as compared with wild type PDGFRα-Fc (Fig. 3 A and B). The replacement with glutamic acid decreased the efficiency of HCMV inhibition 5fold, comparable to the alanine exchange mutant. For measurement of the effect on PDGF sequestration, the protocol of the assay was also adjusted to obtain higher sensitivity which was important because the alanine exchange mutants had already reduced PDGF sequestration close to the limits of detection. As a basis for optimization, the dose-response relationship of cellular Akt phosphorylation after treatment of cells with different concentrations of PDGF-B was determined (Fig. 4). A concentration of 6 ng/ml PDGF-B (about its concentration in human blood) was found to result in strong Akt phosphorylation below saturation, increasing the assays sensitivity by limiting the amount of stimulant. It is important to note that consistently strong Akt phosphorylation after treatment with 6 ng/ml serumB was only achieved when the time of stimulation was reduced from 1.5 hours to 15 min, possibly due to cellular feedback mechanisms. This improved protocol was used to test the I139 mutants regarding their inhibitory effect on PDGF-dependent Akt phosphorylation (Fig. 3 C and D). The results of these analyses showed that replacement of I139 with glutamic acid decreased PDGF sequestration even beyond the effect of the alanine exchange mutation. Measurement of immunoblot signal intensities of p-Akt after treatment of 6 ng/ml PDGF-B with 4000 ng/ml of PDGFRα-Fc I139E showed only about 50% reduction in signaling, while the I139A mutant reduced the signal by about 80% when tested under these more sensitive conditions.

To validate the results obtained by the phospho-Akt immunoblots, the impact of the soluble receptors on cellular signaling was additionally tested with a commercially available phospho-Akt ELISA. The pretreatment of PDGF-BB was performed as for the immunoblot readout. With this assay dose-response curves for the impact of the PDGFRα-Fc fusion proteins on PDGF-dependent phosphorylation of Akt were generated (Fig. 3E). While wild type PDGFRα-Fc blocked Akt phosphorylation at a concentration of 60 ng/ml, the I139E mutation decreased PDGF sequestration so strongly that full inhibition of Akt phosphorylation was not even observed at 36,000 ng/ml. It is also noteworthy that the slope of the curve for the PDGFRα-Fc I139E mutant was much more gradual, with a lower Hill coefficient, indicating that the dynamics of the competition between the soluble and the cellular receptors for the ligands has changed profoundly. In line with this, the EC50 of I139E for inhibition of PDGF signaling was significantly increased by 2 log steps from 13 ng/ml for the wild type molecule to about 2000 ng/ml for the I139E mutant (Fig. 3F). It is noted that these numbers may not be completely precise due to the incomplete inhibition that precludes a very precise calculation of the EC50 for I139E. We also compared the effect of the PDGFRα-Fc variants on Akt phosphorylation at 562 ng/ml. This concentration is sufficient for full HCMV inhibition by PDGFRα wild type and I139E and therefore reflects a plausible concentration to aim at. PDGF-dependent Akt phosphorylation was almost completely blocked upon pretreatment with 562 ng/ml wild type PDGFRα-Fc, but only about 50% reduced with the I139E mutant. This demonstrated that I139E reduced PDGF sequestration but still interferes with cellular signaling. Taken together, these results demonstrate that a change of charge at position 139 by replacing isoleucine with glutamic acid decreases the ability of PDGFRα-Fc to sequester PDGFs and to a lesser degree the inhibition of HCMV.

The alanine exchange scanning had identified tyrosine 206 as another important site for interaction with PDGF-B. Therefore, analogous to the permutation strategy employed for isoleucine 139, the influence of size and charge at position 206 of PDGFRα-Fc on its interactions with HCMV and PDGFs was investigated. Tyrosines have polar, aromatic side chains that are often involved in stacking interactions. Phenylalanine similarly has an aromatic side chain that is however non-polar, whereas serine is polar but much smaller without an aromatic ring. As described for the I139 permutations, the Y206F and Y206S PDGFRα-Fc mutants were analyzed regarding inhibition of HCMV and interference with PDGF-dependent signaling. Pretreatment of HCMV with PDGFRα-Fc Y206F resembled the effect seen with the wild type protein, with regard to the overall dose response and the EC50 (Fig. 5 A and B). Exchange of tyrosine with serine (Y206S) reduced the EC50 for HCMV inhibition by 6fold as

compared to wild type, slightly more than the 4fold reduction caused by the alanine exchange at position 206. The same tendencies, yet to a much greater extent were also observed when the effect of those mutations on PDGF sequestration was measured (Fig. 5 C to F). Y206F did not improve interference as compared to the alanine exchange mutant, in contrast Y206S significantly decreased the inhibition of PDGF-dependent cellular signaling as compared to wild type and the alanine mutant. No inhibition of Akt phosphorylation at all was observed even at 36,000 ng/ml. The same was true when Akt phosphorylation was quantified by ELISA. It is noteworthy that PDGFRα-Fc Y206S, when applied at very high concentrations (greater than 4,000 ng/ml), slightly enhanced cellular signaling. Comparison at lower concentrations, that are more likely to be used against HCMV (i.e. 562 ng/ml), however allowed to quantify the difference between PDGFRα-Fc wild type and mutant. PDGFRα-Fc Y206S caused almost no reduction in Akt phosphorylation whereas wild type led to near complete inhibition of cellular signaling. As no actual dose dependent inhibition of cellular signaling was observed, no EC50s could be determined for this PDGF sequestration by PDGFRα-Fc. These results demonstrate that exchange of polarity at position 206 of PDGFRα-Fc strongly impairs the inhibition of cellular signaling while the efficiency of HCMV inhibition was only slightly reduced.

Example 4: Combination of the most promising mutations further decreases PDGF sequestration

After having established that the mutations I139E and Y206S both significantly decrease PDGF sequestration but have only minor impact on HCMV inhibition, we tested whether a combination of these mutations would have desirable additive effects. We hypothesized that combinations of the most promising mutations may further diminish the inhibitory effect of PDGFRα-Fc on cellular signaling, while having only mild effects on HCMV inhibition. Therefore, two more variants of PDGFRα-Fc were generated, a double alanine-exchange mutant (PDGFRα-Fc I139A+Y206A) and a PDGFRα-Fc I139E+Y206S double mutant. The double mutants were analyzed using the established assays for inhibition of HCMV and interference with PDGF-dependent cellular signaling (Fig. 5). Quantification of HCMV inhibition revealed that both combinatory mutations slightly decreased the inhibitory effect on HCMV as compared to wild type PDGFRα-Fc, but not as compared to the respective single amino acid exchange mutants (Fig. 6 A + B, Table 1). Double alanine exchange resulted in a 4fold increased EC50 for HCMV inhibition as compared to wild type while combination of I139E and Y206S resulted in a 5fold increase, to 20 and 25 ng/ml, respectively. In both cases

the increase of the EC50 for inhibition of HCMV was very similar as compared to the individual mutations which also resulted in about 5fold decreased EC50s (compare Fig. 6B with 3B and 5B). When tested for inhibition of PDGF-dependent cellular signaling (Fig. 6 C + D), both double mutants did not inhibit Akt phosphorylation at all, even when applied at 36,000 ng/ml.
5 It was noticeable however that while the I139A + Y206A mutant did not have any effect on cellular signaling, the I139E + Y206S mutant induced an increase in signaling when used at high concentrations, a phenomenon that was already observed for the Y206S single amino acid exchange mutant. The reason remains unclear, but the effect was independent of PDGFs. Importantly, when used at concentrations that promote full neutralization of HCMV, PDGFRα-
10 Fc I139E + Y206S did neither enhance nor reduce cellular signaling induced by human serum. Because PDGFRα-Fc already inhibits HCMV potently at low concentrations it is not necessary to use high concentrations that can lead to unspecific effects on the cells and the same is true for PDGFRα-Fc I139E + Y206S. Furthermore, the inventors observed that this increase in signaling at high concentrations (about 50fold above the EC50 for HCMV inhibition) can be
15 avoided also by combining the Y206 substitution or the I139+Y206 substitutions with a V242 substitution such as V242K, see Example 6.

As a way to actually compare the binding affinities of PDGFRα-Fc wild type and the most promising mutants for PDGFs microscale thermophoresis (MST) was chosen. To allow measurement, PDGF-BB was fluorescently labelled with the dye NT-647. A constant
20 concentration (2.5 ng/ml = 0.1 nmol/l) of labelled PDGF-BB was mixed with various concentrations, covering 5 log steps, of PDGFRα-Fc. Already prior to heating of the sample, a dose-dependent decrease of fluorescence was observed (Fig. 7 A to C and Fig. 8 A to D), which can be explained by quenching of the ligand's fluorescence upon binding of the receptor. Using the dose-dependent decrease of PDGF fluorescence upon PDGFRα-Fc binding, a K_D of 0.14
25 nmol/l was calculated for the wild type receptor molecule. When full MST analysis was performed, comparing the fluorescence of PDGF bound by PDGFRα-Fc before and after sample heating, the same affinity of 0.14 nmol/l was determined (compare Fig. 7 D and F) which demonstrates that both readouts are equally valid. Based on these results the affinity of the PDGFRα-Fc mutants for PDGFs was determined using the direct change in fluorescence
30 over time when applicable. The single amino acid exchange mutants I139E and Y206S as well as the double alanine exchange mutant decreased the affinity for PDGF-BB by 30 to 50fold to 4.0, 7.3 and 4.6 nmol/l, respectively (Fig. 7 A to D). The combination of I139E and Y206S however did not cause any measurable change in PDGF-BB fluorescence, indicating loss of binding or strongly reduced affinity. To be able to distinguish between those two possibilities,

thermophoresis was performed to calculate the affinity of I139E + Y206S mutant for PDGF-BB (Fig. 7 E, F and Fig. 8 E, F). Evaluation of the MST signals demonstrated that this PDGFRα-Fc mutant interfered with the thermophoretic mobility of PDGFs only when used at very high concentrations of more than 10 nmol/l (1,600 ng/ml). The K_D calculated from the resulting dose response curve was 200 nmol/l, about 1500fold higher than that of wild type PDGFRα-Fc and 30- to 50fold higher as compared to the individual mutations.

Taken together, these experiments demonstrated that combination of selected mutations in PDGFRα-Fc acts synergistically to reduce the affinity for PDGFs while retaining the strong potency for HCMV inhibition. The combination of the two most promising individual mutations resulted in a strong synergism regarding PDGF binding which increased the selectivity index to about 1300, i.e. the concentration of 50% binding to PDGF is 1300fold higher than the concentration needed for 50% inhibition of HCMV. Not only was this synergism unexpected, but also the strength of the synergistic effect was entirely surprising to the inventors. This suggests that combined mutation of I139E + Y206S in PDGFRα-Fc renders this molecule inert with respect to human growth factors while maintaining its efficiency for HCMV neutralization. No alteration of cellular signaling was detectable at concentrations that completely blocked HCMV infection. With regard to interference with PDGFs, this is sufficient for safe application at concentrations that almost completely prevent HCMV entry, given that other unpredicted side effects can be excluded in further preclinical evaluations.

While the efficiency of HCMV inhibition was slightly decreased with PDGFRα-Fc I139E+Y206S as compared to the wild type molecule, with an EC50 of 25 ng/ml for fibroblast its potency is still higher than what has been reported for antibodies that are currently in clinical trial. Importantly, PDGFRα-Fc can be assumed to inhibit all target cell types of HCMV, because its viral target the gH/gL/gO complex is needed for infection of all cell types. This is not the case for pooled immunoglobulin preparations which are much less potent in neutralization of fibroblast infection than of infection of epithelial cells. Also, antibodies directed against gB or gH, which should in principle inhibit entry into different cell types similarly well, are often less effective against infection of fibroblasts, indicating that virus entry mediated by the gH/gL/gO trimer is less susceptible to neutralization. In contrast, PDGFRα-Fc inhibits infection of all cell types efficiently and is even more potent against fibroblast infection than infection of endothelial or epithelial cells

Table 1: Comparison of PDGFRα-Fc wild type and mutants regarding HCMV inhibition and PDGF affinity

	HCMV inhibition EC50 [nmol/l] (SEM)		PDGF binding K_D [nmol/l] (K_D confidence)	
PDGFRα-Fc wild type	0.031	(± 0.003)	0.14	(± 0.03)
PDGFRα-Fc I139E	0.198	(± 0.050)	4.02	(± 1.51)
PDGFRα-Fc Y206S	0.188	(± 0.022)	7.32	(± 3.13)
PDGFRα-Fc I139E+Y206S	0.152	(±0.008)	200	(± 0.74)

Note: 100 ng/ml of PDGFRα-Fc correspond to 0.604 nmol/l.

5 **Example 5: Serum-induced cellular signaling is unaffected by treatment with the modified PDGFRα-Fc**

To get a picture of the effect of PDGFRα-Fc variants on the naturally induced cellular signaling, the effect of PDGFRα-Fc variants was tested on cellular signaling induced by human serum. For this, a pool of human sera from 3 different donors was diluted in cell culture medium 10 to a final concentration of 5%. To test the effect of PDGFRα-Fc wild type and I139E + Y206S mutant, the serum pool was pretreated with 562 ng/ml of either PDGFRα-Fc soluble receptor for 2 hours before fibroblasts were stimulated for 15min. The effect of serum with or without pretreatment was assessed using a phospho-Akt ELISA (Fig. 9). Stimulation with 5% serum alone did induce a strong cellular response which was 50% reduced if the serum was pretreated 15 with PDGFRα-Fc wild type. In contrast, no inhibition of cellular signaling was observed when the serum was pretreated with PDGFRα-Fc I139E + Y206S. This demonstrates that the PDGFRα-Fc double mutant did not sequester any of the growth factors contained in the serum pool to a measurable extent. This set of experiments demonstrates that combination of the two most promising amino acid exchanges identified in this study is sufficient to abolish 20 interference of PDGFRα-Fc with cellular signaling.

It is noted with regard to the other examples testing PDGF-AA that the mutated residues were all predicted to be important for binding to PDGF-AA and PDGF-BB, but the less pronounced cellular response to PDGF-AA at physiological concentration precluded testing PDGF-AA sequestration in experiments analogous to those performed with PDGF-BB. The use 25 of serum circumvented this experimental problem as it can be presumed that a pool of sera contains a mixture of all the growth factors which together elicit a strong cellular response. The

lack of interference with serum-induced signaling indicates that the mutations abrogated sequestration of all relevant ligands, not only PDGF-BB.

All HCMV strains rely on PDGFRα as their receptor for entry into fibroblasts and it can therefore be expected that PDGFRα-Fc is less prone to development of resistance by the virus.

5 Taken together, the improved PDGFRα-Fc presented here is a promising molecule for neutralization of HCMV because it is can be expected to have desirable pharmacokinetics, it is most likely non-immunogenic, probably unlikely to induce resistance mutations, and it neutralizes various strains of HCMV and different cell types efficiently. It is conceivable that PDGFRα-Fc alone or in combination with anti-pentamer antibodies may be favorable as
10 compared to known antibody combinations. The identification of mutations which prevent interference with PDGFs now pave the way for further development of this decoy receptor against HCMV infection.

Example 6: Mutation of V242K in PDGFRα-Fc variants further increases the specificity
15 **of the soluble receptor**

To investigate the influence of further mutations, a set of PDGFRα-Fc mutants was generated: a V242K single mutant, as well as combinations of V242K with I139E and Y206S. Like with the previous PDGFRα-Fc mutants, all of them were expressed as efficient as wild type and did not show any signs of degradation or aggregation. After purification, these mutants
20 were first tested regarding their ability to inhibit HCMV infection.

The V242K mutation did not affect HCMV inhibition (Figure 10). Introduction of V242K into PDGFRα-Fc I139E or Y206S did also not significantly alter the efficiency of HCMV inhibition. The I139E + V242K mutant had an EC50 of 27 ng/ml as compared to 33 ng/ml for I139E, and Y206S + V242K caused half-maximal inhibition of HCMV at 47 ng/ml
25 as compared to 31 ng/ml with Y206S. To test for sequestration of PDGF by the mutants, PDGF-BB was pretreated with different concentrations of the PDGFRα-Fc mutants (2–500 ng/ml) and the effect on PDGF-dependent signaling was assessed with a p-Akt ELISA. As expected, based on the results with the previous combination of mutations, none of the PDGFRα-Fc V242K mutations caused any reduction in Akt phosphorylation.

30 To quantify the increase in specificity, the V242K mutations were included into the MST PDGF-BB binding experiments (Figure 11). Indicative of a strongly reduced affinity for PDGF-BB, no photobleaching was observed with the V242K mutant. Using 1000 nmol/l of PDGFRα-Fc V242K and 0.1 nmol/l of fluorescently labeled PDGF-BB, a Kd of 92 nmol/l was determined. Taken together with the unchanged efficiency for HCMV inhibition, this results in

an 657fold increase in specificity as compared to wild type. Based on the pronounced reduction in binding affinity observed with the combination of I139E and Y206S, it was likely that the combinations of V242K with I139E and/or Y206S would decrease the affinity for PDGF-BB even further. Therefore, the inventors adjusted the MST setup for higher MST sensitivity and used maximal available PDGFRα-Fc concentrations. Nonetheless, only for PDGFRα-Fc I139E + V242K a curve fit was possible, resulting in a roughly estimated Kd of 2,400 nmol/l. However, no distinct binding curves could be generated for any of the V242K combinations, indicating an extensive loss of binding affinity.

Furthermore, it is noteworthy that while PDGFRα-Fc Y206S alone and the combination with I139E both enhanced cellular signaling rather than reducing it when applied to cells at high concentrations, the combination with V242K avoided this effect.

Importantly, however, combinations of I139E and/or V206S with V242K reduced the affinity to PDGF-BB beyond the limit of detection, which is defined by the highest available PDGFRα concentrations (about 1 mg/ml). It can be assumed that interactions with affinities so low that they are not measurable at 1 mg/ml are highly unlikely to cause any biological interference. Even the least potent (regarding PDGF-BB affinity) of the combination mutants, PDGFRα-Fc I139E + Y206S + V242K, fully blocked HCMV infection at 2000 ng/ml, while no binding to PDGF was observed even at the highest concentration tested (720,000 ng/ml = 4.35×10^{-6} nmol/l). This demonstrates that concentrations higher than those tested are neither necessary nor reasonable.

Taken together, these findings further emphasize the potential of combined mutations in PDGFRα-Fc, as a way to generate a soluble receptor which is inert to its natural ligand.

CLAIMS

1. A PDGFRα polypeptide comprising (i) at least a PDGFRα D2 domain and a PDGFRα D3 domain, and (ii) mutations at positions 139 and 206 corresponding to the PDGFRα sequence according to SEQ ID NO: 1.

5

2. The PDGFRα polypeptide of claim 1, wherein the PDGFRα polypeptide comprises a functional virus binding domain, preferably a CMV binding domain.

3. The PDGFRα polypeptide of claim 1 or 2, wherein the mutations are substitutions, and
10 wherein the substitution at position 139 is to a polar amino acid and preferably is I139E, and/or the substitution at position 206 is a smaller or to a non-polar amino acid, and preferably is Y206S.

4. The PDGFRα polypeptide of any one of claims 1 to 3, wherein the PDGFRα polypeptide
15 further comprises (iii) a PDGFRα D1 domain, and optionally (iv) a PDGFRα D4 domain and/or a PDGFRα D5 domain.

5. The PDGFRα polypeptide of any one of claims 1 to 4, wherein the PDGFRα polypeptide lacks (a) at least a part of the transmembrane domain, preferably (b) the signal peptide, and
20 optionally (c) at least a part of the intracellular domain.

6. The PDGFRα polypeptide of any one of claims 1 to 5, wherein the PDGFRα polypeptide is a viral decoy receptor, preferably a CMV decoy receptor.

25 7. A fusion protein comprising the PDGFRα polypeptide of any one of claims 1 to 6.

8. The fusion protein of claim 7, wherein the fusion protein further comprises
(i) a moiety facilitating the crossing of the blood-brain-barrier (BBB moiety),
(ii) a moiety facilitating the crossing of the placenta (placenta moiety),
30 (iii) a multimerization moiety, preferably a dimerization moiety,
(iv) a half-life extending moiety, and/or
(v) an extracellular anti-viral agent (EAA), preferably an EAA active against CMV;
preferably an Fc domain.

9. The fusion protein of claim 7 or 8, wherein the fusion protein comprises an amino acid sequence according to positions 26-530 of SEQ ID NO: 2 and an amino acid sequence according to positions 533-759 of SEQ ID NO: 2, or variants of these amino acid sequences with at least 80% sequence identity to positions 26-530 and 533-759 of SEQ ID NO: 2.

10. A multimer, preferably a dimer, comprising at least two fusion proteins of any one of claims 7 to 9.

11. A nucleic acid or vector comprising a nucleic acid, wherein the nucleic acid encodes the PDGFRα polypeptide of any one of claims 1 to 6, the fusion protein of any one of claims 7 to 9, or one or more fusion proteins forming the multimer of claim 10.

12. A cell comprising the nucleic acid or the vector of claim 11, preferably wherein the cell secretes the PDGFRα polypeptide of any one of claims 1 to 6, the fusion protein of any one of claims 7 to 9 or at least two fusion proteins forming the multimer of claim 10.

13. A pharmaceutical composition comprising the PDGFRα polypeptide of any one of claims 1 to 6, the fusion protein of any one of claims 7 to 9, the multimer of claim 10, the nucleic acid or the vector of claim 11 or the cell of claim 12 and a pharmaceutically acceptable carrier, preferably wherein the pharmaceutical composition further comprises an anti-viral agent, more preferably a CMV anti-viral agent.

14. The PDGFRα polypeptide of any one of claims 1 to 6, the fusion protein of any one of claims 7 to 9, the multimer of claim 10, the nucleic acid or the vector of claim 11, the cell of claim 12 or the pharmaceutical composition of claim 13 for use in medicine.

15. The PDGFRα polypeptide of any one of claims 1 to 6, the fusion protein of any one of claims 7 to 9, the multimer of claim 10, the nucleic acid or the vector of claim 11, the cell of claim 12 or the pharmaceutical composition of claim 13 for use as a virus-decoying medicament, preferably for a use selected from the group consisting of:

(i) use in preventing or treating a viral infection, preferably a CMV infection,

(ii) preventing viral transmission, preferably a CMV transmission, to a fetus,

(iii) use in preventing or treating a viral infectious disease, more preferably a CMV infectious disease,

(iv) use in preventing or treating a virus-facilitated, preferably a CMV-facilitated progression of cancer, and/or

5 (v) use in preventing or treating a transplant rejection.

16. An in *vitro* method of treating a device or a composition, comprising the steps of:

(i) providing a device or a composition, and

(ii) contacting the device or composition with a PDGFRα polypeptide or fragment thereof of
10 any one of claims 1 to 6, the fusion protein of any one of claims 7 to 9, the multimer of claim 10, or a pharmaceutical composition claim 13.

FIGURES

Figure 1

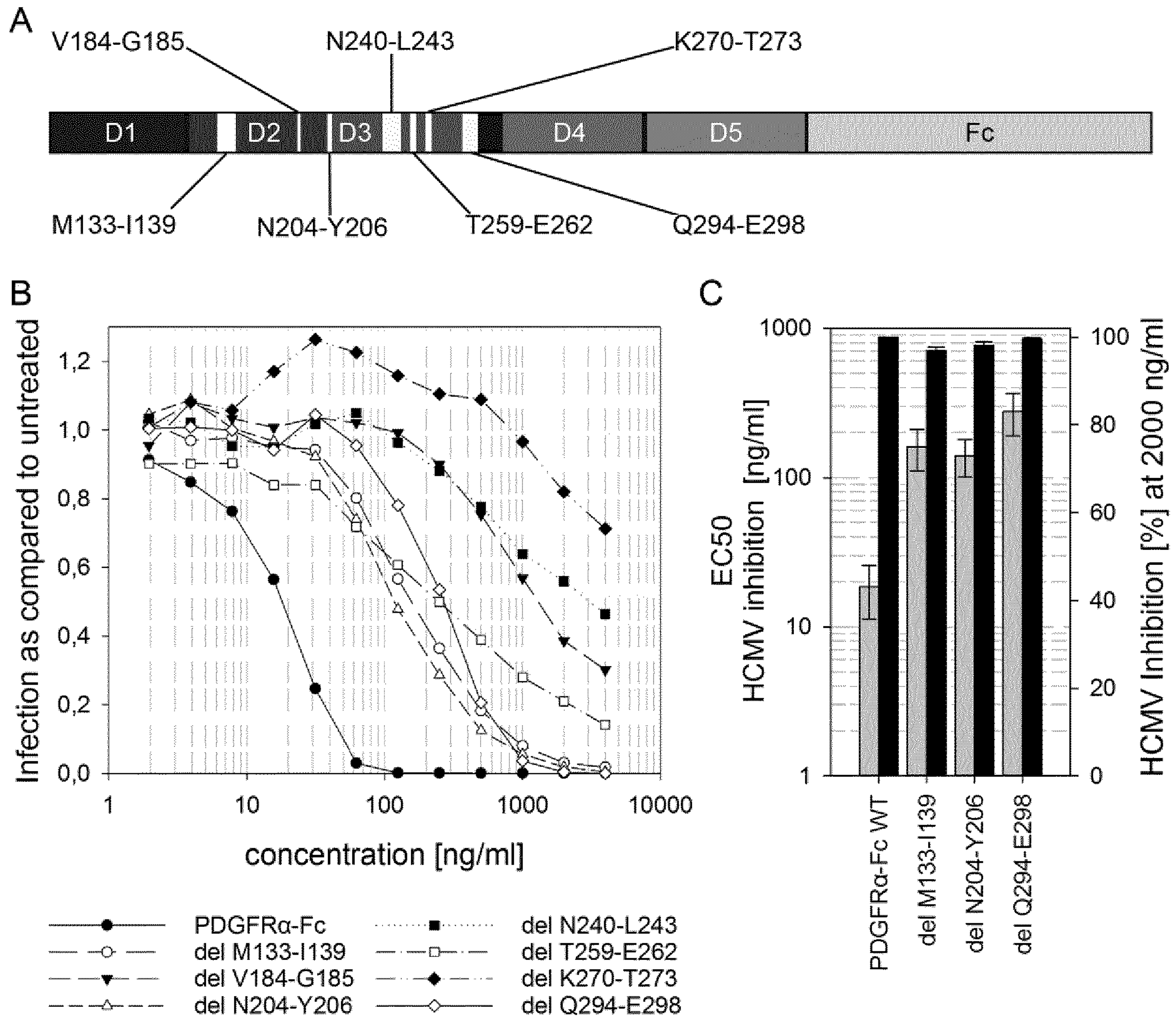


Figure 2

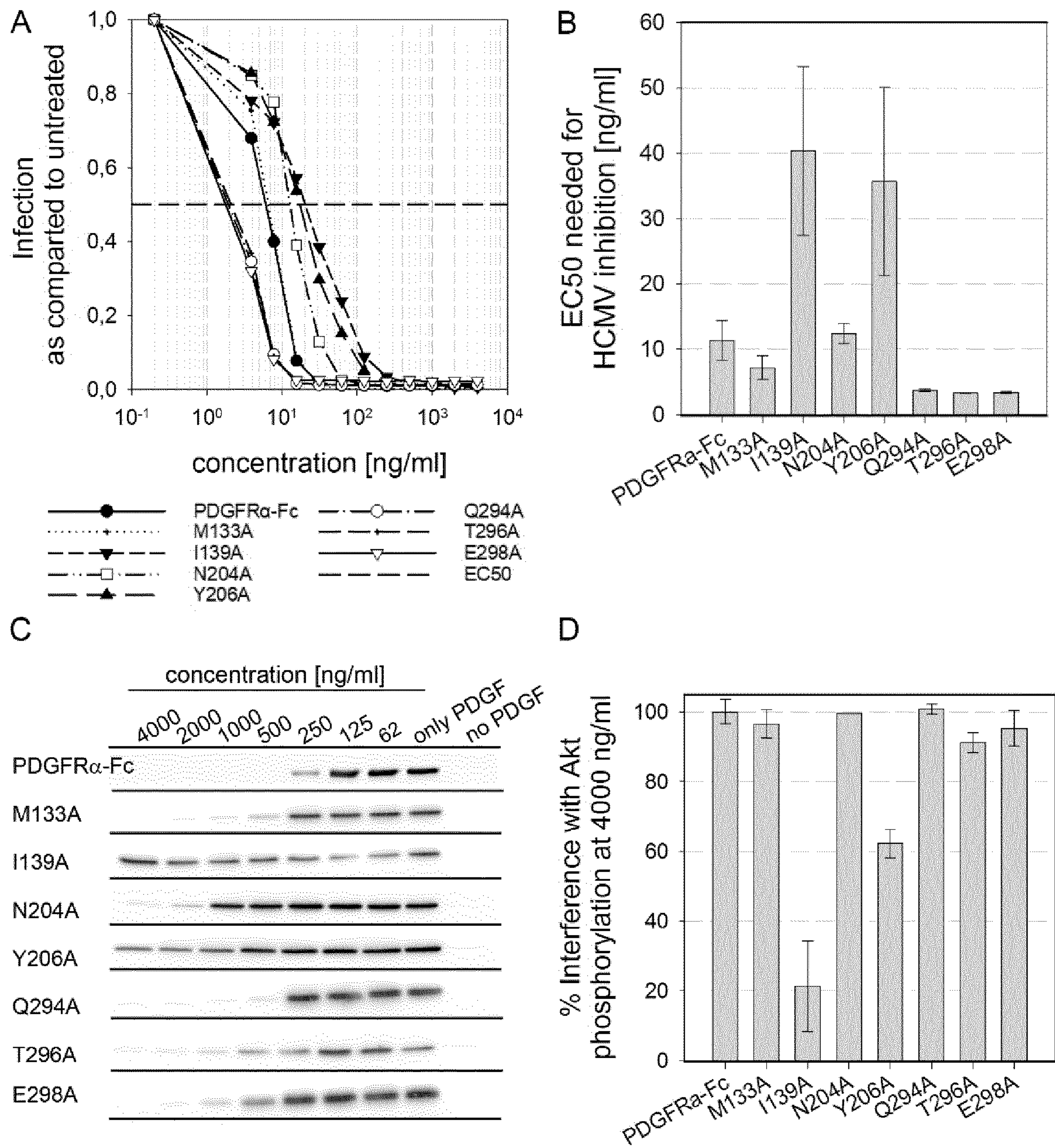


Figure 3

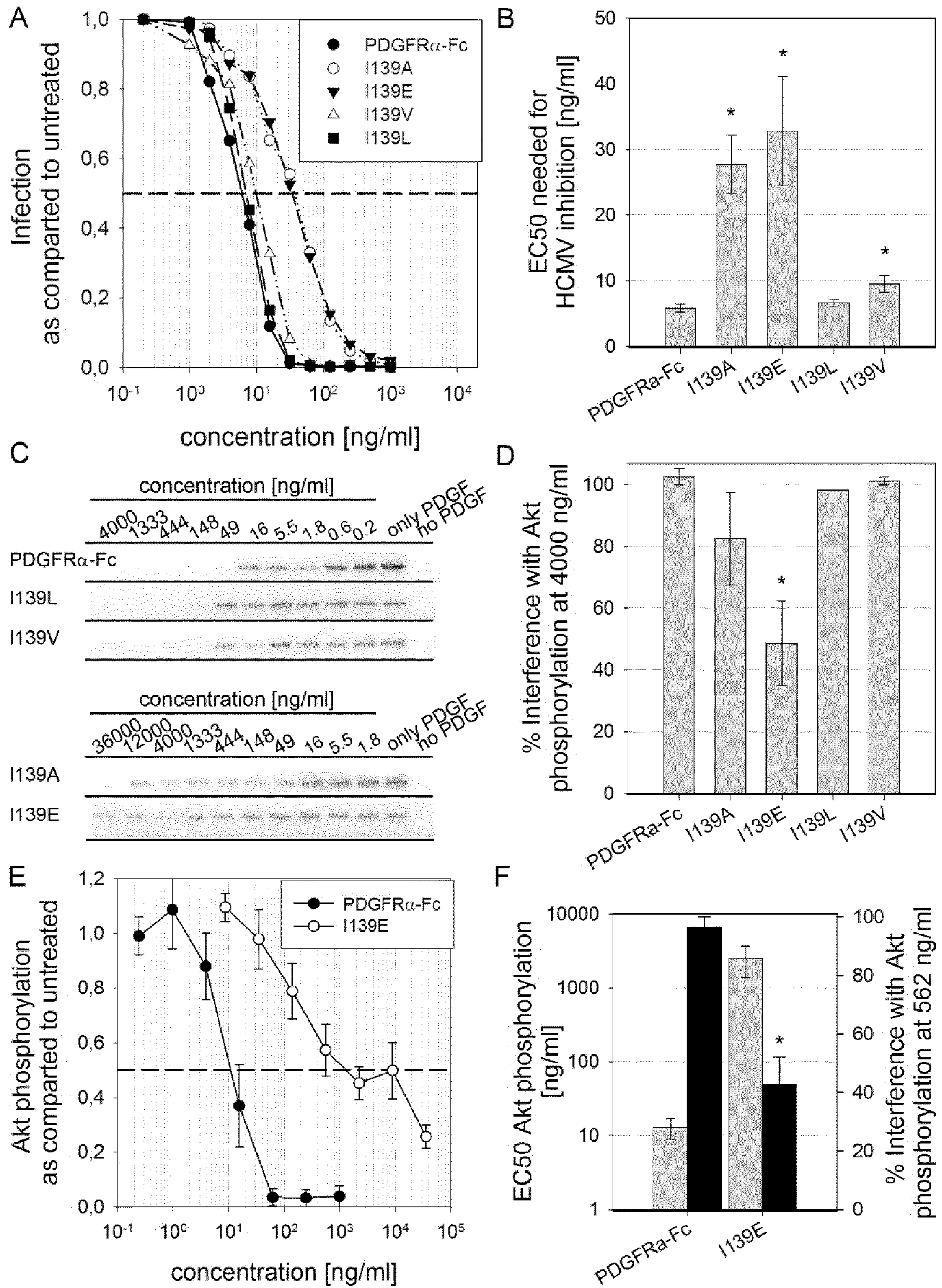


Figure 4

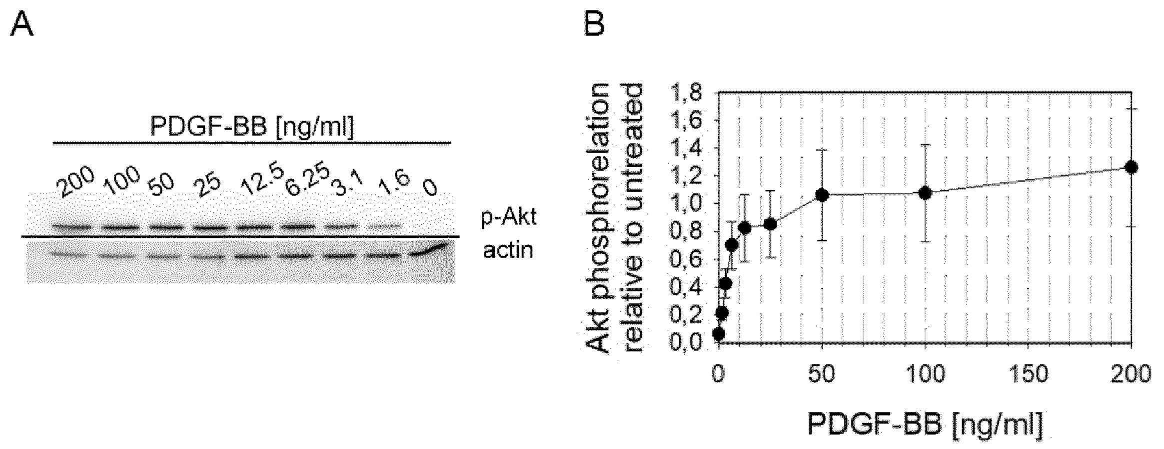


Figure 5

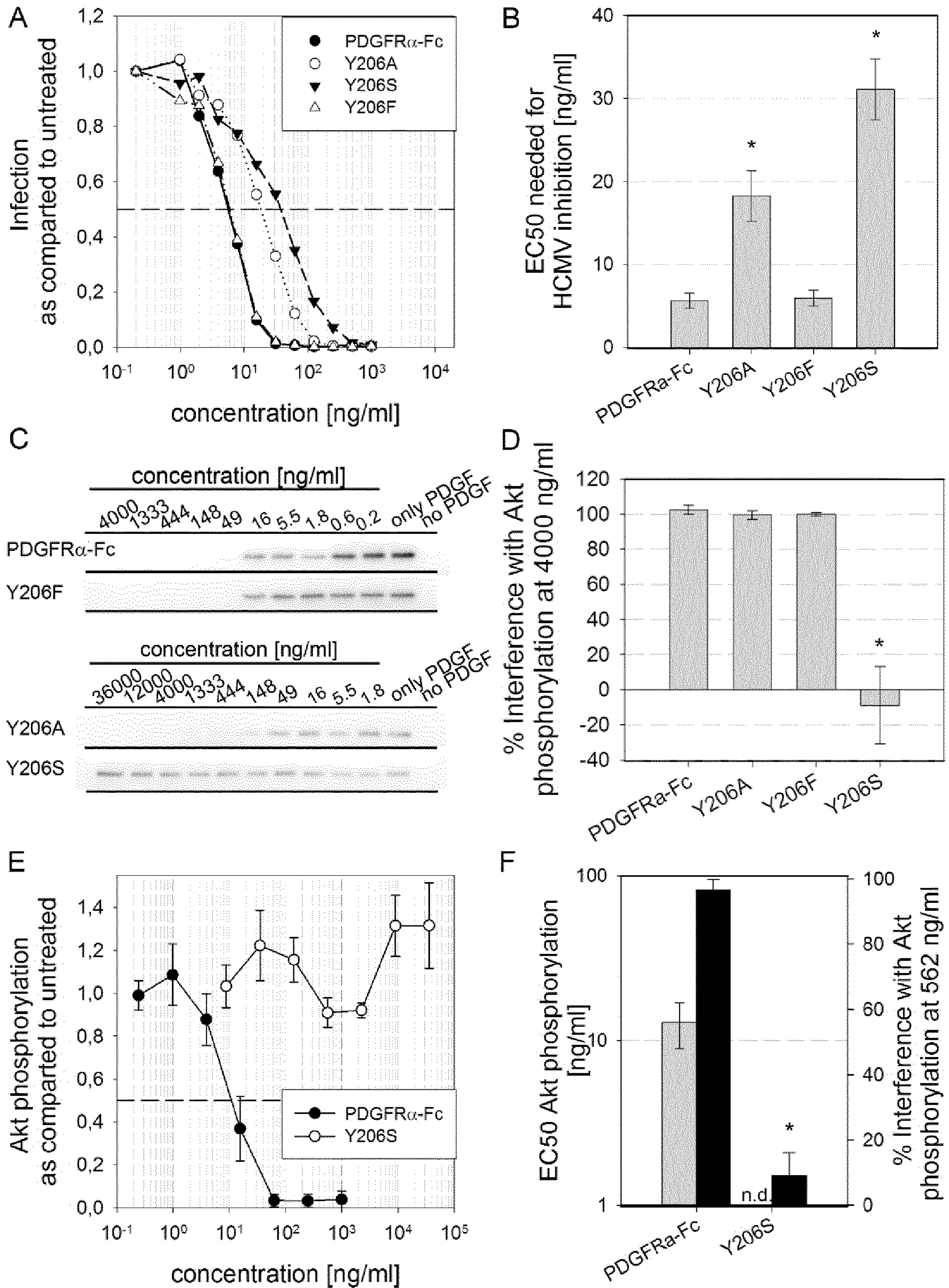


Figure 6

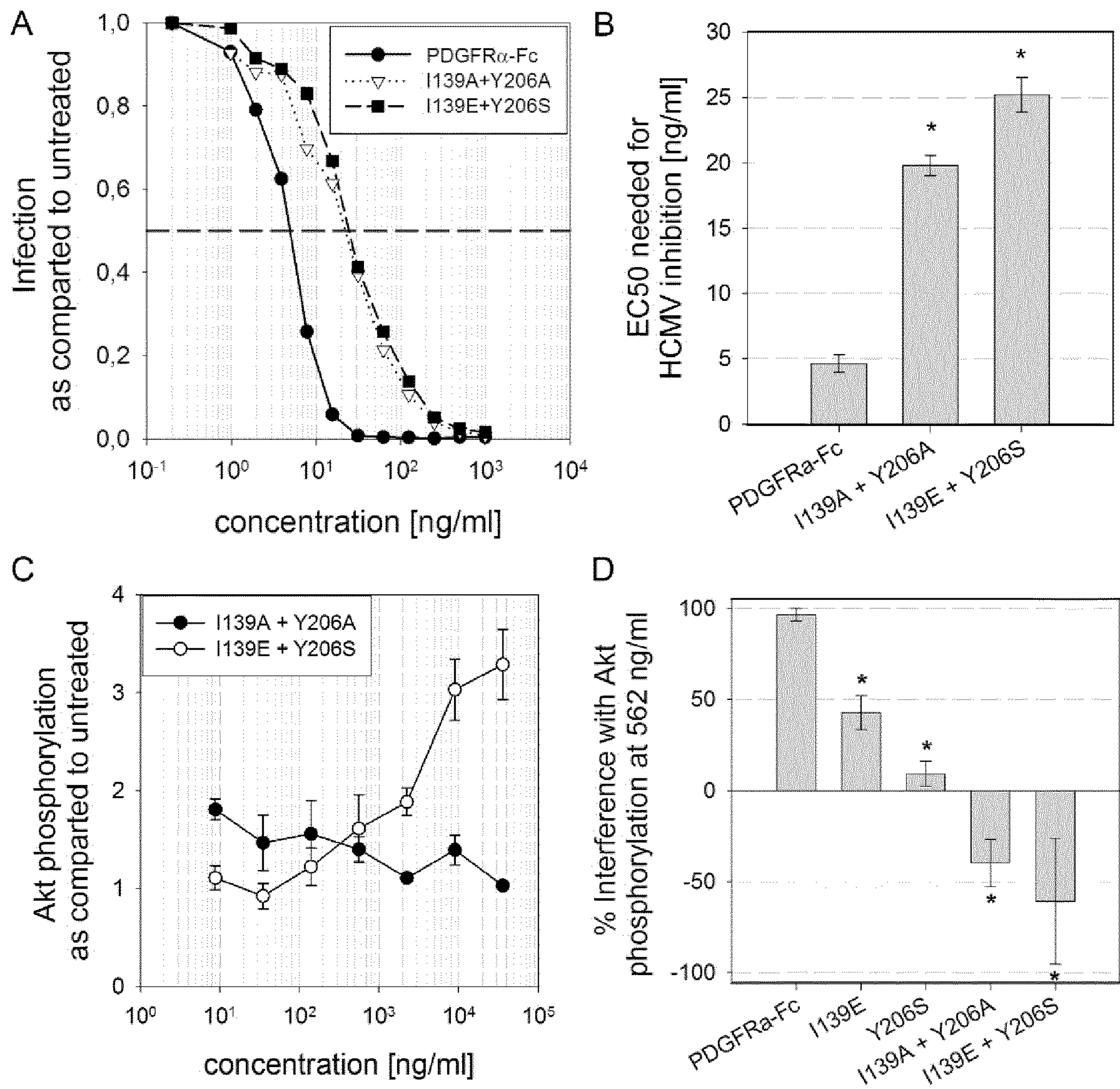


Figure 7

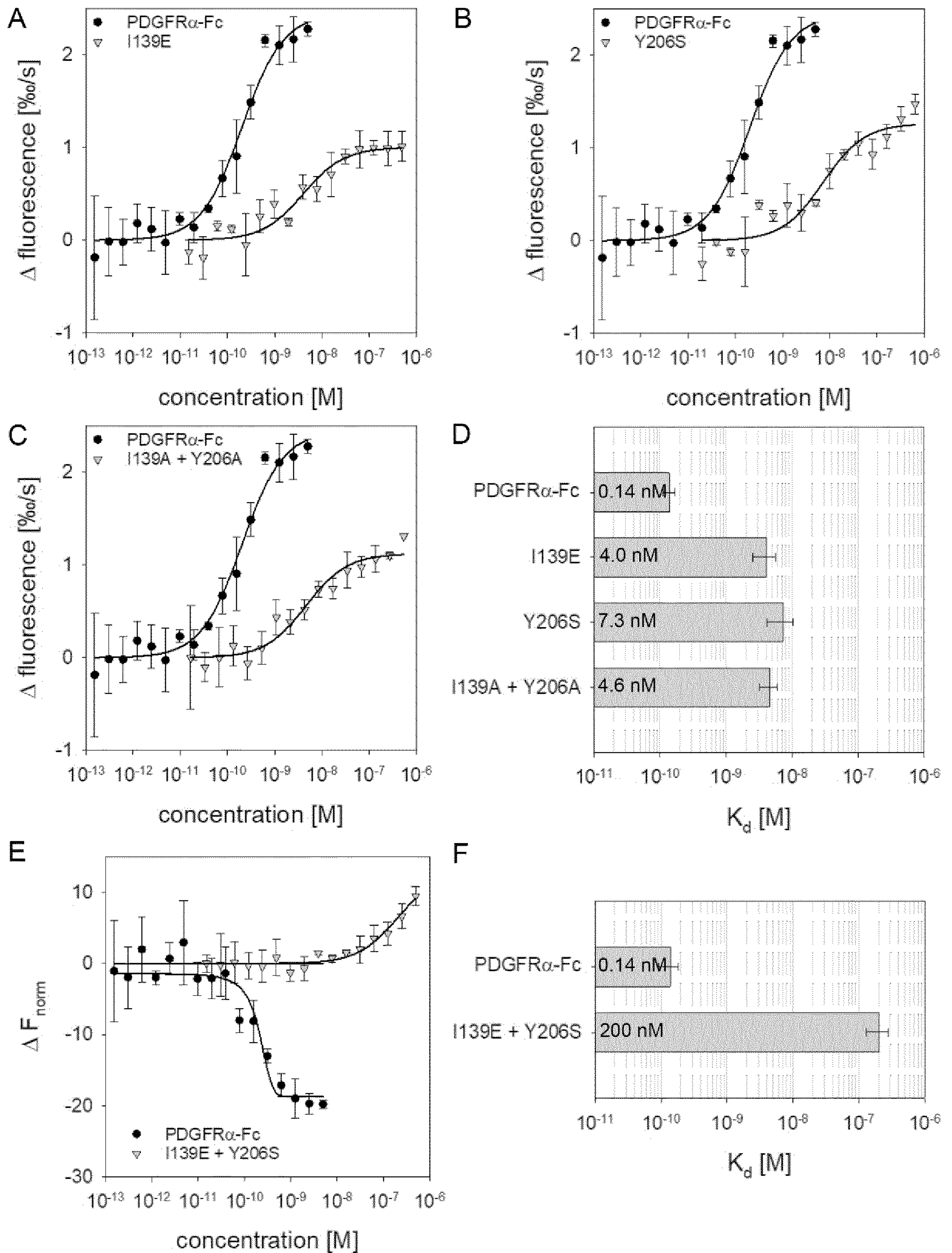


Figure 8

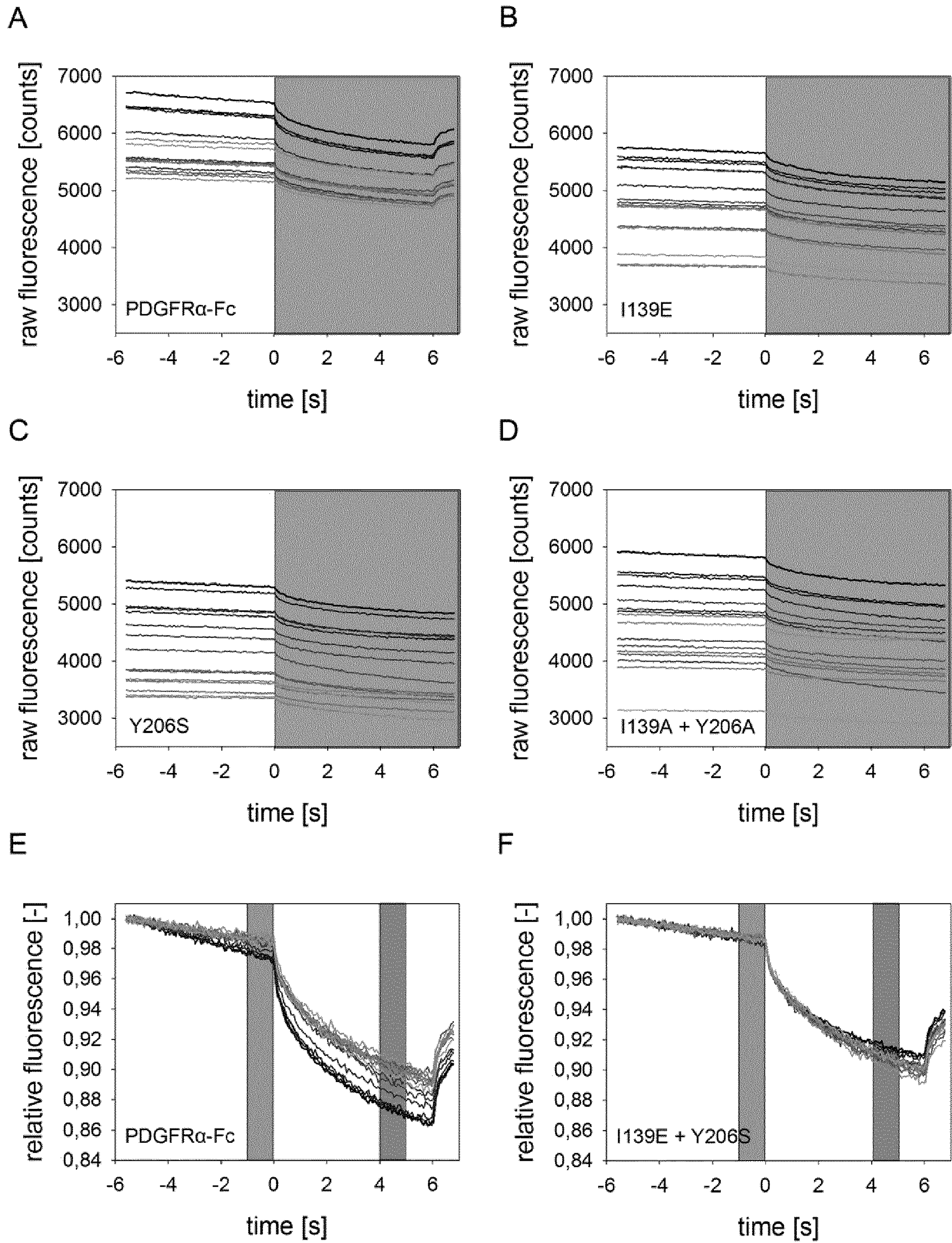


Figure 9

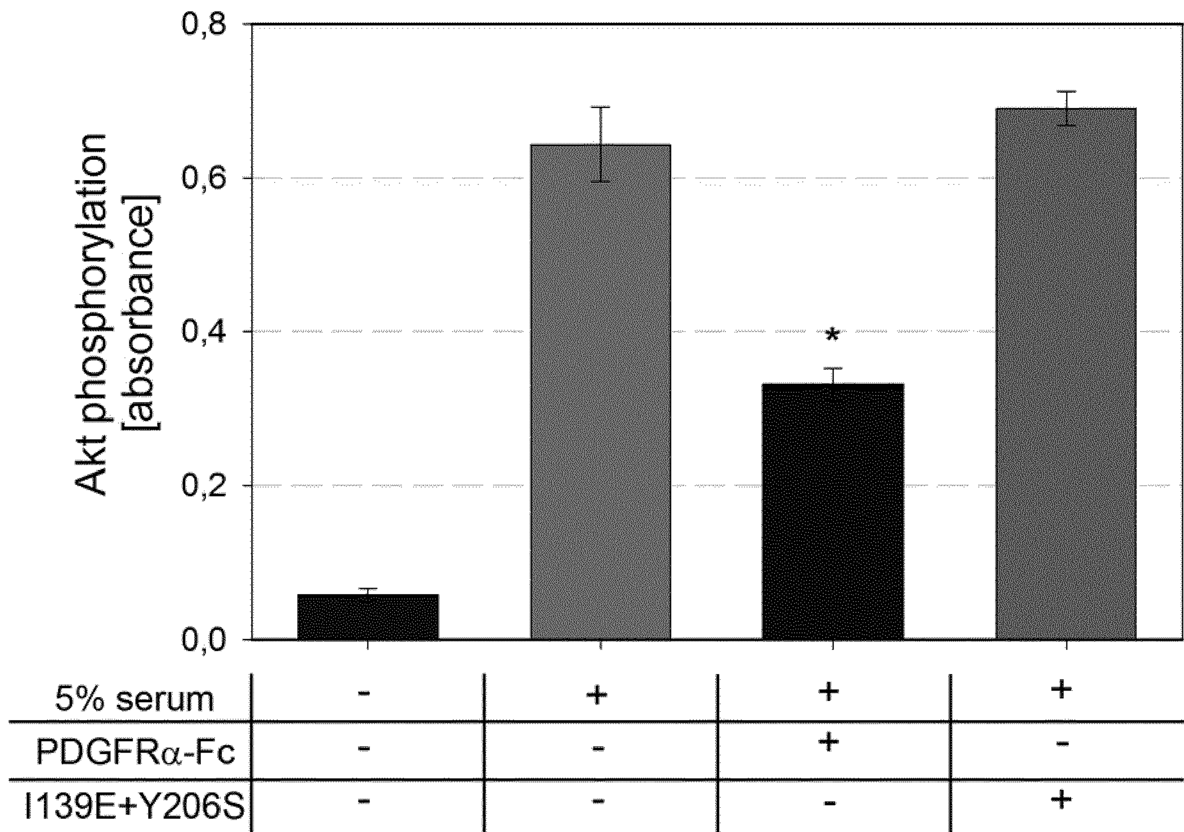


Figure 10

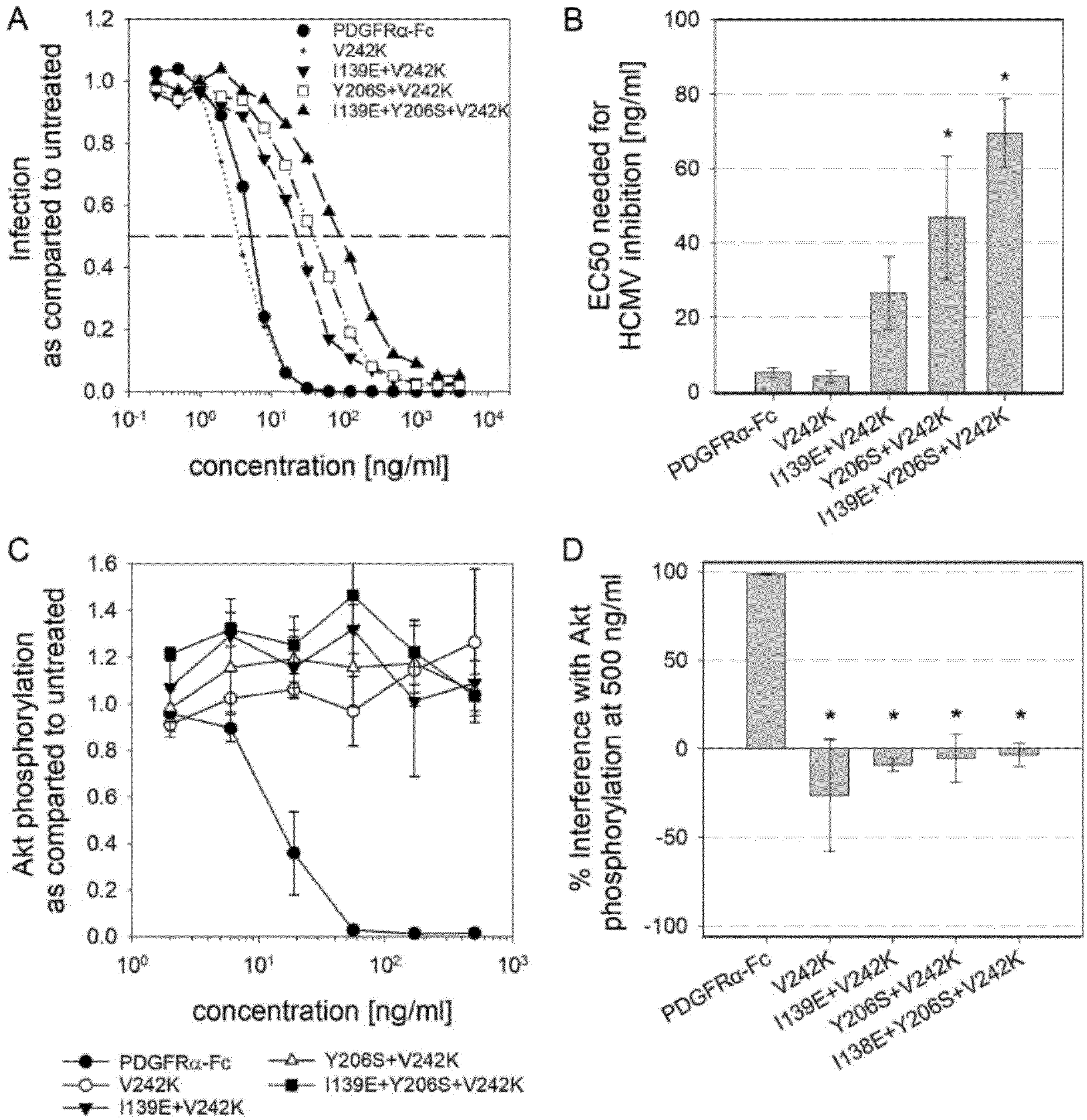
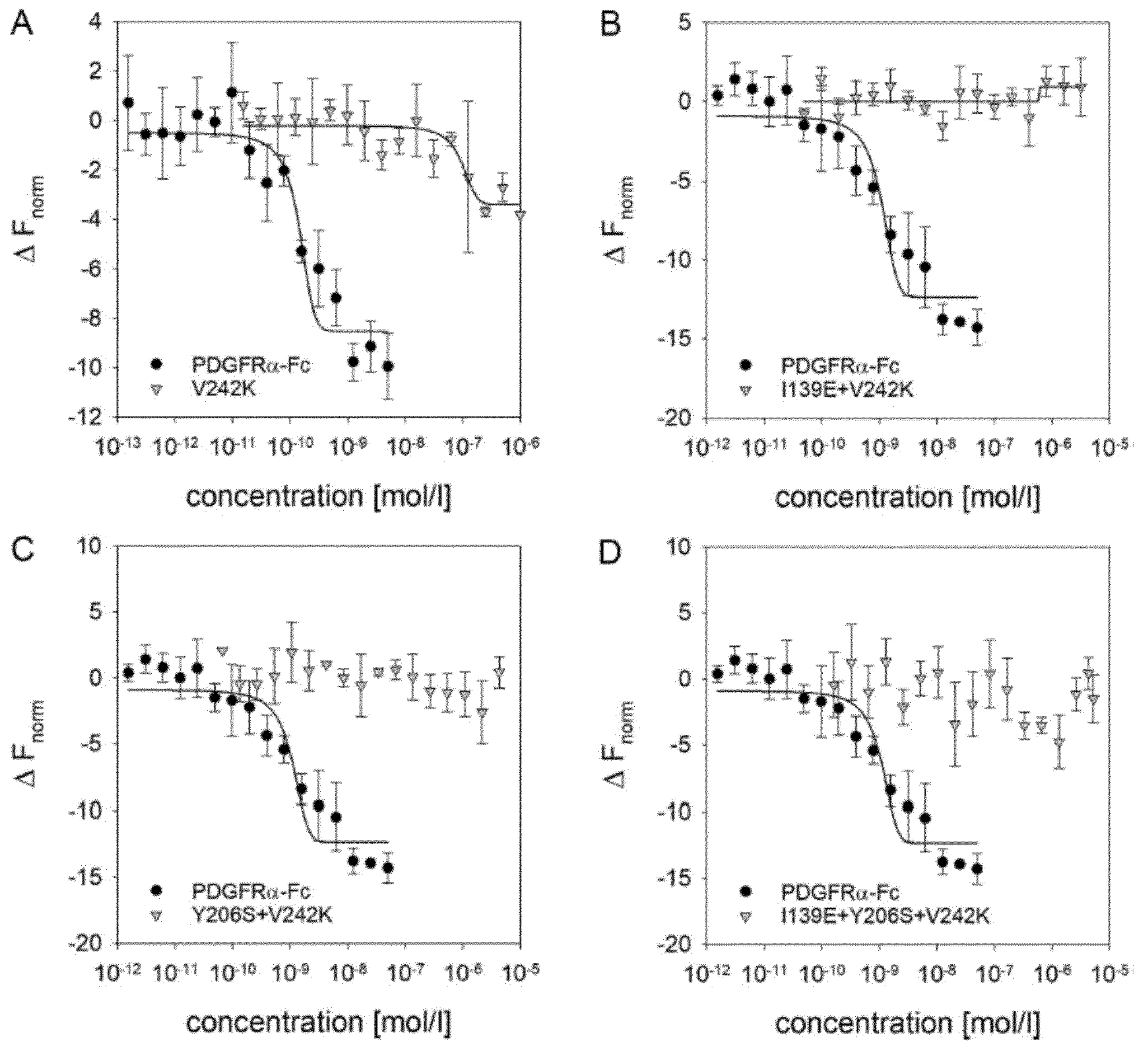


Figure 11



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2021/069644

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/71 A61K39/00
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)
C07K A61K

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, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2018/002081 A1 (AICURIS ANTI-INFECTIVE CURES GMBH [DE]) 4 January 2018 (2018-01-04) the whole document For inventive step of claim 10, see page 15, lines 5-14 -----	1,2,5-16
A	JIHYE PARK ET AL: "Engineered receptors for human cytomegalovirus that are orthogonal to normal human biology", PLOS PATHOGENS, vol. 16, no. 6, 19 June 2020 (2020-06-19), page e1008647, XP055755225, DOI: 10.1371/journal.ppat.1008647 the whole document -----	1-16
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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 September 2021	04/10/2021

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

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE UniProt [Online] 26 November 2014 (2014-11-26), "RecName: Full=Platelet-derived growth factor receptor alpha {ECO:0000256¦PIRNR:PIRNR500950}; Short=PDGF-R-alpha {ECO:0000256¦PIRNR:PIRNR500950}; Short=PDGFR-alpha {ECO:0000256¦PIRNR:PIRNR500950}; EC=2.7.10.1 {ECO:0000256¦PIRNR:PIRNR500950}; AltName: Full=Alpha platelet-derived growth factor receptor", XP002801281, retrieved from EBI accession no. UNIPROT:A0A091D875 Database accession no. A0A091D875 abstract; sequence -----	1,2,4
A	CORA STEGMANN ET AL: "A derivative of platelet-derived growth factor receptor alpha binds to the trimer of human cytomegalovirus and inhibits entry into fibroblasts and endothelial cells", PLOS PATHOGENS, vol. 13, no. 4, 15 May 2019 (2019-05-15), page e1006273, XP055755207, DOI: 10.1371/journal.ppat.1006273 the whole document -----	1-16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2021/069644

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

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

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

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

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2021/069644

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2018002081 A1	04-01-2018	AR 108890 A1	10-10-2018
		CA 3028859 A1	04-01-2018
		EP 3474887 A1	01-05-2019
		MA 45493 A	01-05-2019
		US 2019161532 A1	30-05-2019
		WO 2018002081 A1	04-01-2018