

Claims

1. A urokinase Plasminogen Activator Receptor (uPAR)-targeting peptide
5 conjugate comprising:
 - a fluorophore;
 - a peptide binding to uPAR; and
 - a linker group, wherein the fluorophore, the peptide binding to uPAR and the linker group is connected by covalent bonds, wherein the linker
 - 10 group comprises oligoethylene glycols or other short oligomers such as oligo-glycerol, oligo-lactic acid or carbohydrates which are optionally connected by covalent bonds to at least one amino acid.
2. The uPAR-targeting peptide conjugate according to claim 1, wherein the
15 linker group comprises oligoethylene glycols which are connected by covalent bonds to at least one amino acid.
3. The uPAR-targeting peptide conjugate according to any of claim 1 or 2,
20 wherein the at least one amino acid is selected from proteinogenic amino acids and non-proteinogenic amino acids, which includes natural amino acids and synthetic amino acids.
4. The uPAR-targeting peptide conjugate according to any of claim 3,
25 wherein the natural amino acids include C-alpha alkylated amino acids such as aminoisobutyric acid (Aib), N-alkylated amino acids such as sarcosine and naturally occurring beta-amino acids such as beta-alanine.
5. The uPAR-targeting peptide conjugate according to any of claim 3,
30 wherein the synthetic amino acids include amino acids with non-proteinogenic side-chains such as cyclohexyl alanine, gamma-amino acids, and dipeptide mimics.

6. The uPAR-targeting peptide conjugate according to any of claims 1-5, wherein the linker group is -Glu-Glu-NH-CH₂-CH₂-O-CH₂-CH₂-O-CH₂-CO-NH-CH₂-CH₂-O-CH₂-CH₂-O-CH₂-CO-.
- 5 7. The uPAR-targeting peptide conjugate according to any of claims 1-6, wherein the fluorophore is a near-infrared I fluorophore or a near-infrared II fluorophore.
8. The uPAR-targeting peptide conjugate according to any of claims 1-7, wherein fluorophore is selected from the group consisting of ICG, Methylene blue, 5-ALA, Protoporphyrin IX, IRDye800CW, ZW800-1, Cy5, Cy7, Cy5.5, Cy7.5, IRDye700DX, Alexa fluor 488, Fluorescein isothiocyanate, Flav7, CH1055, Q1, Q4, H1, IR-FEP, IR-BBEP, IR-E1, IR-FGP, IR-FTAP.
- 10 15 9. The uPAR-targeting peptide conjugate according to any of claims 1-7, wherein the fluorophore is a near-infrared I fluorophore selected from the group consisting of ICG, Methylene blue, 5-ALA, Protoporphyrin IX, IRDye800CW, ZW800-1, Cy5, Cy7, Cy5.5, Cy7.5, IRDye700DX, Alexa fluor 488, Fluorescein isothiocyanate.
- 20 10. The uPAR-targeting peptide conjugate according to any of claims 1-7, wherein the fluorophore is a near-infrared II fluorophore selected from the group consisting of Flav7, CH1055, Q1, Q4, H1, IR-FEP, IR-BBEP, IR-E1, IR-FGP, IR-FTAP.
- 25 11. The uPAR-targeting peptide conjugate according to any of claims 1-9, wherein the fluorophore is IRDye800CW.
- 30 12. The uPAR-targeting peptide conjugate according to any of claims 1-11, wherein the receptor binding peptide is selected from the group consisting of:
-Asp-Cha-Phe-ser-arg-Tyr-Leu-Trp-Ser; and

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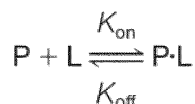
-Asp-Cha-Phe-ser-arg-Tyr-Leu-Trp-Ser-NH₂.

13. The uPAR-targeting peptide conjugate according to any of claims 1-12,
wherein the covalent bonds are selected from the group consisting of an
5 amide, a carbamate, thiourea, an ester, ether, amine, a triazole or any
other covalent bond commonly used to couple chemical moieties by solid-
phase synthesis.
14. The uPAR-targeting peptide conjugate according to any of claims 1-4
10 having the formula:

15. The uPAR-targeting peptide conjugate according to any of claims 1-14,
wherein the fluorophore has a NIR-light absorption in the range of 700-
1200 nm, 700-950 nm (NIR-I), or 1000-1200 nm (NIR-II).
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16. The uPAR-targeting peptide conjugate according to any of claims 1-15,
wherein the fluorophore has a NIR-light emission in the range of 700-
1200 nm, 700-950 nm (NIR-I), or 1000-1200 nm (NIR-II).
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17. The uPAR-targeting peptide conjugate according to any of claims 1-16,
wherein the uPAR-binding affinity is less than 100 nM, preferably less
than 50 nM, preferably less than 25 nM.
18. The uPAR-targeting peptide conjugate according to any of claims 1-17,
15 wherein the uPAR-targeting peptide conjugate is adapted to
- be administered systemically into a human or animal body;
 - provide receptor binding at least within 4,500 minutes, preferably 1,200
minutes, more preferably 600 minutes, even more preferably 300 minutes;
 - be removed from plasma to make the conjugate bound to the receptor
- 20 visible, measured as plasma half-life which is the time it takes for the
concentration in plasma to be reduced with 50%, and wherein the plasma
half-life has a maximum of 4,500 minutes, preferably 1,200 minutes, more
preferably 600 minutes, even more preferably 300 minutes;
- provide receptor binding lasting at least 30 minutes;
- 25 and wherein the receptor binding affinity, the time it takes to reach the desired
receptor binding, the lasting of the receptor binding and plasma half-life
translate into a TBR (tumor-to-background ratio) of at least 1.5 and reaches
that level within 4,500 minutes, preferably 1,200 minutes, more preferably 600
minutes, even more preferably 300 minutes, after administration into the
- 30 human or animal body and stays above 1.5 at least 30 minutes after that this
level has been obtained.

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19. The uPAR-targeting peptide conjugate according to claim 18, wherein the speed of which the protein (P) – ligand (L) complex takes place may be defined as



5 where K_{on} is a constant of the binding reaction and where K_{off} is a constant for the dissociation of the protein-ligand complex, and wherein $K_{\text{on}} > 1 \times 10^3 \text{ M}^{-1}\text{s}^{-1}$ and/or $K_{\text{off}} < 1 \times 10^{-1} \text{ s}^{-1}$.

20. The uPAR-targeting peptide conjugate according to claim 18 or 19,
10 wherein receptor affinity is measured as IC_{50} , which is a measurement of the ligand / receptor binding affinity, on 3000 nM or less.

21. The uPAR-targeting peptide conjugate according to any of claims 18-20,
15 wherein the receptor binding affinity is reached within a time of 30 min measured in vitro.

22. The uPAR-targeting peptide conjugate according to any of claims 18-21,
wherein a receptor binding lasts at least 120 minutes, preferably at least 300 minutes, after administration into the human or animal body measured using
20 a TBR above 1.5.

23. The uPAR-targeting peptide conjugate according to any of claims 1-22 for use in the treatment of a disease.

25 24. The uPAR-targeting peptide conjugate according to any of claims 1-22 for use in diagnosis of a disease.

25. The uPAR-targeting peptide conjugate according to any of claims 1-22 for use in optical imaging or fluorescence imaging (FLI) of a disease.

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26. The uPAR-targeting peptide conjugate for use according to any of claims 23-25, wherein the disease is selected from the group consisting of cancer and inflammatory diseases.
- 5 27. The uPAR-targeting peptide conjugate for use according to any of claims 23-25, wherein the uPAR-targeting peptide conjugate is administered to a subject in a dose of 0.1-1000 mg per person.
28. A pharmaceutical composition comprising the uPAR-targeting peptide
10 conjugate according to any of claims 1-22 together with at least one pharmaceutically acceptable carrier or excipient.
29. The pharmaceutical composition according to claim 28 for use in the treatment of a disease.
- 15 30. The pharmaceutical composition according to claim 28 for use in detection and/or quantification of a disease.
31. The pharmaceutical composition according to claim 28 for use in
20 diagnosis of a disease.
32. The pharmaceutical composition according to claim 28 for use in optical imaging or fluorescence imaging (FLI) of a disease.
- 25 33. The pharmaceutical composition for use according to any of claims 29-32, wherein the disease is selected from the group consisting of cancer and inflammatory diseases.
- 30 34. The pharmaceutical composition for use according to claim 33, wherein the cancer is selected from the group consisting of gliomas, glioblastomas or other brain tumors, pancreatic cancer, oropharyngeal cancer, head-and-neck cancer, breast cancer, lung cancer, colorectal cancer, esophageal cancer,

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gastric cancer, liver cancer, neuroendocrine tumors, neuroendocrine carcinomas, prostate cancer.

35. The pharmaceutical composition for use according to claim 33 or 34,
5 wherein the cancer is selected from the group consisting of gliomas, glioblastomas, pancreatic cancer, head-and-neck cancer, colorectal cancer, lung cancer and breast cancer.

36. The pharmaceutical composition for use according to any of claims 33-35,
10 wherein the cancer is gliomas, glioblastomas or breast cancer.

37. The pharmaceutical composition for use according claim 33, wherein the inflammatory diseases are selected from the group consisting of arthritis and atherosclerosis.

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38. The pharmaceutical composition according to claim 29 for use in delineation of cancer tumors or metastases.

39. The pharmaceutical composition according to claim 29 for use in
20 fluorescence guided tumor or metastasis resection.

40. The pharmaceutical composition for use according to any of claims 29-39, wherein the pharmaceutical composition is administered intravenously, locally or topically.

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41. The pharmaceutical composition for use according to any of claims 29-40, wherein the concentration of the uPAR-targeting peptide conjugate is 0.1-1000 mg per dosage unit, typically in the ranges of 0.001-1000 mg per dosage unit, 0.01-1000 mg per dosage unit, 0.1-1000 mg per dosage unit,
30 0.0001-500 mg per dosage unit, 0.001-500 mg per dosage unit, 0.01-500 mg per dosage unit, 0.1-500 mg per dosage unit, 0.0001-100 mg per dosage unit, 0.001-100 mg per dosage unit, 0.01-100 mg per dosage unit, or 0.1-100 mg per dosage unit.

42. An optical imaging method comprising the steps of:
- (i) administering of a uPAR-targeting peptide conjugate to a target tissue, wherein the uPAR-targeting peptide conjugate comprises a fluorophore, a
5 receptor binding peptide, and a linker group which covalently links the fluorophore to the receptor binding peptide, wherein the receptor binding peptide is binding to a urokinase Plasminogen Activator Receptor (uPAR);
 - (ii) allowing time for the uPAR-targeting peptide conjugate to accumulate
10 in the target tissue and establishing a tumor/target tissue-to-background ratio;
 - (iii) illuminating the target tissue with near infrared light of a wavelength absorbable by the fluorophore;
 - (iv) detecting fluorescence emitted by the fluorophore and forming an
15 optical image of the target tissue.
43. The optical imaging method according to claim 42, wherein the time for the uPAR-targeting peptide conjugate to accumulate in the target tissue to obtain a sufficiently high TBR is at the most 360 minutes, 180 minutes, 120
20 minutes, 90 minutes, 60 minutes, 45 minutes, 30 minutes, 15 minutes, 10 minutes or 5 minutes after administration.
44. The optical imaging method according to claim 42 or 43, wherein the tumor/target tissue-to-background ratio is at least 2.
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45. The optical imaging method according to any of claims 42-44, wherein the fluorophore has a NIR-light absorption in the range of 700-800 nm.
46. The optical imaging method according to any of claims 42-45, wherein the
30 fluorophore has a NIR-light emission in the range of 750-900 nm.
47. The optical imaging method according to any of claims 42-46, wherein the method is performed *in vivo*.

48. The optical imaging method according to any of claims 42-47, wherein the method is performed *in vitro*.

- 5 49. A method for detection of a disease comprising the steps of:
- (i) administering of a uPAR-targeting peptide conjugate according claims 1-22 to a target tissue;
 - (ii) allowing time for the uPAR-targeting peptide conjugate to accumulate
 - 10 administration;
 - (iii) illuminating the target tissue with near infrared light of a wavelength absorbable by the fluorophore of the uPAR-targeting peptide conjugate;
 - (iv) detecting fluorescence emitted by the fluorophore of the uPAR-targeting peptide conjugate; and
 - 15 (v) forming an optical image of the target tissue or quantifying a disease or a combination of both.

50. Use of the uPAR-targeting peptide conjugate according to any of claims 1-22, or the pharmaceutical composition according to claim 28 for optical
- 20 imaging of cancer.

51. Use of the uPAR-targeting peptide conjugate according to any of claims 1-22, or the pharmaceutical composition according to claim 28, for fluorescence guided tumor or metastasis resection.

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52. A kit of parts for preparing a unit dosage of a uPAR-targeting peptide conjugate preparation, comprising:
- a unit dosage amount of the pharmaceutical composition according to claim 28;
 - 30 - optionally a unit dosage amount of at least one a pharmacologically active agent;
 - optionally a unit dosage amount of an adjuvant;

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- optionally a unit dosage of at least one pharmaceutically acceptable carrier or excipient.
- means for separating the unit dosage amounts from each other prior to dispensing; and
- 5 - means for dispensing the unit dosage amounts.

53. The kit of parts according to claim 52, wherein the unit dosage amount of the pharmaceutical composition according to claim 28 is in the range of 0.1-1000 mg, typically in the ranges of 0.001-1000 mg, 0.01-1000 mg, 0.1-1000
10 mg, 0.0001-500 mg, 0.001-500 mg, 0.01-500 mg, 0.1-500 mg, 0.0001-100 mg, 0.001-100 mg, 0.01-100 mg, or 0.1-100 mg.

54. The kit of parts according to claim 52 or 53, wherein the unit dosage amounts are in a total volume range of 0.1-10.0 mL, preferably in a range of
15 0.1-5.0 mL, preferably in a range of 0.5-5.0 mL, preferably in a range of 0.5-1.0 mL.