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(54) Title: THERAPEUTIC COMBINATION OF AN ANTI-EDB FIBRONECTIN DOMAIN ANTIBODY IL2 OR IL12 FUSION PROTEIN AND A LUTETIUM-177 RADIOCONJUGATE

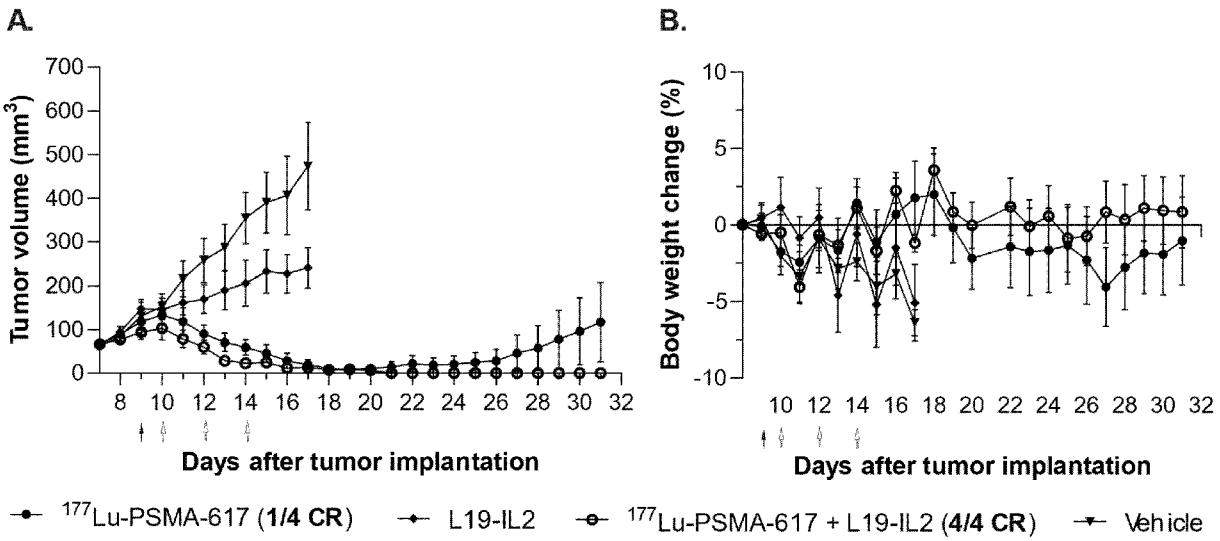

Fig. 8

(57) Abstract: The invention relates to a combination comprising (i) a recombinant protein comprising interleukin-2 (IL2) or interleukin-12 (IL12) and an antibody binding the extra-domain B (ED-B) of fibronectin, or a target binding fragment or derivative thereof, and (ii) a conjugate radiolabelled with 177Lutetium, which conjugate is 177Lu-PSMA-617, and its use for treatment of cancer. In a preferred embodiment, the antibody is L19.

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Therapeutic combination of an anti-EDB fibronectin domain antibody IL2 or IL12 fusion protein and a Lutetium-177 radioconjugate

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Field of the invention

The invention relates to the field of immunoconjugates.

Incorporation by Reference

All publications, patents, patent applications and other documents cited in this application are hereby incorporated by reference in their entireties for all purposes to the same extent as if each individual publication, patent, patent application or other document were individually indicated to be incorporated by reference for all purposes. In the event that there are any inconsistencies between the teachings of one or more of the references incorporated herein and the present disclosure, the teachings of the present specification are intended.

Background

Cytokines are key mediators of innate and adaptive immunity. Many cytokines have been used for therapeutic purposes in patients with advanced cancer, but their administration is typically associated with severe toxicity, hampering dose escalation to therapeutically active regimens and their development as anticancer drugs. To overcome these problems, the use of 'immunocytokines' (i.e. cytokines fused to antibodies or antibody fragments) has been proposed, with the aim to concentrate the immune-system stimulating activity at the site of disease while sparing normal tissues (Neri & Bicknell, 2005). However, genetically fusing a

cytokine to an antibody or to an antibody fragment creating an "immunocytokine", does not always result in an immunocytokine that retains the ability to target the tumor of the antibody. For example, in certain Interleukin-7 fusions (Pasche *et al.* (2011) J Biotechnology, 154, 84-92) the tumor targeting was completely abrogated, while in certain GM-CSF fusions (Kaspar *et al.* (2007) Cancer Res, 67, 4940-4948) the tumor targeting ability was found to be dose dependent.

Interleukin-2 (IL2) is a four α helix bundle cytokine produced by T helper 1 cells and plays an essential role in the activation phases of both specific and natural immune responses. IL2 promotes proliferation and differentiation of activated T and B lymphocytes and of natural killer (NK) cells and induces cytotoxic T cell (CTL) activity and NK/lymphokine-activated killer (LAK) cell antitumor cytotoxicity. IL2 has been approved for the treatment of several human cancers. Administration of recombinant IL2 (rIL2) alone or in combination with adoptively transferred lymphoid cells has been shown to result in the regression of established tumors in both animal models and patients. However, the *in vivo* therapeutic efficacy of IL2 is limited by its rapid clearance and, at high doses severe toxicity mainly related to a vascular leak syndrome.

Interleukin-12 (IL12) is a heterodimeric cytokine with multiple biological effects on the immune system. It is made up of two subunits, p35 and p40, both of which are required for the secretion of the active form of IL12, p70. Interleukin-12 acts on dendritic cells (DC), leading to increased maturation and antigen presentation, which can allow for the initiation of a T cell response to tumor specific antigens. It also drives the secretion of IL12 by DCs, creating a positive feedback mechanism to amplify the response. Once a response is initiated, IL12 plays a fundamental role in directing the immune system towards a Th1 cytokine profile, inducing CD4+ T cells to secrete interferon-gamma (IFN-γ) and leading to a CD8+ cytotoxic T cell response.

IL12 is also a strong pro-inflammatory cytokine that leads to the secretion of other cytokines including tumor necrosis factor-alpha (TNF-α) which, combined with IFN-γ, is a prerequisite for the development of CD4+ cytotoxic T lymphocytes (CTL). Furthermore, IL12 can promote the activation of innate immune cells such as macrophages and eosinophils through its induction of IFN-γ and other cytokines. This activation then leads to IL12 secretion by these cells and further amplification of both the innate and acquired responses. However, high levels of IL12,

and consequently IFN-γ, have also been associated with induction of antagonistic molecules such as IL-10 and the depletion of signaling molecules downstream of IL12, such as STAT4.

Previous attempts at utilizing IL12 as a therapeutic agent were unsuccessful as IL12 showed at best modest anti-tumor effects which were often accompanied by unacceptably toxic side effects, including fever, fatigue, hematological changes, hyperglycemia, and/or organ dysfunction.

To overcome the drawbacks associated with IL2 or IL12 therapy, delivery of IL2 or IL12 to the tumor site by means of an antibody directed against tumor-associated marker to increase local concentrations of IL2 or IL12 at the tumour site, as well as reduce toxicities associated with systemic administration of IL2 or IL12 has been proposed. In particular, the concentration of cytokines at the level of tumour blood vessels is an attractive therapeutic strategy as the tumour neovasculature is more accessible to intravenously administered therapeutic agents than tumour cells, which helps avoid problems associated with the interstitial hypertension of solid tumours. In addition, angiogenesis is characteristic of most aggressive solid tumours. Angiogenesis describes the growth of new blood vessels from existing blood vessels. Tumours can induce angiogenesis through secretion of various growth factors (e.g. Vascular Endothelial Growth Factor). Tumour angiogenesis allows tumours to grow beyond a few millimetres in diameter and is also a prerequisite for tumour metastasis. New blood vessels formed as the result of angiogenesis form the neovasculature of the tumour or the tumour metastases. Targeting IL2 or IL12 to the neovasculature should allow the immunotherapy of a variety of different tumour types.

The alternatively spliced extra domain B (ED-B) of fibronectin represent one of the best-characterised markers of angiogenesis and has been reported to be expressed around the neo-vasculature and in the stroma of virtually all types of aggressive solid tumours. Furthermore, even non-solid cancers, such as leukaemia, may be amenable to treatment by targeting antigens of the neovasculature. WO2011/015333 described treating leukaemia, including acute myeloid leukaemia, by targeting the bone marrow neovasculature.

A human monoclonal antibody specific to this target named L19 has been extensively described (WO1999/058570, WO2003/076469, WO2005/023318).

In addition, immunocytokines based on L19 are currently being investigated in Phase I, Phase II and Phase III clinical trials in patients with cancer. These immunocytokines include several cytokines, comprising IL2 or IL12.

It is one object of the present invention to broaden the scope of therapeutic applications of the above identified immunocytokines.

It is another object of the present invention to provide new therapeutic options for conditions for which so far no adequate treatment option exists.

It is another object of the present invention to improve efficacy of a therapy using the above described immunocytokines.

Brief description of the figures

Fig. 1: Therapeutic activity of 177Lu-ESV6-DOTAGA, 177Lu-Bi-ESV6-DOTAGA, L19-IL2, 177Lu-ESV6-DOTAGA + L19-IL2, 177Lu-Bi-ESV6-DOTAGA + L19-IL2 or saline in athymic Balb/c AnNRj-Foxn1 mice bearing SK-RC-52.hFAP tumors (FAP = Fibroblast activation protein). The efficacy of the different treatments was assessed by daily measurement of tumor volume (mm3) during and after administration of the drugs. Data points represent mean tumor volume±SEM (n=4 per group). ****P<0.0001; *P<0.1 (two-way ANOVA test, followed by Bonferroni post-test).

Fig. 2: The tolerability of the different treatments reported in Fig. 1 was assessed by the evaluation of changes (%) in body weight during the experiment in mice bearing SK-RC-52.hFAP tumors.

Fig. 3: The therapeutic activity of 177Lu-ESV6-DOTAGA, 177Lu-Bi-ESV6-DOTAGA, L19-IL2, 177Lu-ESV6-DOTAGA + L19-IL2, 177Lu-Bi-ESV6-DOTAGA + L19-IL2 or saline was further confirmed in a second tumor model. The efficacy of the different treatments in athymic Balb/c AnNRj-Foxn1 mice bearing HT-1080.hFAP was assessed by daily measurement of tumor volume (mm3) during and after administration of the drugs. Data points represent mean tumor volume±SEM (n=4 per group). ****P<0.0001; *P<0.1 (two-way ANOVA test, followed by Bonferroni post-test).

Fig. 4: Volcano plots representing up- and down-regulated proteins for each treatment group compared the treatment with saline in the HT-1080.hFAP tumor model. **A**. Treatment with 177Lu-Bi-ESV6-DOTAGA resulted in the identification of 1 statistically significant down-regulated protein and 8 statistically significant up-regulated proteins. **B**. Treatment with L19-IL2 resulted in the identification of 179 statistically significant down-regulated proteins and 178 statistically significant up-regulated proteins. **C**. Treatment with "177Lu-Bi-ESV6-DOTAGA + L19-IL2" resulted in the identification of 634 statistically significant down-regulated proteins and 804 statistically significant up-regulated proteins.

Fig. 5: Specific protein intensities expressed as relative abundance compared the saline group in the HT-1080.hFAP tumor model. Expression of human antigen describing tumoral cells as FAP (Fibroblast Activation Protein) and CAIX (Carbonic Anhydrase IX) (A-B). Expression of proteins involved in NK cells activity as Perforin-1 (C) and different Granzymes (G). Expression of immune cells chemotactic protein pro-IL16 (D), marker of lymphatic cells as B, T and NK cells CD48 (E), and marker of macrophages CD68 (F). Data are reported as mean ± SEM.

Fig. 6: *Ex vivo* immunofluorescence analysis on SK-RC-52.hFAP tumor sections following administration of vehicle (saline), 177Lu-Bi-ESV6-DOTAGA (250 nmol/kg, 250 MBq/kg), L19-IL2 (2.5 mg/kg), or 177Lu-Bi-ESV6-DOTAGA (250 nmol/kg, 250 MBq/kg) + L19-IL2 (2.5 mg/kg) or with the schedule indicated in Figure 1. Representative immunofluorescence analysis of tumor samples are depicted at a 20X magnification. Green = NKp46 staining; Blue = DAPI staining. (scale bars: 100 μm).

Fig. 6A: Original color reproduction. Fig. 6B: Grey scale reproduction of the NKp46 staining only. Fig. 6C: Grey scale reproduction of the DAPI staining only.

Fig. 7: Radio-HPLC profile of 177Lu-PSMA-617 after radiolabelling.

Fig. 8: *In vivo* therapy experiment with 177Lu-PSMA-617 (black arrow) and L19-IL2 (white arrows) in Balb/c nude mice bearing HT-1080.hPSMA xenografts. Graph (A) compares the therapeutic activity of the single agent (L19-IL2 or 177Lu-PSMA-617), combination, and

vehicle groups. Graph (B) outlines the percentage change in body weight over the course of the experiment. The study was performed with randomized groups of 4 tumor-bearing mice.

Fig. 9: A. Volcano plot representing the up- and down- regulated proteins in comparison with the vehicle treatment in the HT-1080.hPSMA tumor model treated with L19-IL2, 177Lu-PSMA-617, or the combination of the two. B. Statistically significant up-regulated proteins in the combination therapy group indicating activation of the host immune system. Data are presented as mean ± standard deviation.

Fig. 10: *In vivo* therapy experiment with 177Lu-PSMA-617 (black arrow) and L19-murineIL12 (grey arrows) in C57BL/6J mice bearing MC38.hPSMA tumors. The graph compares the therapeutic activity of the single agent (L19-murineIL12 or 177Lu-PSMA-617), the combination of the two, and the vehicle groups. The combination with L19-murineIL12 strongly potentiates the *in vivo* anti-cancer activity of 177Lu-PSMA-617.

Fig. 11: A. Volcano plot representing the up- and down- regulated proteins in comparison with the vehicle treatment in the MC38.hPSMA tumor model treated with L19-murineIL12, 177Lu-PSMA-617, or the combination of the two. B. Statistically significant up-regulated proteins in the combination therapy group indicating activation of the host immune system. Data are presented as mean ± standard deviation.

Summary of the Invention

The present invention provides, among other things, compositions comprising L19-IL2 or L19-IL12 in combination with Lutetium 177-labelled radioconjugates.

According to one aspect of the invention, a pharmaceutical composition is provided, comprising

(a) a recombinant protein comprising

(i) interleukin-2 (IL2) or interleukin-12 (IL12) and

(ii) an antibody binding the extra-domain B (ED-B) of fibronectin, or a target binding fragment or derivative thereof, and

(b) a conjugate radiolabelled with 177Lutetium.

As used herein, the term “a pharmaceutical composition” relates to an embodiment that may consist of

(i) at least one entity that comprises, in a solid or liquid form, the recombinant protein of (a) and the conjugate of (b), and/or
(ii) at least two entities, one of which comprising, in a solid or liquid form, the recombinant protein of (a), and the second of which comprising, in a solid or liquid form, the conjugate of (b),

In addition, said composition may optionally comprise additional pharmaceutically acceptable excipients, including diluents, tonifiers, cryoprotectants, surfactants and the like.

The inventors have surprisingly shown that such combination has a superior therapeutic activity as compared to the single agents. Furthermore, unexpectedly, the combination maintains a comparable or superior safety profile as compared to the single agents.

Lutetium 177 is a gamma and beta emitter with a half-life of 6.7 days and low energy beta-particles emissions with a mean range of 0.7 mm and maximum range of 2.1 mm in soft tissue. It finds application in several therapies, most notably radiolabelled somatostatin analogues for neuroendocrine tumors and PMSA-ligands prostate cancer and it is particularly suitable for radiolabelling of biologically active tracer molecules.

The inventors have surprisingly found out that the two components, i.e., (a) the recombinant protein comprising interleukin-2 (IL2) or interleukin-12 (IL12) and an antibody binding the extra-domain B (ED-B) of fibronectin, or a target binding fragment or derivative thereof, and (b) the conjugate radiolabelled with 177Lutetium develop a synergism that has so far not been discussed or even considered. In such way, the inventors were able to provide an improvement to current Lutetium-177 based therapies, and, in particular, broaden its potential indications.

A number of Lutetium 177-labelled radioconjugates have already received marketing approval or are in clinical development including:

(i) Lutathera™, a Lutetium 177-labelled somatostatin analogue peptide which has received marketing authorizations in Europe and in the United States (Hennrich & Kopka, (2019)

Pharmaceuticals, 12, 114) for the treatment of gastroenteropancreatic neuroendocrine tumors.

(ii) "PSMA-617", a Lutetium 177-labelled radioconjugate based on vipivotide tetraxetan, an inhibitor specific for Prostate-specific Membrane Antigen (PMSA) (Sanli et al. (2021) Biomedicines, 9, 430) for the treatment of metastatic castration-resistant prostate cancer.

(iii) "NeoBOMB1" a Lutetium 177-labelled radioconjugate based on a high-affinity gastrin-releasing peptide receptor (GRPR) antagonist (Nock et al., (2017) J Nucl Med, 58, 75-80). It can also be radiolabelled with Gallium 68 and is studied in patients with prostate and breast cancer.

(iv) "DOTA-JR11" also known as "OPS201", a Lutetium 177-labelled radioconjugate based on satoreotide tetraxane a somatostatin antagonistic peptide (Nicolas et al., (2017) J Nucl Med, 58. 1425-1431) which is being studied in neuroendocrine tumors.

(v) "PMSA-R2" an equivalent of "PSMA-617", a Lutetium 177-labelled, urea-based PSMA antigen carboxypeptidase-II which is being studied as a prostate cancer therapeutic agent.

(vi) "CTT1403" a Lutetium 177-labelled radioconjugate, phosphoramidate-based inhibitor with an albumin binding motif (Choy et al. (2017) Theranostics, 7, 1928-1939) which is being studied for treatment of metastatic prostate cancer.

(vii) "177Lu-RM2" a Lutetium 177-labelled radioconjugate, based on a high affinity GRPR antagonist (Kurth et al. (2020), Eur J Nucl Med Mol Imaging, 47, 123-125) for the treatment of metastatic castration-resistant prostate cancer.

(viii) "177Lu.DOTA.SA.FAPi" a Lutetium 177-labelled radioconjugate, based on a small molecule (4-quinolinoyl)glycyl-2-cyano-4,4-difluoropyrolidine Fibroblast Activation Protein (FAP) inhibitor (Ballal et al. (2021), Eur J Nucl Med Mol Imaging, 48, 942-944) for the treatment of tumors expressing FAP

(ix) "177Lu-ESV6-DOTA" a Lutetium 177-labelled radioconjugate based on a 2,2',2''-(10-(1-carboxy-4-((2-(4-((4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl) quinolin-8-yl)amino)-4-oxobutanamido)ethyl)amino)-4-oxobutyl)-1,4,7,10-

tetraazacyclododecane-1,4,7-triyl)triacetic acid, FAP inhibitor (WO2021/160825)

(x) "177Lu-Bi-ESV6-DOTA" which is a bivalent version of "177Lu-ESV6-DOTA" (WO2022/171811) not yet in clinical trial

(xi) "177Lu-ESV6-DOTAGA" a Lutetium 177-labelled radioconjugate based on a 2,2',2''-(10-(1-carboxy-4-((2-(4-((4-((2-((S)-2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl) quinolin-8-yl)amino)-4-oxobutanamido)ethyl)amino)-4-oxobutyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid, a small molecule FAP inhibitor for the treatment of tumors expressing FAP (WO2021/160825)

(xii) "177Lu-Bi-ESV6-DOTAGA" which is a bivalent version of "177Lu-ESV6-DOTAGA" not yet in clinical trial (WO2022/171811).

Combination of L19-IL2 or L19-IL12 and Lutetium 177-labelled radioconjugate

L19-IL2 has been studied in combination with a variety of therapeutic modalities generating different results, including in combination with Stereotactic Ablative Body Radiotherapy (SABR) in patients with Stage IV Non-Small Cells Lung Cancer (NSCLC's) (Lieverse et al., (2019) BMC Cancer, 20, 557), but to our knowledge it has never been tried in combination with a Lutetium 177-labelled radioconjugate.

L19-IL12 is being studied in a Phase I clinical trial (NCT04471987), but to our knowledge it has never been tried with a Lutetium 177-labelled radioconjugate.

According to another aspect of the invention, a dosage form is provided, comprising

(a) a recombinant protein comprising

(i) interleukin-2 (IL2) or interleukin-12 (IL12) and

(ii) an antibody binding the extra-domain B (ED-B) of fibronectin, or a target binding fragment or derivative thereof,

(b) a conjugate radiolabelled with 177Lutetium

in a pharmaceutically acceptable carrier.

As used herein, the term "dosage form" relates to an embodiment that may consist of

(i) at least one entity that comprises, in a solid or liquid form, the recombinant protein of (a) and the conjugate of (b), and/or

(ii) at least two entities, one of which comprising, in a solid or liquid form, the recombinant protein of (a), and the second of which comprising, in a solid or liquid form, the conjugate of (b),

Said dosage form can adopt the form of a vial, syringe, injection pen, infusion, tablet, lozenge, sachet, capsule, powder, aerosol or the like.

In addition, said dosage form may optionally comprise additional pharmaceutically acceptable excipients, including diluents, tonifiers, cryoprotectants, surfactants and the like.

According to another aspect of the invention, a combination is provided comprising at least

(a) a recombinant protein comprising

(i) interleukin-2 (IL2) or interleukin-12 (IL12) and

(ii) an antibody binding the extra-domain B (ED-B) of fibronectin, or a target binding fragment or derivative thereof, and

(b) a conjugate radiolabelled with 177Lutetium.

According to another aspect of the invention, a kit of dosage forms is provided, which kit comprises at least

(a) a first dosage form comprises a recombinant protein comprising

(i) interleukin-2 (IL2) or interleukin-12 (IL12) and

(ii) an antibody binding the extra-domain B (ED-B) of fibronectin, or a target binding fragment or derivative thereof,

in a pharmaceutically acceptable carrier and

(b) a second dosage form comprises a conjugate radiolabelled with 177Lutetium in a pharmaceutically acceptable carrier.

According to another aspect of the invention, a pharmaceutical composition is provided comprising

(a) a recombinant protein comprising

(i) interleukin-2 (IL2) or interleukin-12 (IL12) and, fused or conjugated thereto

(ii) an antibody binding the extra-domain B (ED-B) of fibronectin, or a target binding fragment or derivative thereof,

which antibody or fragment or derivative comprises a set of three heavy chain and three light chain complementarity determining regions (CDR) as set forth in SEQ ID NOs 7-12, and

(b) a conjugate radiolabelled with 177Lutetium,

which conjugate is 177Lu-PSMA-617

The inventors have surprisingly shown that such combination has even better therapeutic activity as compared to the single agents. Furthermore, unexpectedly, the combination maintains a comparable or superior safety profile as compared to the single agents.

177Lu-PSMA-617 (INN: Lutetium (177Lu) vipivotide tetraxetan, sold under the brand name Pluvicto®, is a radiopharmaceutical medication used for the treatment of prostate-specific membrane antigen (PSMA)-positive metastatic castration-resistant prostate cancer (mCRPC).

It comprises PSMA-617, a human prostate-specific membrane antigen (PSMA)-targeting ligand, conjugated to the beta-emitting radioisotope Lutetium177, and has antineoplastic activity against PSMA-expressing tumor cells. Upon intravenous administration of lutetium, the conjugate targets and binds to PSMA-expressing tumor cells. Upon binding, the PSMA expressing cells are destroyed by Lutetium177 through the specific delivery of beta particle radiation.

The inventors have found that the two components given together are better tolerated in terms of body weight change, than the single agents.

This is surprising considering that administration of two therapeutic agents is expected to increase toxicity rather than reducing toxicity.

Even more surprisingly, the inventors have found that the recombinant protein comprising interleukin-2 (IL2) or interleukin-12 (IL12) and an antibody binding the extra-domain B (ED-B) and a conjugate radiolabelled with 177Lutetium given together activate the immune system better than the recombinant protein comprising interleukin-2 (IL2) or interleukin-12 (IL12) and an antibody binding the extra-domain B (ED-B) of fibronectin alone.

This is surprising considering that while the recombinant protein comprising interleukin-2 (IL2) or interleukin-12 (IL12) and an antibody binding the extra-domain B (ED-B) of fibronectin (L19-IL2 or L19-IL12) are well-known immune system activators, the conjugate radiolabelled with 177Lutetium (177Lu-PSMA617) is not expected to activate the immune system, since its therapeutic activity on cancer cells is promoted by the β emissions.

One antibody that comprises the complementarity determining regions (CDR) as set forth in SEQ ID NOs 7-12 is L19, discussed elsewhere herein.

According to another aspect of the invention, a dosage form is provided, comprising

(a) a recombinant protein comprising

(i) interleukin-2 (IL2) or interleukin-12 (IL12) and, fused or conjugated thereto

(ii) an antibody binding the extra-domain B (ED-B) of fibronectin, or a target binding fragment or derivative thereof,

which antibody or fragment or derivative comprises a set of three heavy chain and three light chain complementarity determining regions (CDR) as set forth in SEQ ID NOs 7-12, and

(b) a conjugate radiolabelled with 177Lutetium,

which conjugate is 177Lu-PSMA-617

in a pharmaceutically acceptable carrier.

According to another aspect of the invention, a combination is provided, comprising at least

(a) a recombinant protein comprising

(i) interleukin-2 (IL2) or interleukin-12 (IL12) and, fused or conjugated thereto

(ii) an antibody binding the extra-domain B (ED-B) of fibronectin, or a target binding fragment or derivative thereof,

which antibody or fragment or derivative comprises a set of three heavy chain and three light chain complementarity determining regions (CDR) as set forth in SEQ ID NOs 7-12, and

(b) a conjugate radiolabelled with 177Lutetium,

which conjugate is 177Lu-PSMA-617

According to another aspect of the invention, a kit of dosage forms is provided, comprising at least:

(a) a first dosage form comprising a recombinant protein comprising

(i) interleukin-2 (IL2) or interleukin-12 (IL12) and, fused or conjugated thereto

(ii) an antibody binding the extra-domain B (ED-B) of fibronectin, or a target binding fragment or derivative thereof,

which antibody or fragment or derivative comprises a set of three heavy chain and three light chain complementarity determining regions (CDR) as set forth in SEQ ID NOs 7-12,

in a pharmaceutically acceptable carrier and

(b) a second dosage form comprising a conjugate radiolabelled with 177Lutetium,

which conjugate is 177Lu-PSMA-617

in a pharmaceutically acceptable carrier.

According to embodiments, in the pharmaceutical composition, dosage form, combination, or kit according to the above description, the antibody or fragment or derivative comprises:

a) the heavy chain/light chain variable domain (HCVD/LCVD) pair set forth in SEQ ID NOs 2 and 3,

b) the heavy chain/light chain variable domains (HCVD/LCVD) pairs of a), with the proviso that

- the HCVD has a sequence identity of ≥ 80 % to the respective SEQ ID NO, and/or
- the LCVD has a sequence identity of ≥ 80 % to the respective SEQ ID NO, or

c) the heavy chain/light chain variable domains (VD) pairs of a) or b), with the proviso that at least one of the HCVD or LCVD has up to 10 amino acid substitutions relative to the respective SEQ ID NO.

Said antibody or fragment is still capable to bind to the extra-domain B (ED-B) of fibronectin

One antibody that comprises the heavy chain/light chain variable domain (HCVD/LCVD) pair set forth in SEQ ID NOs 2 and 3 is L19, discussed elsewhere herein.

According to embodiments of the pharmaceutical composition, dosage form, combination, or kit according to the above description, at least one amino acid substitution in the antibody is a conservative amino acid substitution.

According to embodiments of the pharmaceutical composition, dosage form, combination, or kit according to the above description, the antibody or fragment or derivative is provided in the single-chain Fv (scFv) format or in the single-chain diabody (scDb) format.

According to embodiments, the single-chain Fv format is provided for L19-IL2, and the single-chain diabody format is provided for L19-IL12.

In one embodiment, the L19-IL2 single-chain Fv format has the following structure, in N->C orientation: VH-linker-VL-linker-IL2.

In one embodiment, the L19-IL12 single-chain diabody format has the following structure, in N->C orientation: p40-linker-p35-linker-VH-linker-VL-linker-VH-linker-VL.
According to embodiments of the pharmaceutical composition, dosage form, combination, or kit according to the above description, the recombinant protein is Darleukin or Dodekin.

Darleukin (INN: bifikafusp alpha) is an antibody-cytokine fusion protein consisting of a single-chain Fv (scFv) antibody fragment directed against the extra-domain B (ED-B) of fibronectin, called L19, and human IL-2. The antibody binds to the tumor and deliver IL-2 into the tumor vasculature and microenvironment, thereby directly stimulating immune effector cells at the tumor site.

Darleukin is disclosed in, *inter alia*, WO2001/062298, the content of which is incorporated herein by reference. The complete amino acid sequence of Darleukin is given in SEQ ID NO: 1.

Dodekin or Dodeka is an antibody-cytokine fusion protein consisting of a single-chain diabody (scDb) antibody fragment directed against the extra-domain B (ED-B) of fibronectin, called L19, and human IL12. Dodekin is disclosed in, inter alia, WO2019/154986 the content of which is incorporated herein by reference. The complete amino acid sequence of Dodekin is given in SEQ ID NO: 14.

According to several embodiments of the invention (pharmaceutical composition, dosage form, combination, kit of dosage forms)

(a) the recombinant protein comprising

(i) interleukin-2 (IL2) or interleukin-12 (IL12) and

(ii) an antibody binding the extra-domain B (ED-B) of fibronectin, or a target binding fragment or derivative thereof, and

(b) the conjugate radiolabelled with 177Lutetium

are administered or taken simultaneously.

According to several embodiments of the invention (pharmaceutical composition, dosage form, combination, kit of dosage forms),

(a) the recombinant protein comprising

(i) interleukin-2 (IL2) or interleukin-12 (IL12) and

(ii) an antibody binding the extra-domain B (ED-B) of fibronectin, or a target binding fragment or derivative thereof, and

(b) the conjugate radiolabelled with 177Lutetium

are administered or taken sequentially.

In this embodiment, the recombinant protein comprising interleukin-2 (IL2) or interleukin-12 (IL12) and an antibody binding the extra-domain B (ED-B) of fibronectin, or a target binding fragment or derivative thereof, is administered or taken either before the conjugate radiolabelled with 177Lutetium, or *vice versa*.

According to several embodiments of the invention, the pharmaceutical composition, dosage form, combination, or kit is provided for (the manufacture of a medicament consisting of at least one dosage form for) use in the treatment of a human or mammalian patient

(i) being diagnosed for,

(ii) suffering from or

(iii) being at risk of developing

cancer.

This language is deemed to encompass both the Swiss type claim language accepted in some countries (in this case, brackets are deemed absent) and EPC2000 language (in this case, brackets and content within the brackets are deemed absent).

According to one aspect of the invention, a method for treating a human or mammalian patient is provided, which method comprises administration of one or more effective amounts of the pharmaceutical composition, dosage form combination or kit according to the above description.

According to one embodiment of that method, the human or mammalian patient

(i) is diagnosed for,

(ii) suffers from or

(iii) is at risk of developing

cancer.

According to several embodiments of the invention, the cancer is at least one selected from the group consisting of

(i) Neuroendocrine tumors such as: thyroid cancers including medullary carcinoma, pulmonary neuroendocrine tumors including pulmonary carcinoid tumor, bronchus cancer, large cell neuroendocrine carcinoma of the lung, extrapulmonary small cell carcinomas, gastroenteropancreatic neuroendocrine tumors, prostate cancer, pheochromocytoma, peripheral nervous system tumor including schwannoma, paraganglioma, neuroblastoma, liver and gallbladder cancer, adrenal tumors, genitourinary tract cancers including ovary tumor, testes tumor, neuroendocrine tumor of the cervix, Merkel cell carcinoma.

(ii) GRPR positive tumors such as: small cell lung cancer, breast cancer, colorectal cancer, pancreatic cancer, renal cell carcinoma, head and neck cancers including squamous cell carcinoma, esophageal squamous cell carcinoma, nonatral gastric adenocarcinoma, leiomyomas,

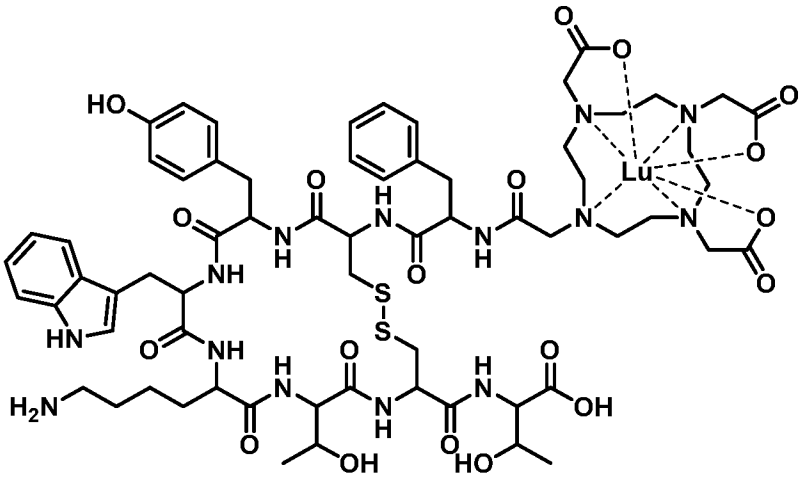
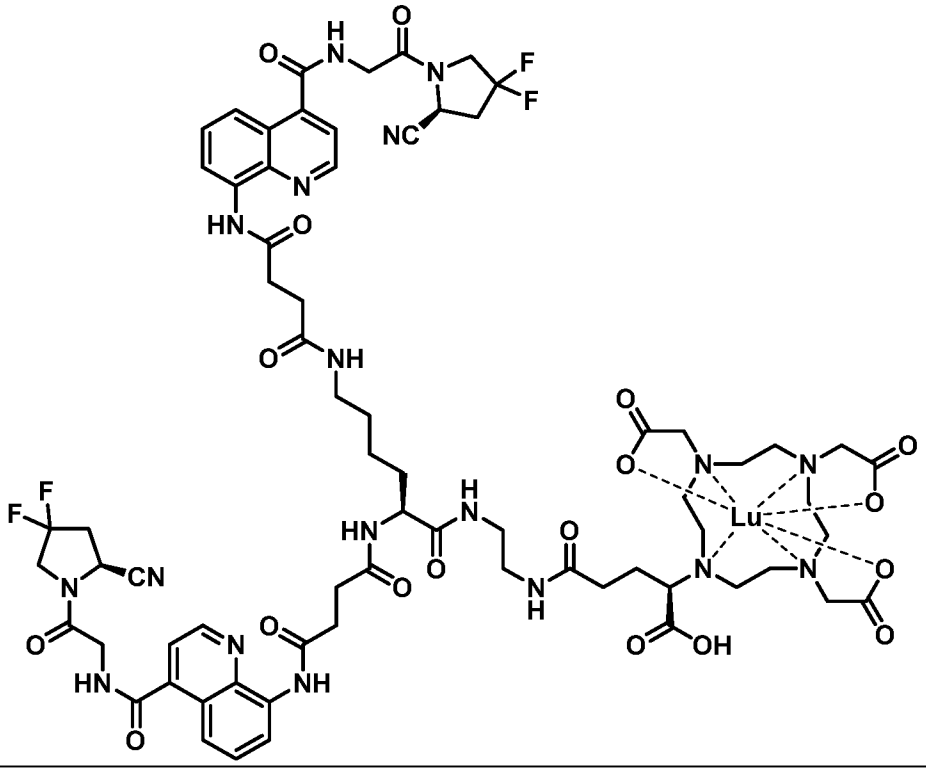
(iii) FAP positive tumors such as: soft tissue sarcomas, brain tumors including glioblastoma, intrahepatic bile duct tumors, ovarian cancer, melanoma, myeloma including multiple myeloma, gastric cancer,

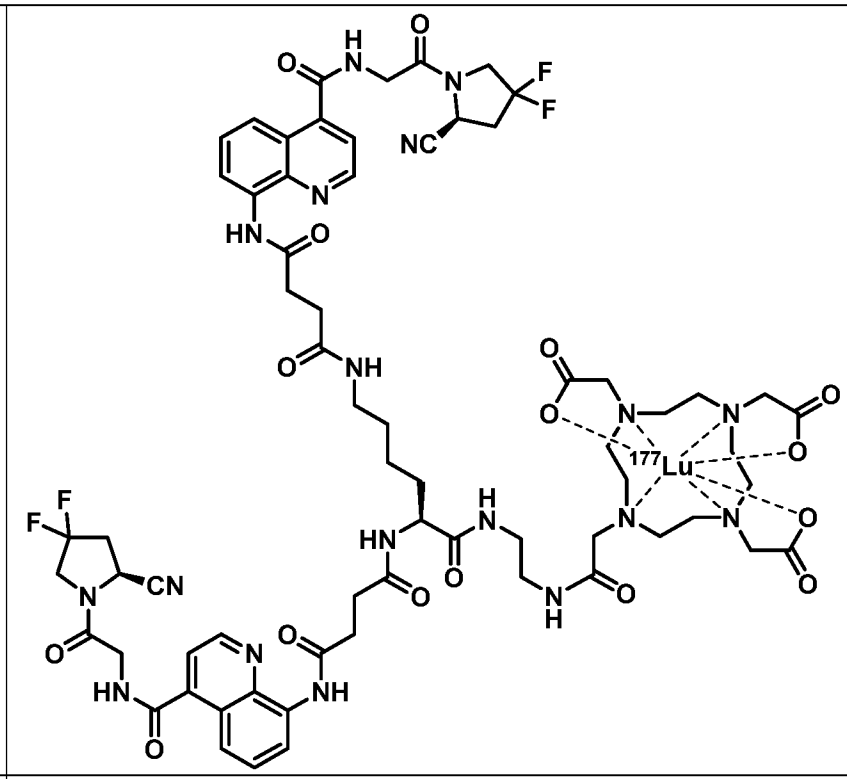
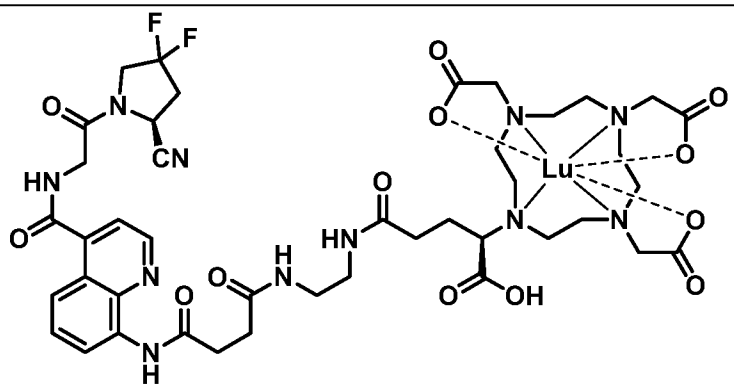
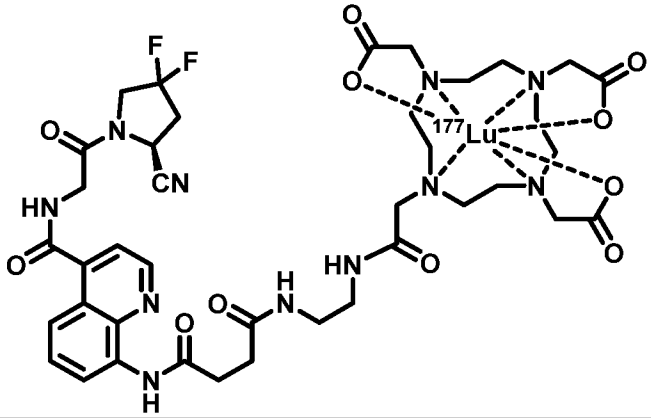
(iv) other tumors such as: small intestine cancer, multi-drug resistant colon cancer, rectal cancer, lung cancer, non-small cell lung cancer, hepatocellular cancer, hypopharynx cancer, nasopharynx cancer, larynx cancer, myeloma cells, bladder cancer, cholangiocarcinoma, clear cell renal carcinoma, oncogenic osteomalacia, sarcoma, CUP (carcinoma of unknown primary), thymus cancer, desmoid tumours, glioma, astrocytoma, cervix cancer and skin cancer.

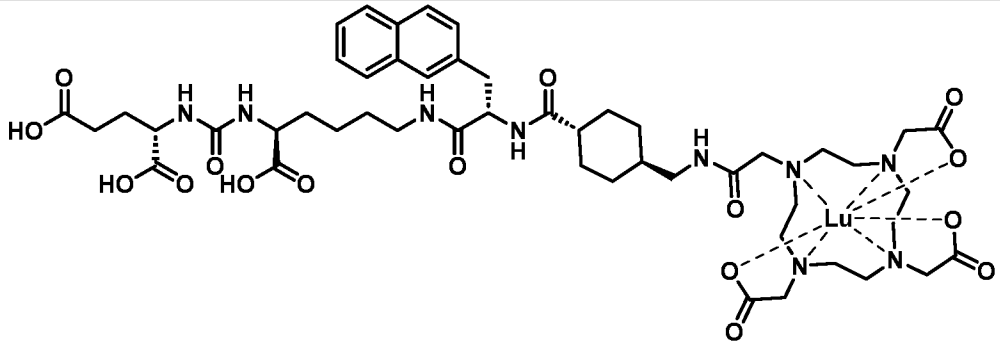
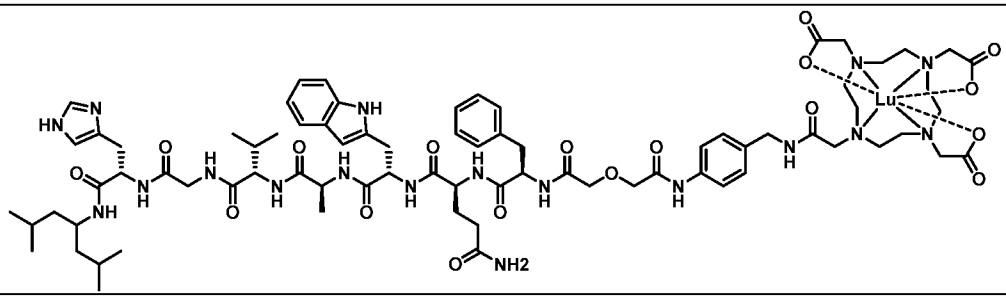
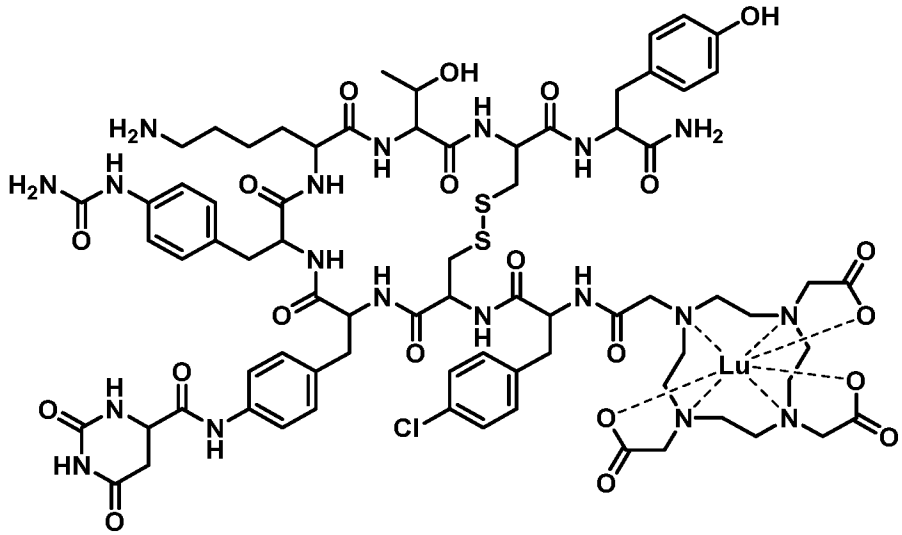
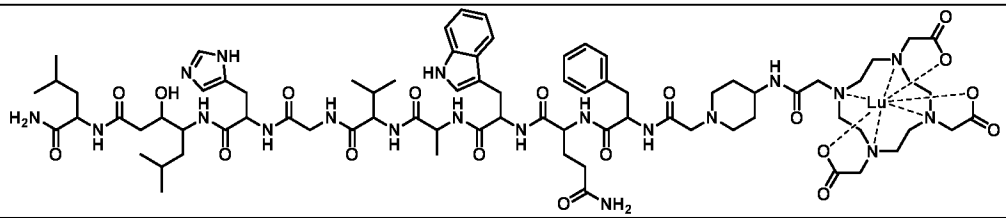
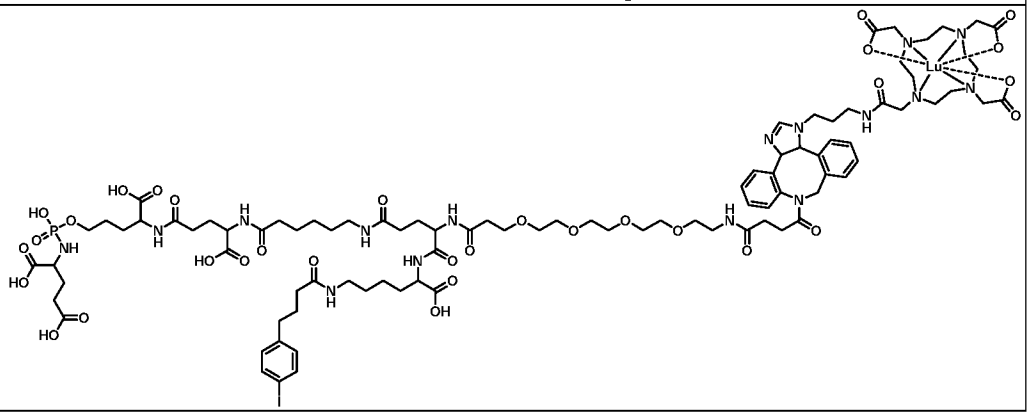
(v) PSMA positive tumors such as: prostate cancer, metastatic prostate cancer, prostate adenocarcinoma, ductal prostate cancer, ductal adenocarcinoma, clear cell adenocarcinoma, acinar adenocarcinoma, urothelial cancer, neuroendocrine prostate cancer, small cell prostate cancer, hormone sensitive prostate cancer (HSPC), and castration resistant, prostate cancer (CRPC). In some embodiments, the metastatic prostate cancer may be metastatic hormone sensitive prostate cancer (mHSPC) or metastatic castration resistant prostate cancer (mCRPC). According to several embodiments of the invention, the conjugate radiolabelled with 177Lutetium comprises one or more of the conjugates show in Table 1.

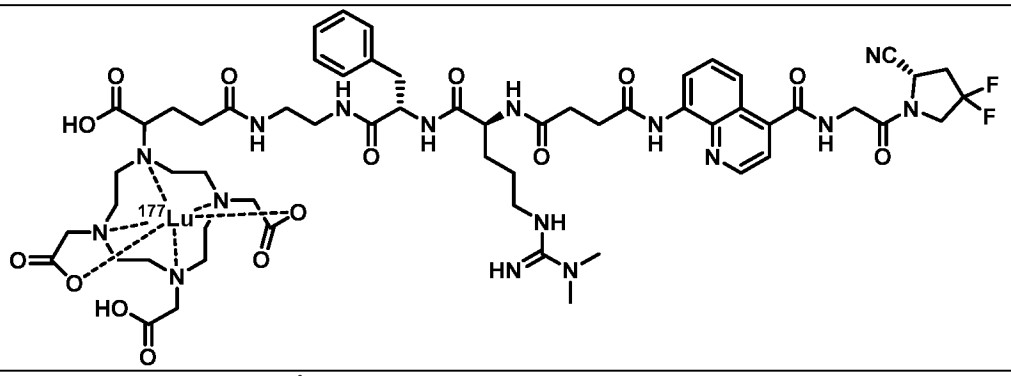
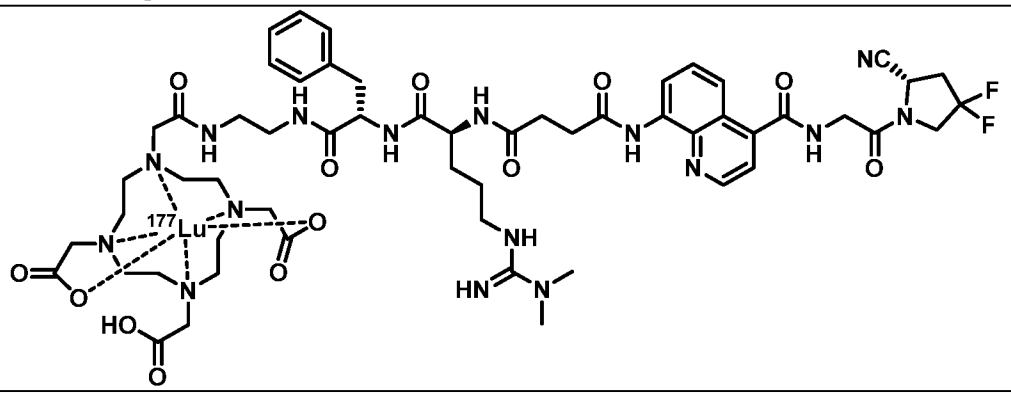
The names and the structures of Lutetium 177-labelled radioconjugates disclosed herein are summarized in the following Table 1. 177Lu-PSMA-617 is one of them.

Table 1: structures of Lutetium 177-labelled radioconjugates

Compound	Chemical structure
Lutathera	
177Lu-BiOncoFAP-DOTAGA (177Lu-Bi-ESV6-DOTAGA)	

177Lu-BiOncoFAP-DOTA (177Lu-Bi-ESV6-DOTA)	
177Lu-OncoFAP-DOTAGA (177Lu-ESV6-DOTAGA)	
177Lu-OncoFAP-DOTA (177Lu-ESV6-DOTA)	

Pluvicto (177Lu-PSMA-617)	
NeoBomb1	
DOTA-JR11	
RM2	
CTT1403	

177Lu-OncoFAP11-DOTAGA (177Lu-ESV6-11-DOTAGA)	
177Lu-OncoFAP11-DOTA (177Lu-ESV6-11-DOTA)	

Fibronectin isoform B-FN and L19

Fibronectin isoform B-FN is one of the best-known markers of angiogenesis (WO97/45544). An extra domain "ED-B" of 91 amino acids is found in the B-FN isoform and is identical in mouse, rat, rabbit, dog and man. B-FN accumulates around neovascular structures in aggressive tumours and other tissues undergoing angiogenesis, such as the endometrium in the proliferative phase and some ocular structures in pathological conditions, but is otherwise undetectable in normal adult tissues.

The extra-domain B (ED-B) of fibronectin is an attractive target for anti-cancer therapy, including the use of immunocytokines as discussed herein.

L19 is a human monoclonal scFv specific alternatively spliced ED-B domain of fibronectin and has been previously described (WO1999/058570; WO2003/076469, WO2005/023318).

"L19 antibody" as used herein means any antibody that binds to EDB Fibronectin or any portion thereof and comprises an amino acid sequence having at least seventy-five percent (75%) identity to one or more of the following amino acid sequences:

L19 VH (SEQ ID NO: 2)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAPGKGLEWVSSISGSSGTTYYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKPFPYFDYWGQGTLVTVSS

L19 VL (SEQ ID NO: 3)

EIVLTQSPGTLSLSPGERATLSCRASQSVSSSFLAWYQQKPGQAPRLLIYYASSRATGIPDR
FSGSGSGTDFTLTISRLEPEDFAVYYCQQTGRIPPTFGQGTKVEIK

CDR1 VH (SEQ ID NO: 7)

SFSMS

CDR2 VH (SEQ ID NO: 8)

SISGSSGTTYYADSVKG

CDR3 VH (SEQ ID NO: 9)

PFPYFDY

CDR1 VL (SEQ ID NO: 10)

RASQSVSSSFLA

CDR2 VL (SEQ ID NO: 11)

YASSRAT

CDR3 VL (SEQ ID NO: 12)

QQTGRIPPT

L19 scFv (SEQ ID NO: 13)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAPGKGLEWVSSISGSSGTTYYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKPFPYFDYWGQGTLVTVSSGDGSSGGS
GGASEIVLTQSPGTLSLSPGERATLSCRASQSVSSSFLAWYQQKPGQAPRLLIYYASSRATG
IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQTGRIPPTFGQGTKVEIK

Interleukin-2 (IL2)

IL2 in the L19-IL2 conjugate retains a biological activity of IL2, e.g. an ability to promote proliferation and differentiation of activated T and B lymphocytes and natural killer (NK) cells,

induce cytotoxic T cell (CTL) activity, and/or NK/lymphokine-activated killer (LAK) cell antitumor cytotoxicity. The amino acid sequence of human IL-2 is set out in SEQ ID NO: 6.

L19-IL2 conjugate

L19-IL2 is disclosed in WO2001/062298 by the current applicants, the content of which is incorporated herein by reference in its entirety, and has been tested in a variety of therapeutic regimens, modalities, molecular formats and combinations for treatment of different types of cancer. These embodiments are disclosed in *inter alia* WO2007/115837, WO2009/089858, WO2013/010749, WO2013/045125, WO2018/115377, WO2018/154517, WO2020/070150 the content of which is incorporated herein by reference in its entirety, with good results. The complete amino acid sequence of L19-IL2 is given in SEQ ID NO: 1.

Interleukin-12 (IL12)

IL12 in the L19-IL12 conjugate retains a biological activity of IL12, e.g. an ability to inducing CD4+ T cells to secrete interferon-gamma (IFN-γ) and leading to a CD8+ cytotoxic T cell response. It is made up of two subunits, p35 and p40, both of which are required for the secretion of the active form of IL12. The amino acid sequence of p40 is set out in SEQ ID NO: 16. The amino acid sequence of p35 is set out in SEQ ID NO: 17 and the linker between the p40 and p35 subunits in the L19-IL12 immunoconjugate is set out in SEQ ID NO: 18.

L19-IL12 conjugate

L19-IL12 is disclosed in WO2019/154986 by the current applicants, the content of which is incorporated herein by reference in its entirety, and has been tested in a variety of therapeutic regimens, modalities, molecular formats and combinations for treatment of different types of cancer. These embodiments are disclosed in *inter alia* in WO2021/209452, WO2023/131611 the content of which is incorporated herein by reference in its entirety, with good results. The complete amino acid sequence of L19-IL12 is given in SEQ ID NO: 14. The complete amino acid sequence of L19-murineIL12 used in the examples below is given in SEQ ID NO: 15.

Examples

While the invention has been illustrated and described in detail in the drawings and foregoing description, such illustration and description are to be considered illustrative or exemplary and not restrictive; the invention is not limited to the disclosed embodiments. Other variations to the disclosed embodiments can be understood and effected by those skilled in the art in practicing the claimed invention, from a study of the drawings, the disclosure, and the appended claims. In the claims, the word "comprising" does not exclude other elements or steps, and the indefinite article "a" or "an" does not exclude a plurality. The mere fact that certain measures are recited in mutually different dependent claims does not indicate that a combination of these measures cannot be used to advantage. Any reference signs in the claims should not be construed as limiting the scope.

All amino acid sequences disclosed herein are shown from N-terminus to C-terminus.

Example 1: Therapy Studies in mice on SK-RC-52.hFAP cancer model

Studies were conducted injecting subcutaneously SK-RC-52.hFAP (cancer cells expressing FAP) in mice.

1.1 Cell culture

SK-RC-52.hFAP cells were grown to 80% confluence in RPMI-1640 medium with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic and detached with Trypsin-EDTA (ethylenediaminetetraacetic acid) 0.05%. Tumour cells were resuspended in Hanks' Balanced Salt Solution medium. Aliquots of 5 million cells (100 µL of suspension) were injected subcutaneously in the right flank of female athymic Balb/c AnNRj-Foxn1 mice (6 to 8 wk of age).

1.2 Radiolabelling with 177Lu

Radiolabeling of ESV6-DOTAGA and Bi-ESV6-DOTAGA with lutetium-177 was performed as follows: precursors (25 nmol) were dissolved in 25 µL of milliQ water, then sodium acetate buffer (75 µL, 1 M in water, pH 4.5) and 25 MBq of 177Lu solution were added. The mixture was heated at 90°C for 1 minutes followed by dilution with 400 µL of PBS to afford a final

volume of 500 µL (5 doses of 100 µL each). Quality control of radiosynthesis was performed using radio-HPLC.

1.3 Therapeutic efficacy evaluation in tumour bearing mice

The anti-cancer efficacy of 177Lu-ESV6-DOTAGA, 177Lu-Bi-ESV6-DOTAGA, L19-IL2 and of the combinations 177Lu-ESV6-DOTAGA + L19-IL2 and 177Lu-Bi-ESV6-DOTAGA + L19-IL2 was assessed in athymic Balb/c AnNRj-Foxn1 mice bearing SK-RC-52.hFAP tumor in the right flank. Intravenous injections of 177Lu-ESV6-DOTAGA (250 nmol/kg, 250 MBq/kg), 177Lu-Bi-ESV6-DOTAGA (250 nmol/kg, 250 MBq/kg), L19-IL2 (2.5 mg/kg), or vehicles were performed with the schedule indicated in Figure 1. Therapy experiments started when the average volume of established tumours had reached 100-150 mm^3. Body weight of the animals (Figure 2) and tumour volume were daily measured and recorded (Figure 1). Tumour dimensions were measured with an electronic caliper and tumour volume was calculated with the formula (long side, mm) × (short side, mm) × (short side, mm) × 0.5. Animals were euthanized when one or more termination criterium indicated by the experimental license was reached. Prism 7 software (GraphPad Software) was used for data analysis.

1.4 Results

Therapeutic efficacy of 177Lu-ESV6-DOTAGA and of 177Lu-Bi-ESV6-DOTAGA as monotherapy or administered in combination with L19-IL2 was assessed in mice bearing SK-RC-52.hFAP tumors on the right flank (**Figure 1**). Systemic administration of 177Lu-ESV6-DOTAGA and of 177Lu-Bi-ESV6-DOTAGA (5 MBq/mouse, 250 nmol/kg) and of L19-IL2 (2.5 mg/kg) resulted in selective and potent anti-cancer activity against the growth of SK-RC-52.hFAP as compared to mice injected with saline. L19-IL2 potently synergizes with radioligand therapeutics (177Lu-ESV6-DOTAGA and 177Lu-Bi-ESV6-DOTAGA). The most active treatment in the therapy study was 177Lu-Bi-ESV6-DOTAGA in combination with L19-IL2, which showed superior anti-cancer activity compared to monotherapies based on single agents (177Lu-Bi-ESV6-DOTAGA at 5 MBq/mouse, or L19-IL2 at 2.5 mg/kg). All mice administered with 177Lu-Bi-ESV6-DOTAGA + L19-IL2 were cured after treatment. No significant body weight loss and no signs of toxicity were observed during and after injection of 177Lu-ESV6-DOTAGA, 177Lu-Bi-ESV6-DOTAGA, L19-IL2 administered as monotherapy or in combination (**Figure 2**).

Example 2: Therapy Studies in mice on HT-1080.hFAP cancer model

Studies were conducted injecting subcutaneously HT-1080.hFAP (cancer cells expressing FAP) in mice.

2.1 Cell culture

HT-1080.hFAP cells were grown to 80% confluence in DMEM with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic and detached with Trypsin-EDTA (ethylenediaminetetraacetic acid) 0.05%. Tumour cells were resuspended in Hanks' Balanced Salt Solution medium. Aliquots of 2 million cells (50 µL of suspension) were injected subcutaneously in the right shoulder of female athymic Balb/c AnNRj-Foxn1 mice (6 to 8 wk of age).

2.2 Radiolabelling with 177Lu

Radiolabeling of ESV6-DOTAGA and Bi-ESV6-DOTAGA with lutetium-177 was performed as follows: precursors (~650 nmol) were dissolved in 34.3 mL of sodium acetate buffer (34.3 mL, 0.5 M in water, pH 5.2), then 700 µL of a sterile filtered ascorbic acid solution (250 g/L in ultrapure water) were added. 700 µL of above solutions were separately added to 1 GBq of $^{177}LuCl_3$. The mixture was heated to 90°C for 20 min. After cooling, radiochemical purity was verified by radio-HPLC to confirm complete Lutetium-177 incorporation. A premixed solution of 150 µL phosphate buffer and 50 µL Sodium Ascorbate solution (100 mg/mL) was added. The solutions were then diluted with saline (NaCl solution) to a final dose of 5 MBq/100 µL.

2.3 Therapeutic efficacy evaluation in tumour bearing mice

The anti-cancer efficacy of 177Lu-ESV6-DOTAGA, 177Lu-Bi-ESV6-DOTAGA, L19-IL2 and of the combinations 177Lu-ESV6-DOTAGA + L19-IL2 and 177Lu-Bi-ESV6-DOTAGA + L19-IL2 was assessed in athymic Balb/c AnNRj-Foxn1 mice bearing HT-1080.hFAP tumor in the right shoulder.

The aforementioned radiolabelled preparations of 177Lu-ESV6-DOTAGA and 177Lu-Bi-ESV6-DOTAGA were transferred into 1 mL syringes (100 µL/syringe; 1mL Sub-Q, 0,45mm x 12,7mm). The full volume (100 µL) was injected through a tail vein catheter with the syringe adapted with a 5 cm long hose. The syringe and hose were flushed with 50 µL of NaCl solution. Injections of 177Lu-ESV6-DOTAGA (i.v., 250 MBq/kg), 177Lu-Bi-ESV6-DOTAGA (i.v., 250 nmol/kg, 250 MBq/kg), L19-IL2 (s.c., 2.5 mg/kg), or saline were performed with the schedule indicated in Figure 3.

Tumor volume of the animals was daily measured and recorded. Tumour dimensions were measured with an electronic caliper and tumour volume was calculated with the formula (long side, mm) × (short side, mm) × (short side, mm) × 0.5. Animals were euthanized when one or more termination criterium indicated by the experimental license was reached. Prism 7 software (GraphPad Software) was used for data analysis.

2.4 Results

Therapeutic efficacy of 177Lu-ESV6-DOTAGA and of 177Lu-Bi-ESV6-DOTAGA as monotherapy or administered in combination with L19-IL2 was assessed in mice bearing HT-1080.hFAP tumors on the right flank (**Figure 3**). Systemic administration of 177Lu-ESV6-DOTAGA and of 177Lu-Bi-ESV6-DOTAGA (5 MBq/mouse, 250 nmol/kg) and of L19-IL2 (2.5 mg/kg) resulted in selective and potent anti-cancer activity against the growth of HT-1080.hFAP as compared to mice injected with saline. L19-IL2 potently synergizes with radioligand therapeutics (177Lu-ESV6-DOTAGA and 177Lu-Bi-ESV6-DOTAGA). The most active treatment in the therapy study was 177Lu-Bi-ESV6-DOTAGA in combination with L19-IL2, which showed superior anti-cancer activity compared to monotherapies based on single agents (177Lu-Bi-ESV6-DOTAGA at 5 MBq/mouse, or L19-IL2 at 2.5 mg/kg). All mice administered with 177Lu-Bi-ESV6-DOTAGA + L19-IL2 were cured after treatment.

Example 3: Proteomics Analysis in the SK-RC-52.hFAP tumor model

3.1 Materials and Methods

Balb/c AnNRj-Foxn1 mice were implanted with SK-RC-52.hFAP tumor cells as mentioned in Example 1 and then treated with either 177Lu-Bi-ESV6-DOTAGA, L19-IL2 or with the

combinations 177Lu-Bi-ESV6-DOTAGA + L19-IL2 following the schedule presented in Fig. 1. Mice were sacrificed at day 15 after tumor implantation (7 days after beginning of therapeutic treatments) by CO_2 asphyxiation, tumors were harvested and snap-frozen with liquid nitrogen. Tissues were resuspended at final concentration of 0.1 mg (wet tissue)/mL in an aqueous solution containing Urea 8 M, Tris-HCl 50 mM, 100 mM NaCl at pH = 8, with protease inhibitors. Samples were homogenized with a tissue lyser (TissueLyser II, QIAGEN) for 1 minutes at 30 Hz two times at 4 °C. The homogenised samples were sonicated for 2 minutes at 50 % intervals and 36 % intensity. After sonication samples were centrifugated for 10 minutes at 15000 g and protein concentration of the supernatant was measured with BCA kit following kit instructions.

20 µg of proteins for each sample were diluted to the final volume of 200 µL with an aqueous solution containing 50 mM Tris-HCl, 1 mM $CaCl_2$ pH 8.0. Proteins were reduced with TCEP for 15 min at RT followed by 30 min at 65 °C and alkylated with iodoacetamide for 30 mins in the dark. Proteins were then digested by trypsin (enzyme-protein ratio 1:50) at 37 °C overnight. After digestion, samples were acidified with 10% formic acid and then subjected to C18 purification and desalting (Macro Spin Columns). Purified samples were dried under vacuum and resuspended in 120 µL of an aqueous solution containing 3% acetonitrile and 0.1% formic acid. 1 µL of the sample was then subjected to nanoHPLC-HRMS analysis. All samples were analysed on an Orbitrap Q-Exactive mass spectrometer coupled to an EASY nanoLC 1000 system via a Nano Flex ion source. Chromatographic separation was carried out at room temperature on an Acclaim PepMap RSLC column (50 µm x 15 cm, particle size 2 µm, pore size, 100 Å), using 120 min linear gradient with 5-35% solvent B (0.1% formic acid in acetonitrile) at a flow rate of 300 nL/min. Ionization was carried out in positive ion mode, with 2 kV of spray voltage, 250 °C of capillary temperature, 60 S-lens RF level. The mass spectrometer was working in a data-dependent mode. MS1 scan range was set from 350 to 1650 m/z, the 10 most abundant peptides were subjected to HCD fragmentation with NCE of 25. A dynamic exclusion was set at 20 seconds. Raw files were processed with Proteome Discoverer 2.5 (Thermo Fisher) for quantitative analysis. Database searches were performed with Sequest as search engine using a FASTA file containing the human and *Mus musculus* and *Homo sapiens* reference proteome, Carbamidomethylation of cysteines was set as a fixed modification, and trypsin was set as cleavage specificity allowing a maximum of 2 missed cleavages. Data filtering was performed using percolator, resulting in 1% false discovery rate (FDR). The output file was exported as excel and data mining was carried out with Python and

Prism (GraphPad). Intensities were transformed in logarithm base 2, missing values were imputed with the minimum value observed in all samples. Intensities for each analysis were normalized on the median of each sample. Average and standard deviation for each protein of each group of treatment were calculated and multiple t-student test was performed. FDR (two stage set up method from Benjiamini, Kriger, Yekutieli) was set at 1% (q value < 0.001). Statistically significant up or down regulated proteins were identified setting the fold change at ± 1.

Results of the proteomics analysis are reported in Figures 4 and 5.

3.2 Results

The impact on the regulation of the expression of proteins in the "177Lu-Bi-ESV6-DOTAGA + L19-IL2" group (Figure 4C) was surprisingly much higher than the simple combination of the results obtained in the single treatments (Figures 4A and 4B).

Levels of proteins expressed on surface of tumor cells (i.e., human FAP (Fibroblast Activation Protein) and human CAIX (Carbonic Anhydrase IX)) were lower in the "177Lu-Bi-ESV6-DOTAGA + L19-IL2" group than the simple addition of the single treatments (Figures 5A and 5B), due to tumor cell death. Biomarkers of host NK cells activity as Perforin-1 and Granzymes (i.e., A, C, B, G, E, F, D, K) were surprisingly higher in the "177Lu-Bi-ESV6-DOTAGA + L19-IL2" group than the simple addition of the single treatments (Figures 5C and 5G). Similarly, markers of the engagement of immune cells such as B cells, T cells, NK (CD48) cells and Macrophages (CD68) were surprisingly higher in the "177Lu-Bi-ESV6-DOTAGA + L19-IL2" group than the simple addition of the single treatments (Figures 5E and 5F).

Hence, the combination as set forth in the patent claims has a synergistic effect over the administration of the individual components alone.

Example 4: Immunofluorescence in the SK-RC-52.hFAP tumor model

4.1 Materials and Methods

SK-RC-52.hFAP tumors were excised, snap-frozen in Optimal Cutting Temperature medium and stored at - 80°C. Cryostat sections (10 µm) were cut, fixed with acetone, blocked with 20% FBS in 3% BSA/PBS for 1h, washed twice with PBS, and subjected to immunofluorescence. Primary staining for NK cells was performed with a rat antimurine NKp46 IgG (NCR1) (green, 1:200 in 3% BSA/PBS). Donkey anti-rat IgG-AlexaFluor594 was then used for microscopic detection as secondary antibodies. Cell nuclei were stained with 150 µL/slide of DAPI (D1306) (blue, 1 µg/mL in PBS). After washing three times with PBS in the dark, slides were mounted with fluorescent mounting medium and analyzed with a Leica DMI6000B microscope equipped with 20 x 0.7 NA HC PlanApo objective (20x magnification) using 405 nm, 488 nm laser lines. Pictures were collected using Andor iXon EM CCD camera and elaborated using Leica LAS AF software and Fiji.

4.2 Results

Monotherapy with 177Lu-Bi-ESV6-DOTAGA or L19-IL2 resulted in a low to moderate increase of infiltrating NK cells in the SK-RC-52.hFAP tumors. Contrarily, treatment with a combination of 177Lu-Bi-ESV6-DOTAGA and L19-IL2 resulted in a surprisingly high infiltration of NK-cells in the tumors. Interestingly, in the slice of "177Lu-Bi-ESV6-DOTAGA and L19-IL2" group, cell nuclei (DAPI, blue) appeared to be smaller, which is due to reduced presence of SK-RC-52.hFAP cells, characterized by big nuclei, as shown in the saline slice, and increased presence of NK cells. Those data were in line with the outcome of the proteomic analysis (i.e., reduced tumor cells and increased infiltrating inflammatory cells).

Results of the immunofluorescence analysis are shown in Figures 6A - 6C. "POS" stands for staining with a target specific primary antibody and a labelled secondary antibody. "NEG" stands for staining with a labelled secondary antibody only.

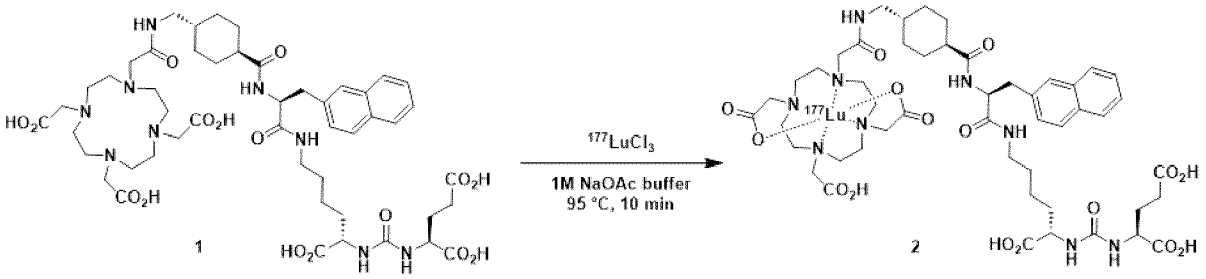
In Figure 6A, it can be seen that treatment with 177Lu-Bi-ESV6-DOTAGA alone results in a few green dots, representing a few attracted NK cells. Treatment with L19-IL2 likewise results in a few green dots, while treatment with 177Lu-Bi-ESV6-DOTAGA and L19-IL2 potentiates the concentration of NK cells in the sample.

Figure 6B shows only the NKp46 staining. NKp46 stains NK cells. It can be seen how the staining increases for the treatment with 177Lu-Bi-ESV6-DOTAGA and L19-IL2.

Figure 6C shows only the DAPI staining. As DAPI stains cell nuclei, one can appreciate nuclei, how the treatment with 177Lu-Bi-ESV6-DOTAGA and L19-IL2 or with L19-IL2 alone results in a reduction of the size of the nuclei, which means less tumor cells and more NK-cells.

Example 5: Therapy Studies in HT-1080.hPSMA tumor-bearing mice

5.1. Radiolabelling of PSMA-617 (1) to generate 177Lu-PSMA-617 (2)



PSMA-617 (10 mL, 1mM solution in mQ Water, 2% DMSO, 10 nmol) was added to a 1.5 mL Eppendorf tube and diluted with 1M NaOAc buffer (pH 4.5, 170 mL). $177LuCl_3$ (20 mL, aqueous solution, 50 MBq) was added, and the mixture was incubated in a thermomixer for 10 min at 95 °C. The 177Lu incorporation was followed by HPLC using a radiometric detector. Complete incorporation was afforded as evidenced by radio-HPLC analysis as shown in Figure 7.

5.2 Combination therapy

5.2.1. Implantation of subcutaneous tumors

HT-1080.hPSMA tumor cells were grown to 90% confluence and detached with Trypsin-EDTA 0.05%. Cells were resuspended in Hank's Balanced Salt Solution (HBSS) at a 5 x 10^7 cells/mL density. 100 µL of HT1080.hPSMA cells (5 x 10^6 cells) were injected subcutaneously in the right flank of female athymic Balb/c AnNRj-Foxn1 mice (6 to 8 weeks of age). Tumors were grown until volumes of ~100 mm^3.

5.2.2. Schedule of treatment

Tumor-bearing Balb/c nude mice were randomized into groups of 4 and 0.15 mL of a 6.7 µM solution (0.03% DMSO, 9% Acetate Buffer pH 4.5, PBS, 50 nmol/kg, 250 MBq/kg) of 177Lu-PSMA-617, 0.1 mL solution of L19-IL2 (0.5 mg/mL, 2.5 mg/kg), 0.1 mL PBS solution, or 0.1 mL L19-IL2 buffer (NaH_2PO_4 6.7 mM, NaCl 20 mM, KCl 1.8 mM, Mannitol 133 mM, Tween80 0.1% v/v, Glycerol 1% w/v) was injected intravenously (i.e., systemic administration through tail-vein) with the following schedule:

177Lu-PSMA-617 group: 1 injection (day 9 after tumor implantation).
L19-IL2 group: 3 injections with a 1-day break in between (days 10, 12, and 14 after tumor implantation).

Combination therapy group: 1 injection of 177Lu-PSMA-617 (day 9) and 3 injections of L19-IL2 with a 1-day break in between (days 10, 12, and 14 after tumor implantation).

Vehicle group: 1 injection of PBS (day 9 after tumor implantation) and 3 injections of L19-IL2 buffer with a 1-day break in between (days 10, 12, and 14 after tumor implantation).

5.3 Proteomics analysis

Animals were treated according to the above-stated therapy conditions and sacrificed after 1 week (n=2 per therapy group). The tumors were extracted and processed as follows:

Tumor tissues were resuspended at a final concentration of 0.1 mg/mL in an aqueous solution containing Urea 8 M, Tris-HCl 50 mM, 100 mM NaCl at pH = 8, with proteases inhibitors. Homogenization was performed with a tissue lyser (TissueLyser II, QIAGEN) for 1 minute at 30Hz two times at 4°C, after that the samples were sonicated for 2 minutes, 36 % power, and 50 % cycle. After sonication samples were centrifuged for 10 minutes at 21000 g, the supernatant was recovered, and the protein concentration was measured with BCA kit following kit instructions. 20 µg of proteins for each sample were reduced with TCEP, alkylated with Iodoacetamide, and finally overnight digested with trypsin. Digested peptides were then subjected to C18 purification and desalting. Purified samples were dried under vacuum and resuspended in 120 µL of an aqueous solution containing 3% acetonitrile and 0.1% formic acid. 1 µL of the sample was then subjected to nanoHPLC-HRMS analysis on an Orbitrap Q-Exactive mass spectrometer coupled to an EASY nanoLC 1000 system via a Nano Flex ion

source. Chromatographic separation was carried out at room temperature on an Acclaim PepMap RSLC column (50 µm x 15 cm, particle size 2 µm, pore size, 100 Å), using a 60 min linear gradient with 5-35% solvent B (0.1% formic acid in acetonitrile) at a flow rate of 300 nL/min. Ionization was carried out in positive ion mode, with 2 kV of spray voltage, 250 °C capillary temperature, and 60 S-lens RF level. The mass spectrometer was working in a data-dependent (DDA) top 12 mode with the following parameters: MS1 scan range: from 350 to 1650 m/z, HCD NCE: 25, Dynamic exclusion: 15 sec. Raw files were processed with Proteome Discoverer 2.5 (Thermo Fisher) for quantitative analysis. Database searches were performed with Sequest as a search engine using a FASTA file containing the *Mus musculus* and *Homo sapiens* reference proteomes. Carbamidomethylation of cysteines was set as a fixed modification, methionine oxidation as a variable modification, and trypsin as cleavage specificity allowing a maximum of 2 missed cleavages. A rescoring of PSM was carried out with the Inferys node. Data filtering was performed using a percolator, resulting in 1% false discovery rate (FDR). Data mining was carried out with an in-house built Python script. Briefly, only identified and not quantified proteins were filtered out. Proteins without abundance values in at least 2 replicates for at least one condition were removed. Abundances were median-normalised, NaNs were imputed to the absolute minimum and finally abundances were log2 transformed. Fold change analysis was carried out to compare protein abundances in all treatment groups against the saline. The statistical significance of the fold change was evaluated with multiple Welch t-test corrected for multiple comparisons (using the Benjamini-Hochberg correction with 5% FDR). The abundances of selected proteins involved in inflammatory and immune processes were compared among groups on the log10 transformed protein abundances normalized on the Saline condition. Samples were analyzed in quadruplicates (2 biological plus 2 tryptic digestion replicas).

5.4. Results

Figure 8A presents changes in tumor volume over the course of the combination therapy, outlining the therapeutic efficacy of 177Lu-PSMA-617 and its therapeutic synergy with L19-IL2. Monotherapy with 177Lu-PSMA-617 was efficacious but did not lead to complete and long-lasting cancer regression in all treated animals, with only 1 out of 4 mice cured at the end of the experiment. In contrast, all mice administered with the 177Lu-PSMA-617 + L19-IL2 combination treatment showed complete responses (cancer cures) after treatment. L19-IL2 single agent induced modest tumor-growth retardation.

Figure 8B presents body weight changes (%) of the therapy groups associated with single agent (L19-IL2 or 177Lu-PSMA-617), combination, and the vehicle-treated groups. In the combination no significant body weight loss was observed unlike in the other three arms.

Figure 9. Bottom-up label-free quantitative proteomics was carried out to further investigate the mechanism by which the combination of L19-IL2 and 177Lu-PSMA-617 successfully produces a complete and durable response. A total number of 3'466 proteins were globally identified. Of these, 207 were nonredundant proteins dysregulated with FDR of 5% and Fold Change threshold of ±2 when comparing vehicle treatment with the other three groups of treatments (i.e., L19-IL2, 177Lu-PSMA-617, and 177Lu-PSMA-617 + L19-IL2). 177Lu-PSMA-617 alone does not alter the expression of the proteome compared to the vehicle treatment.

Conversely, L19-IL2 and the combination therapy produce an alteration of the proteome (Fig. 9A). Many proteins associated with the immune system are upregulated, highlighting the activation of the host defense mechanisms. Among these, granzyme B (Gzmb), Cd16a, Cd90, and Spp1 are significantly upregulated in the combination therapy compared to the single agents alone. (Fig. 9B).

Hence, the combination as set forth in the patent claims has a synergistic effect over the administration of the individual components alone.

Example 6: Therapy Studies in MC38.hPSMA tumor-bearing mice

6.1. Radiolabelling of PSMA-617 to generate 177Lu-PSMA-617

177Lu-PSMA-617 was produced as described in Example 5.1

6.2. Production of L19-murineIL12

L19-murineIL12 was produced as described by Puca et al., Int J Cancer, 2020 146:2518-2530.

6.3 Combination therapy

6.3.1. Implantation of subcutaneous tumors

MC38.hPSMA tumor cells were grown to 90% confluence and detached with Trypsin-EDTA 0.05%. Cells were resuspended in Hank's Balanced Salt Solution (HBSS) at a 5 x 10^7 cells/mL density. 100 µL of MC38.hPSMA cells (5 x 10^6 cells) were injected subcutaneously in the right flank of female C57BL/6J mice (6 to 8 weeks of age). Tumors were grown until volumes of ~100 mm^3.

6.3.2. Schedule of treatment

Tumor-bearing mice were randomized into groups of 4 and 177Lu-PSMA-617 (250 MBq/kg, 50 nmol/kg), L19-murineIL12 (0.4 nmol/kg), the combination of the two (L19-murineIL12 at 0.4 nmol/kg + 177Lu-PSMA-617 at 250 MBq/kg), or saline were injected intravenously (i.e., systemic administration through tail-vein) with the following schedule:

- 177Lu-PSMA-617 group: 1 injection (day 5 after tumor implantation).
- L19-murineIL12 group: 3 injections with a 1-day break in between (days 6, 8, and 10 after tumor implantation).
- Combination group: 1 injection of 177Lu-PSMA-617 (day 5) and 3 injections of L19-murineIL12 with a 1-day break in between (days 6, 8, and 10 after tumor implantation).
- Vehicle group: 4 injections of saline (day 5, 6, 8, and 10 after tumor implantation).

6.3.3 Proteomics analysis

Animals were treated according to the above-stated therapy conditions and sacrificed after 9 days from the begin of the treatment week (n=2 per therapy group). The tumors were extracted and processed as follows:

Tumor tissues were resuspended at a final concentration of 0.1 mg/mL in an aqueous solution containing Urea 8 M, Tris-HCl 50 mM, 100 mM NaCl at pH = 8, with proteases inhibitors. Homogenization was performed with a tissue lyser (TissueLyser II, QIAGEN) for 1 minute at 30Hz two times at 4°C, after that the samples were sonicated for 2 minutes, 36 % power, and 50 % cycle. After sonication samples were centrifuged for 10 minutes at 21000 g, the supernatant was recovered, and the protein concentration was measured with BCA kit following

kit instructions. 20 µg of proteins for each sample were reduced with TCEP, alkylated with Iodoacetamide, and finally overnight digested with trypsin. Digested peptides were then subjected to C18 purification and desalting. Purified samples were dried under vacuum and resuspended in 120 µL of an aqueous solution containing 3% acetonitrile and 0.1% formic acid. 1 µL of the sample was then subjected to nanoHPLC-HRMS analysis on an Orbitrap Q-Exactive mass spectrometer coupled to an EASY nanoLC 1000 system via a Nano Flex ion source. Chromatographic separation was carried out at room temperature on an Acclaim PepMap RSLC column (50 µm x 15 cm, particle size 2 µm, pore size, 100 Å), using a 60 min linear gradient with 5-35% solvent B (0.1% formic acid in acetonitrile) at a flow rate of 300 nL/min. Ionization was carried out in positive ion mode, with 2 kV of spray voltage, 250 °C capillary temperature, and 60 S-lens RF level. The mass spectrometer was working in a data-dependent (DDA) top 12 mode with the following parameters: MS1 scan range: from 350 to 1650 m/z, HCD NCE: 25, Dynamic exclusion: 15 sec. Raw files were processed with Proteome Discoverer 2.5 (Thermo Fisher) for quantitative analysis. Database searches were performed with Sequest as a search engine using a FASTA file containing the *Mus musculus* reference proteomes. Carbamidomethylation of cysteines was set as a fixed modification, methionine oxidation as a variable modification, and trypsin as cleavage specificity allowing a maximum of 2 missed cleavages. A rescoring of PSM was carried out with the Inferys node. Data filtering was performed using a percolator, resulting in 1% false discovery rate (FDR). Data mining was carried out with an in-house built R script. Briefly, only identified and not quantified proteins were filtered out. Proteins without abundance values in at least 2 replicates for at least one condition were removed. Abundances were median-normalised and log2 transformed, NaNs were imputed as follow: for each protein if the abundance values were absent in more than 50% of the replicates NaN were imputed with a left censored normal distribution with $\mu_i = \mu_m - 2.5\sigma_m$ and $\sigma_i = 0.3\ \sigma_m$ (μ = mean, σ = standard deviation, i = values for imputation, m = measured values), otherwise NaN were imputed with a random forest approach with missForest function of the "missForest" R package. Fold change analysis was carried out to compare protein abundances in all treatment groups against the saline. The statistical significance of the fold change was evaluated with multiple Welch t-test corrected for multiple comparisons (using the Benjamini-Hochberg correction with 5% FDR). The abundances of selected proteins involved in inflammatory and immune processes were compared among groups on the absolute protein abundances normalized on the Saline condition. Samples were analyzed in quadruplicates (2 biological plus 2 tryptic digestion replicas).

6.4 Results

Figure 10 presents changes in tumor volume over the course of the combination therapy, outlining the therapeutic efficacy of 177Lu-PSMA-617 and its therapeutic synergy with L19-IL12. Monotherapy with 177Lu-PSMA-617 induced modest tumor-growth retardation, while L19-IL12 was efficacious but did not lead to complete cancer regression. In contrast, mice administered with the 177Lu-PSMA-617 + L19-IL12 combination treatment showed potent responses.

Figure 11. Bottom-up label-free quantitative proteomics was carried out to further investigate the mechanism by which the combination of L19-mIL12 and 177Lu-PSMA-617 successfully produces a potent response. A total number of 3'480 proteins were globally identified. As highlighted by the volcano plot (11A) L19mIL12 and 177Lu-PSMA-617 induce minor proteomics changes in the tumor, with only few statistically significant up-and down-regulated proteins. Surprisingly, the combination of L19mIL12 and 177Lu-PSMA-617 produces a striking effect on the tissue proteome with 408 and 373 proteins up and down-regulated respectively).

Markers of cell apoptosis (i.e., Casp1 and Adamtsl4) and immune response (i.e., Cd38, Cd180, granzyme F (Gzmf), and granzyme C (Gzmc)) are upregulated in the combination treatment, highlighting the activation of the host defense mechanisms (Figure 11B).

Hence, the combination as set forth in the patent claims has a synergistic effect over the administration of the individual components alone.

Further items

The following set of items forms also part of this disclosure:

1. A pharmaceutical composition comprising
   (a) a recombinant protein comprising
      (i) interleukin-2 (IL2) and
      (ii) an antibody binding the extra-domain B (ED-B) of fibronectin, or a target binding fragment or derivative thereof, and
   (b) a conjugate radiolabelled with 177Lutetium.

2. A dosage form comprising

(a) a recombinant protein comprising

(i) interleukin-2 (IL2) and

(ii) an antibody binding the extra-domain B (ED-B) of fibronectin, or a target binding fragment or derivative thereof,

(b) a conjugate radiolabelled with 177Lutetium

in a pharmaceutically acceptable carrier.

3. A combination comprising at least

(a) a recombinant protein comprising

(i) interleukin-2 (IL2) and

(ii) an antibody binding the extra-domain B (ED-B) of fibronectin, or a target binding fragment or derivative thereof, and

(b) a conjugate radiolabelled with 177Lutetium

4. A kit of dosage forms, comprising at least

(a) a first dosage form comprises a recombinant protein comprising

(i) interleukin-2 (IL2) and

(ii) an antibody binding the extra-domain B (ED-B) of fibronectin, or a target binding fragment or derivative thereof,

in a pharmaceutically acceptable carrier and

(b) a second dosage form comprises a conjugate radiolabelled with 177Lutetium in a pharmaceutically acceptable carrier.

5. The pharmaceutical composition, dosage form, combination, or kit according to any of items 1 - 4, wherein

(a) the recombinant protein comprising

(i) interleukin-2 (IL2) and

(ii) an antibody binding the extra-domain B (ED-B) of fibronectin, or a target binding fragment or derivative thereof, and

(b) the conjugate radiolabelled with 177Lutetium

are administered or taken simultaneously.

6. The pharmaceutical composition, dosage form, combination, or kit according to any of items 1 - 5, wherein

(a) the recombinant protein comprising
(i) interleukin-2 (IL2) and
(ii) an antibody binding the extra-domain B (ED-B) of fibronectin, or a target binding fragment or derivative thereof, and
(b) the conjugate radiolabelled with 177Lutetium

are administered or taken sequentially.

7. The pharmaceutical composition, dosage form, combination, or kit according to any one of items 1 - 6 for (the manufacture of a medicament consisting of at least one dosage form for) use in the treatment of a human or mammalian patient
(i) being diagnosed for,
(ii) suffering from or
(iii) being at risk of developing
cancer.

8. A method for treating a human or mammalian patient, which method comprises administration of one or more effective amounts of the pharmaceutical composition, dosage form combination or Kit of any of items 1 – 6.

9. The method according to item 8, wherein the human or mammalian patient
(i) is diagnosed for,
(ii) suffers from or
(iii) is at risk of developing cancer.

10. The pharmaceutical composition, dosage form, combination, kit or method of treatment according to any of items 1 - 9, wherein the cancer is at least one selected from the group consisting of Neuroendocrine tumors, GRPR expressing tumors, PSMA expressing tumors, and/or FAP expressing tumors

11. The pharmaceutical composition, dosage form, combination, kit or method of treatment according to any of items 1 - 10, wherein the antibody binding the extra-domain B (ED-B) of fibronectin comprises the complementarity determining regions (CDR's) of the L19 antibody as shown in SEQ ID Nos: 7-12.

12. The pharmaceutical composition, dosage form, combination, kit or method of treatment according to any of items 1 - 11, wherein the antibody binding the extra-domain B (ED-B) of fibronectin comprises the L19 VH as shown in SEQ ID NO: 2 and the L19 VL as shown in SEQ ID NO: 3.

13. The pharmaceutical composition, dosage form, combination, kit or method of treatment according to any of items 1 - 12, wherein the conjugate radiolabelled with 177Lutetium comprises one or more of the conjugates show in Table 1.

14. The conjugate radiolabelled with 177Lutetium according to item 13, wherein the conjugate is at least one selected from the group consisting of

(i) Lutathera
(ii) PSMA-617
(iii) NeoBOMB
(iv) DOTA-JR11
(v) PMSA-R2
(vi) CTT1403
(vii) 177Lu-RM2
(viii) 177Lu.DOTA.SA.FAPi
(ix) 177Lu-ESV6-DOTA
(x) 177Lu-Bi-ESV6-DOTA
(xi) 177Lu-ESV6-DOTAGA, and/or
(xii) 177Lu-Bi-ESV6-DOTAGA.

The following set of items form also part of this disclosure:

1. A pharmaceutical composition comprising

(a) a recombinant protein comprising

(i) interleukin-2 (IL2) or interleukin-12 (IL12) and

(ii) an antibody binding the extra-domain B (ED-B) of fibronectin, or a target binding fragment or derivative thereof, and

(b) a conjugate radiolabelled with 177Lutetium.

2. A dosage form comprising

(a) a recombinant protein comprising

(i) interleukin-2 (IL2) or interleukin-12 (IL12) and

(ii) an antibody binding the extra-domain B (ED-B) of fibronectin, or a target binding fragment or derivative thereof,

(b) a conjugate radiolabelled with 177Lutetium

in a pharmaceutically acceptable carrier.

3. A combination comprising at least

(a) a recombinant protein comprising

(i) interleukin-2 (IL2) or interleukin-12 (IL12) and

(ii) an antibody binding the extra-domain B (ED-B) of fibronectin, or a target binding fragment or derivative thereof, and

(b) a conjugate radiolabelled with 177Lutetium

4. A kit of dosage forms, comprising at least

(a) a first dosage form comprises a recombinant protein comprising

(i) interleukin-2 (IL2) or interleukin-12 (IL12) and

(ii) an antibody binding the extra-domain B (ED-B) of fibronectin, or a target binding fragment or derivative thereof,

in a pharmaceutically acceptable carrier and

(b) a second dosage form comprises a conjugate radiolabelled with 177Lutetium in a pharmaceutically acceptable carrier.

5. The pharmaceutical composition, dosage form, combination, or kit according to any of items 1 - 4, wherein

(a) the recombinant protein comprising

(i) interleukin-2 (IL2) or interleukin-12 (IL12) and

(ii) an antibody binding the extra-domain B (ED-B) of fibronectin, or a target binding fragment or derivative thereof, and

(b) the conjugate radiolabelled with 177Lutetium

are administered or taken simultaneously.

6. The pharmaceutical composition, dosage form, combination, or kit according to any of items 1 - 5, wherein

(a) the recombinant protein comprising

(i) interleukin-2 (IL2) or interleukin-12 (IL12) and

(ii) an antibody binding the extra-domain B (ED-B) of fibronectin, or a target binding fragment or derivative thereof, and

(b) the conjugate radiolabelled with 177Lutetium

are administered or taken sequentially.

7. The pharmaceutical composition, dosage form, combination, or kit according to any one of items 1 - 6 for (the manufacture of a medicament consisting of at least one dosage form for) use in the treatment of a human or mammalian patient

(i) being diagnosed for,

(ii) suffering from or

(iii) being at risk of developing cancer.

8. A method for treating a human or mammalian patient, which method comprises administration of one or more effective amounts of the pharmaceutical composition, dosage form combination or Kit of any of items 1 – 6.

9. The method according to item 8, wherein the human or mammalian patient

(i) is diagnosed for,

(ii) suffers from or

(iii) is at risk of developing cancer.

10. The pharmaceutical composition, dosage form, combination, kit or method of treatment according to any of items 1 - 9, wherein the cancer is at least one selected from the group consisting of Neuroendocrine tumors, GRPR expressing tumors, PSMA expressing tumors, and/or FAP expressing tumors

11. The pharmaceutical composition, dosage form, combination, kit or method of treatment according to any of items 1 - 10, wherein the antibody binding the extra-domain B (ED-B) of fibronectin comprises the complementarity determining regions (CDR's) of the L19 antibody as shown in SEQ ID Nos: 7-12.

12. The pharmaceutical composition, dosage form, combination, kit or method of treatment according to any of items 1 - 11, wherein the antibody or fragment or derivative comprises

a) the heavy chain/light chain variable domain (HCVD/LCVD) pair set forth in SEQ ID NOs 2 and 3,

b) the heavy chain/light chain variable domains (HCVD/LCVD) pairs of a), with the proviso that

- the HCVD has a sequence identity of ≥ 80 % to the respective SEQ ID NO, and/or
- the LCVD has a sequence identity of ≥ 80 % to the respective SEQ ID NO,

c) the heavy chain/light chain variable domains (VD) pairs of a) or b), with the proviso that at least one of the HCVD or LCVD has up to 10 amino acid substitutions relative to the respective SEQ ID NO,

said antibody or fragment still being capable to bind to the extra-domain B (ED-B) of fibronectin.

13. The pharmaceutical composition, dosage form, combination, kit or method of treatment according to item 12, wherein at least one amino acid substitution is a conservative amino acid substitution.

14. The pharmaceutical composition, dosage form, combination, kit or method of treatment according to any one of items 1 - 13, wherein the antibody or fragment or derivative is provided in the single-chain Fv format or in the single-chain diabody format.

15. The pharmaceutical composition, dosage form, combination, kit or method of treatment according to any one of items 1 - 14, wherein the recombinant protein is Darleukin or Dodekin.

16. The pharmaceutical composition, dosage form, combination, kit or method of treatment according to any one of items 1 - 15, wherein the recombinant protein comprises one of the amino acid sequences set selected from SEQ ID NO: 1 and SEQ ID NO: 14.

17. The pharmaceutical composition, dosage form, combination, kit or method of treatment according to any of items 1 - 16, wherein the conjugate radiolabelled with 177Lutetium comprises one or more of the conjugates show in Table 1.

18. The conjugate radiolabelled with 177Lutetium according to item 17, wherein the conjugate is at least one selected from the group consisting of

(i) Lutathera
(ii) PSMA-617
(iii) NeoBOMB
(iv) DOTA-JR11
(v) PMSA-R2
(vi) CTT1403
(vii) 177Lu-RM2
(viii) 177Lu.DOTA.SA.FAPi
(ix) 177Lu-ESV6-DOTA
(x) 177Lu-Bi-ESV6-DOTA
(xi) 177Lu-ESV6-DOTAGA, and/or
(xii) 177Lu-Bi- ESV6-DOTAGA.

Sequences

The following sequences form part of the disclosure of the present application. A WIPO ST 26 compatible electronic sequence listing is provided with this application, too. For the avoidance of doubt, if discrepancies exist between the sequences in the following table and the electronic sequence listing, the sequences in this table shall be deemed to be the correct ones.

In some cases, signal peptides may be encompassed in the reproduced sequences. In such case, the sequences shall be deemed disclosed with and without signal peptides. A readily available tool to identify signal peptides in a given protein sequence is SignalP - 6.0 provided by Dansk Technical University under https://services.healthtech.dtu.dk/service.php?SignalP. The same applies to His tags or C-Myc tags, if existing.

Table 2: Sequence listing

SEQ ID NO	Qualifier	Sequence
1	L19-IL2	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAPG KGLEWVSSISGSSGTTYYADSVKGRFTISRDNSKNTLYLQMN SLRAEDTAVYYCAKPFPYFDYWGQGTLVTVSSGDGSSGGSGG ASEIVLTQSPGTLSLSPGERATLSCRASQSVSSSFLAWYQQK PGQAPRLLIYYASSRATGIPDRFSGSGSGTDFTLTISRLEPE DFAVYYCQQTGRIPPTFGQGTKVEIKEFSSSSGSSSSGSSSS GAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLT FKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPR DLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFC QSIISTLT
2	L19 VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAPG KGLEWVSSISGSSGTTYYADSVKGRFTISRDNSKNTLYLQMN SLRAEDTAVYYCAKPFPYFDYWGQGTLVTVSS
3	L19 VL	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSFLAWYQQKPG QAPRLLIYYASSRATGIPDRFSGSGSGTDFTLTISRLEPEDF AVYYCQQTGRIPPTFGQGTKVEIK
4	VH⇔VL linker	GDGSSGGSGGAS
5	L19⇔IL2 linker	EFSSSSGSSSSGSSSSG
6	Human IL2	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTF KFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRD LISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLT
7	L19 HCDR1	SFSMS
8	L19 HCDR2	SISGSSGTTYYADSVKG
9	L19 HCDR3	PFPYFDY
10	L19 LCDR1	RASQSVSSSFLA
11	L19 LCDR2	YASSRAT
12	L19 LCDR3	QQTGRIPPT
13	L19 scFv	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFSMSWVRQAPG KGLEWVSSISGSSGTTYYADSVKGRFTISRDNSKNTLYLQMN SLRAEDTAVYYCAKPFPYFDYWGQGTLVTVSSGDGSSGGSGG ASEIVLTQSPGTLSLSPGERATLSCRASQSVSSSFLAWYQQK PGQAPRLLIYYASSRATGIPDRFSGSGSGTDFTLTISRLEPE DFAVYYCQQTGRIPPTFGQGTKVEIK
14	L19-IL12	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQ SSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHK

		KEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTTI STDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYS VECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDI IKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFC VQVQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYS SSWSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPGMFPC LHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITKDKT STVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMA LCLSSIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVI DELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIR AVTIDRVMSYLNASGSADGGSSAGGSDAGEVQLLESGGGLVQ PGGSLRLSCAASGFTFSSFSMSWVRQAPGKGLEWVSSISGSS GTTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCA KPFPYFDYWGQGTLVTVSSGSSGGEIVLTQSPGTLSLSPGER ATLSCRASQSVSSSFLAWYQQKPGQAPRLLIYYASSRATGIP DRFSGSGSGTDFTLTISRLEPEDFAVYYCQQTGRIPPTFGQG TKVEIKSSSSGSSSSGSSSSGEVQLLESGGGLVQPGGSLRLS CAASGFTFSSFSMSWVRQAPGKGLEWVSSISGSSGTTYYADS VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKPFPYFDY WGQGTLVTVSSGSSGGEIVLTQSPGTLSLSPGERATLSCRAS QSVSSSFLAWYQQKPGQAPRLLIYYASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQQTGRIPPTFGQGTKVEIK
15	L19-murine IL12	MWELEKDVYVVEVDWTPDAPGETVNLTCDTPEEDDITWTSDQ RHGVIGSGKTLTITVKEFLDAGQYTCHKGGETLSHSHLLLHK KENGIWSTEILKNFKNKTFLKCEAPNYSGRFTCSWLVQRNMD LKFNIKSSSSSPDSRAVTCGMASLSAEKVTLDQRDYEKYSVS CQEDVTCPTAEETLPIELALEARQQNKYENYSTSFFIRDIIK PDPPKNLQMRPLKNSQVEVSWEYPDSWSTPHSYFSLKFFVRI QRKKEKMKETEEGCNQKGAFLVERTSTEVQCKGGNVCVQAQD RYYNSSCSKWACVPCRVRSGGGGSGGGGSGGGGSRVIPVSGP ARCLSQSRNLLKTTDDMVKTAREKLKHYSCTAEDIDHEDITR DQTSTLKTCLPLELHKNESCLATRETSSTTRGSCLPPQKTSL MMTLCLGSIYEDLKMYQTEFQAINAALQNHNHQQIILDKGML VAIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLHAF STRVVTINRVMGYLSSAGSADGEVQLLESGGGLVQPGGSLRL SCAASGFTFSSFSMSWVRQAPGKGLEWVSSISGSSGTTYYAD SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKPFPYFD YWGQGTLVTVSSGSSGGEIVLTQSPGTLSLSPGERATLSCRA SQSVSSSFLAWYQQKPGQAPRLLIYYASSRATGIPDRFSGSG SGTDFTLTISRLEPEDFAVYYCQQTGRIPPTFGQGTKVEIKS SSSGSSSSGSSSSGEVQLLESGGGLVQPGGSLRLSCAASGFT FSSFSMSWVRQAPGKGLEWVSSISGSSGTTYYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCAKPFPYFDYWGQGTLV TVSSGSSGGEIVLTQSPGTLSLSPGERATLSCRASQSVSSSF LAWYQQKPGQAPRLLIYYASSRATGIPDRFSGSGSGTDFTLT ISRLEPEDFAVYYCQQTGRIPPTFGQGTKVEIK
16	Human IL12 p40	IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQ SSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHK KEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTTI STDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEYS VECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDI

		IKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFC VQVQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYS SSWSEWASVPCS
17	Human IL12 p35	RNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPC TSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFIT NGSCLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNAKLLMD PKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYK TKIKLCILLHAFRIRAVTIDRVMSYLNAS
18	p40⇔p35 linker	GGGGSGGGGSGGGGS

What is claimed is:

1. A pharmaceutical composition comprising

(a) a recombinant protein comprising

(i) interleukin-2 (IL2) or interleukin-12 (IL12) and, fused or conjugated thereto

(ii) an antibody binding the extra-domain B (ED-B) of fibronectin, or a target binding fragment thereof,

which antibody or fragment comprises a set of three heavy chain and three light chain complementarity determining regions (CDR) as set forth in SEQ ID NOs 7-12, and

(b) a conjugate radiolabelled with 177Lutetium,

which conjugate is 177Lu-PSMA-617.

2. A dosage form comprising

(a) a recombinant protein comprising

(i) interleukin-2 (IL2) or interleukin-12 (IL12) and, fused or conjugated thereto

(ii) an antibody binding the extra-domain B (ED-B) of fibronectin, or a target binding fragment thereof,

which antibody or fragment comprises a set of three heavy chain and three light chain complementarity determining regions (CDR) as set forth in SEQ ID NOs 7-12, and

(b) a conjugate radiolabelled with 177Lutetium,

which conjugate is 177Lu-PSMA-617

in a pharmaceutically acceptable carrier.

3. A combination comprising at least

(a) a recombinant protein comprising

(i) interleukin-2 (IL2) or interleukin-12 (IL12) and, fused or conjugated thereto

(ii) an antibody binding the extra-domain B (ED-B) of fibronectin, or a target binding fragment thereof,

which antibody or fragment comprises a set of three heavy chain and three light chain complementarity determining regions (CDR) as set forth in SEQ ID NOs 7-12, and

(b) a conjugate radiolabelled with 177Lutetium,

which conjugate is 177Lu-PSMA-617.

4. A kit of dosage forms, comprising at least

(a) a first dosage form comprising a recombinant protein comprising

(i) interleukin-2 (IL2) or interleukin-12 (IL12) and, fused or conjugated thereto

(ii) an antibody binding the extra-domain B (ED-B) of fibronectin, or a target binding fragment thereof,

which antibody or fragment comprises a set of three heavy chain and three light chain complementarity determining regions (CDR) as set forth in SEQ ID NOs 7-12,

in a pharmaceutically acceptable carrier and

(b) a second dosage form comprising a conjugate radiolabelled with 177Lutetium,

which conjugate is 177Lu-PSMA-617

in a pharmaceutically acceptable carrier.

5. The pharmaceutical composition, dosage form, combination, or kit according to any one of claims 1 - 4, wherein the antibody or fragment comprises

a) the heavy chain/light chain variable domain (HCVD/LCVD) pair set forth in SEQ ID NOs 2 and 3,

b) the heavy chain/light chain variable domains (HCVD/LCVD) pairs of a), with the proviso that

- the HCVD has a sequence identity of ≥ 80 % to the respective SEQ ID NO, and/or
- the LCVD has a sequence identity of ≥ 80 % to the respective SEQ ID NO,

c) the heavy chain/light chain variable domains (VD) pairs of a) or b), with the proviso that at least one of the HCVD or LCVD has up to 10 amino acid substitutions relative to the respective SEQ ID NO,

said antibody or fragment still being capable to bind to the extra-domain B (ED-B) of fibronectin.

6. The pharmaceutical composition, dosage form, combination, or kit according to claim 5, wherein at least one amino acid substitution is a conservative amino acid substitution.

7. The pharmaceutical composition, dosage form, combination, or kit according to any one of claims 1 - 6, wherein the antibody or fragment is provided in the single-chain Fv format or in the single-chain diabody format.

8. The pharmaceutical composition, dosage form, combination, or kit according to any one of claims 1 - 7, wherein the recombinant protein is Darleukin or Dodekin.

9. The pharmaceutical composition, dosage form, combination, or kit according to any one of claims 1 - 7, wherein the recombinant protein comprises one of the amino acid sequences set selected from SEQ ID NO: 1 and SEQ ID NO: 14.

10. The pharmaceutical composition, dosage form, combination, or kit according to any one of claims 1 - 9, wherein

(a) the recombinant protein and

(b) the conjugate radiolabelled with 177Lutetium

are administered or taken simultaneously or sequentially.

11. The pharmaceutical composition, dosage form, combination, or kit according to any one of claims 1 - 10 for (the manufacture of a medicament consisting of at least one dosage form for) use in the treatment of a human or mammalian patient

(i) being diagnosed for,

(ii) suffering from or

(iii) being at risk of developing

cancer.

12. A method for treating a human or mammalian patient, which method comprises administration of one or more effective amounts of the pharmaceutical composition, dosage form combination or Kit of any one of claims 1 – 10.

13. The method according to claim 12, wherein the human or mammalian patient

(i) is diagnosed for,

(ii) suffers from or

(iii) is at risk of developing

cancer.

14. The pharmaceutical composition, dosage form, combination, kit or method of treatment according to any one of claims 11 and 13, wherein the cancer is at least one selected from the group consisting of Neuroendocrine tumors, GRPR expressing tumors, PSMA expressing tumors, and/or FAP expressing tumors.

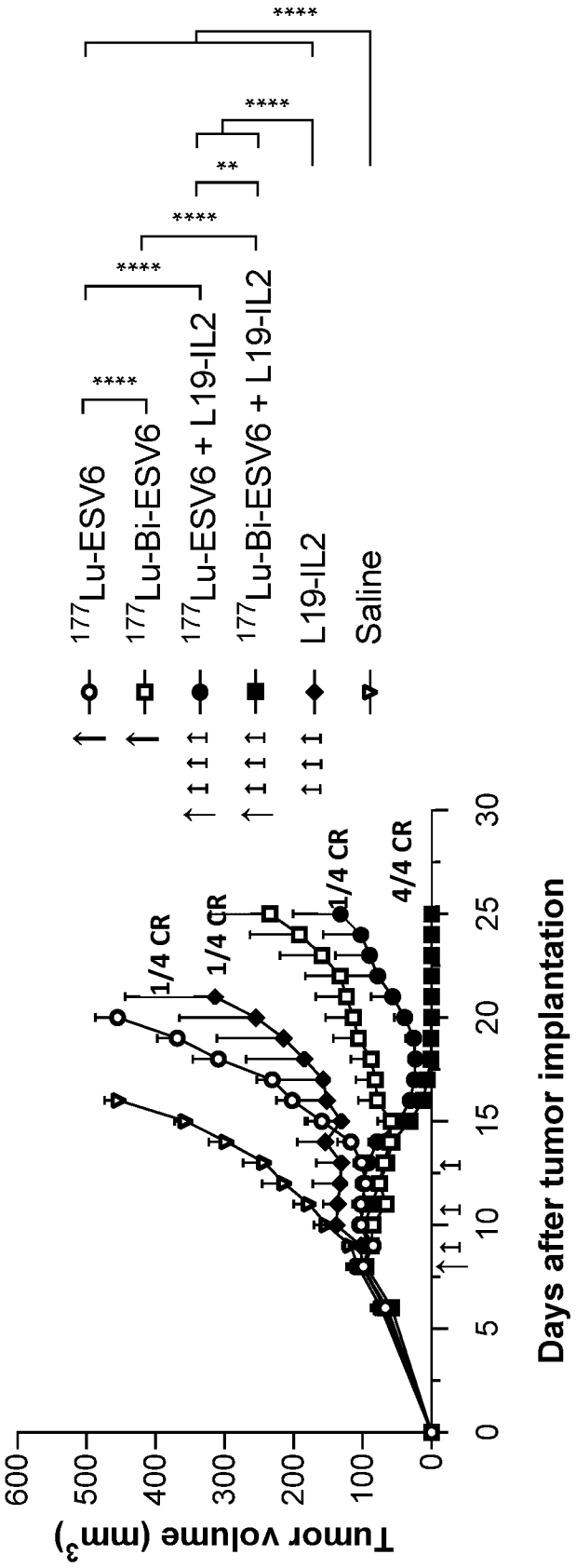

Tumor volume (mm³)
600
500
400
300
200
100
0
0
5
10
15
20
25
30
Days after tumor implantation
1/4 CR
1/4 CR
1/4 CR
4/4 CR
177Lu-ESV6
177Lu-Bi-ESV6
177Lu-ESV6 + L19-IL2
177Lu-Bi-ESV6 + L19-IL2
L19-IL2
Saline
****
****
****
**
****
****

Fig. 1

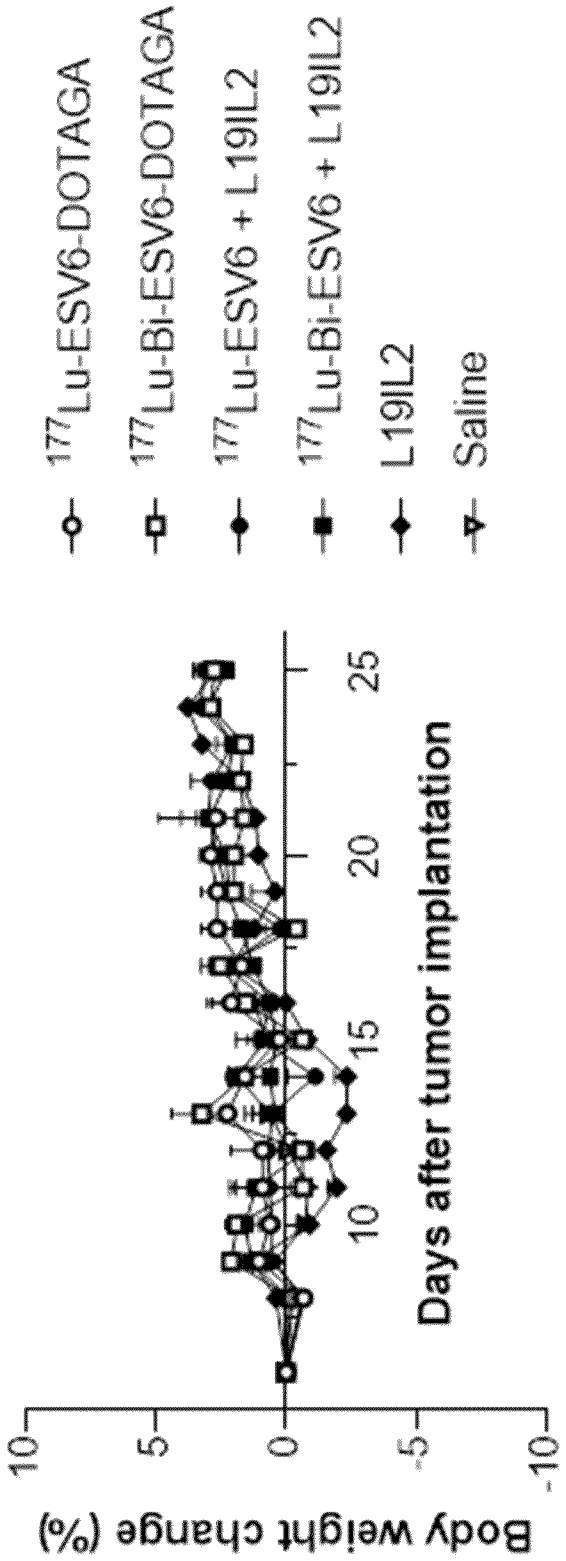

^{177}Lu-ESV6-DOTAGA
^{177}Lu-Bi-ESV6-DOTAGA
^{177}Lu-ESV6 + L19IL2
^{177}Lu-Bi-ESV6 + L19IL2
L19IL2
Saline
Body weight change (%)
10
5
0
-5
-10
10
15
20
25
Days after tumor implantation

Fig. 2

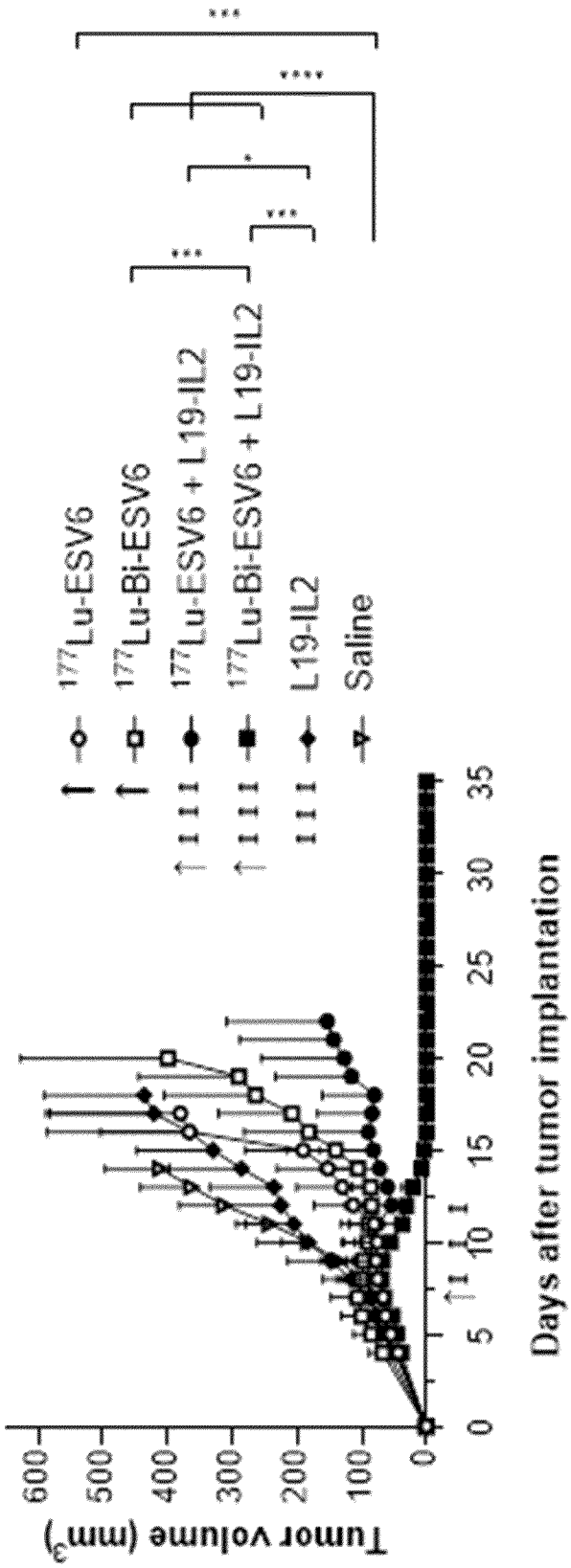

Tumor volume (mm³)
600
500
400
300
200
100
0
0
5
10
15
20
25
30
35
Days after tumor implantation
177Lu-ESV6
177Lu-Bi-ESV6
177Lu-ESV6 + L19-IL2
177Lu-Bi-ESV6 + L19-IL2
L19-IL2
Saline
***
****
***
*
***
***

Fig. 3

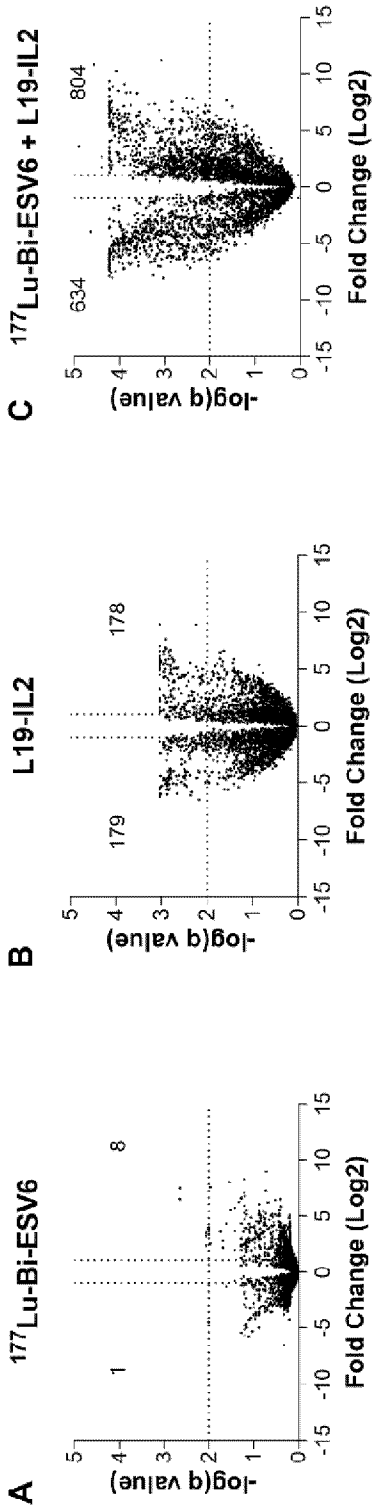

A
^{177}Lu-Bi-ESV6
B
L19-IL2
C
^{177}Lu-Bi-ESV6 + L19-IL2
-log(q value)
Fold Change (Log2)
1
8
179
178
634
804

Fig. 4

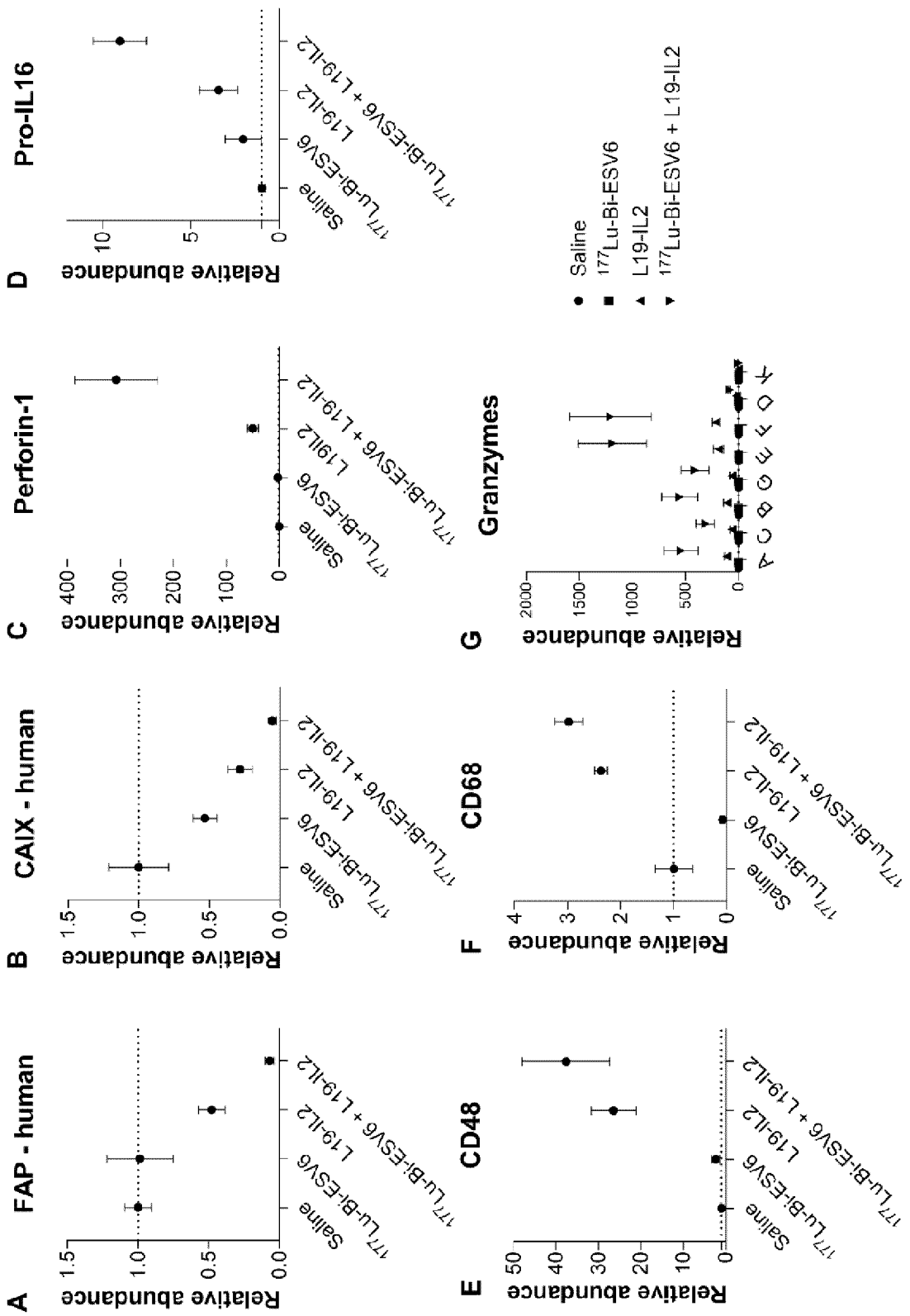

A
FAP - human
B
CAIX - human
C
Perforin-1
D
Pro-IL16
E
CD48
F
CD68
G
Granzymes
Relative abundance
Saline
177Lu-Bi-ESV6
L19-IL2
177Lu-Bi-ESV6 + L19-IL2
Saline
177Lu-Bi-ESV6
L19-IL2
177Lu-Bi-ESV6 + L19-IL2
A
B
C
D
E
F
G
H

Fig. 5

A

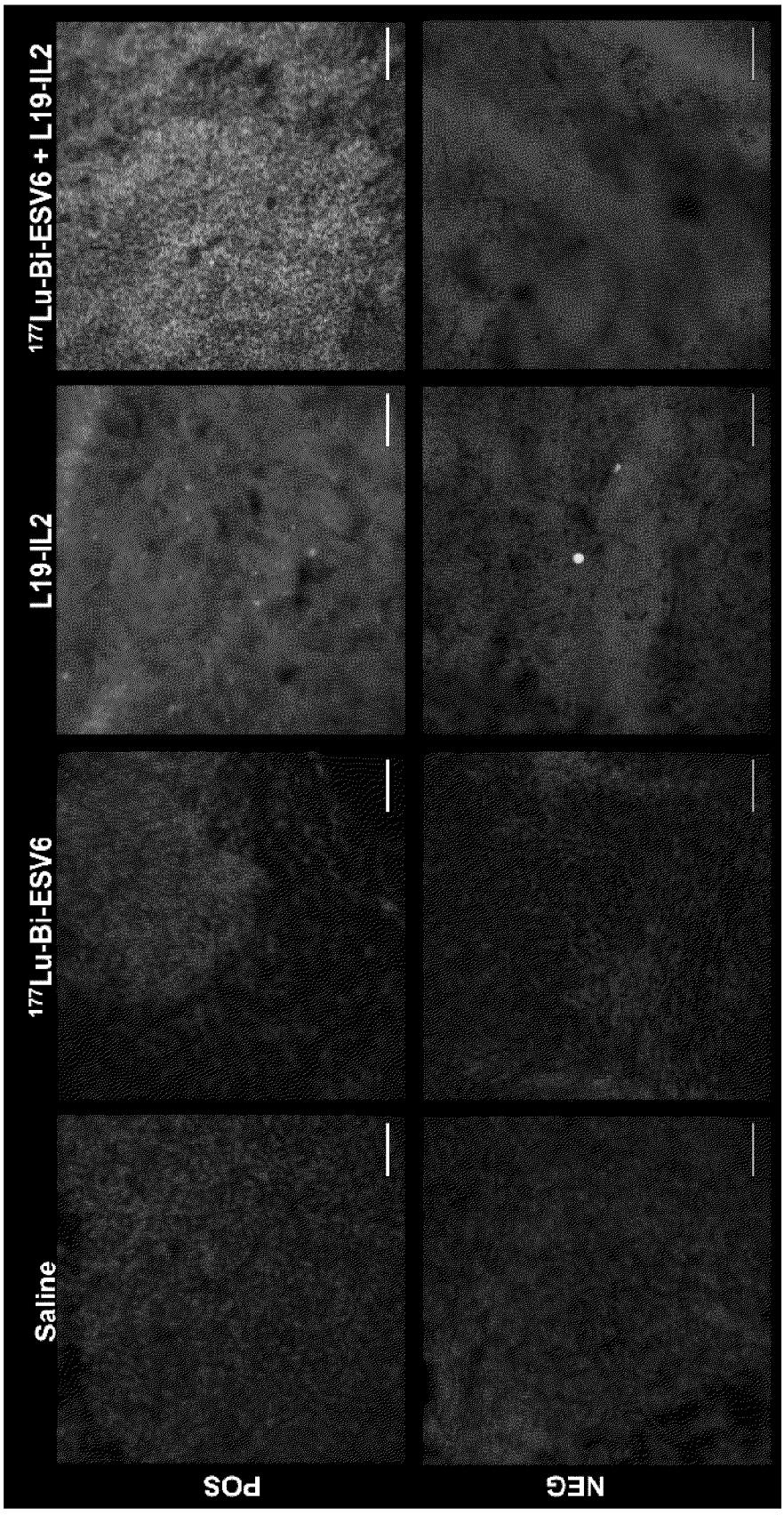


Fig. 6

B

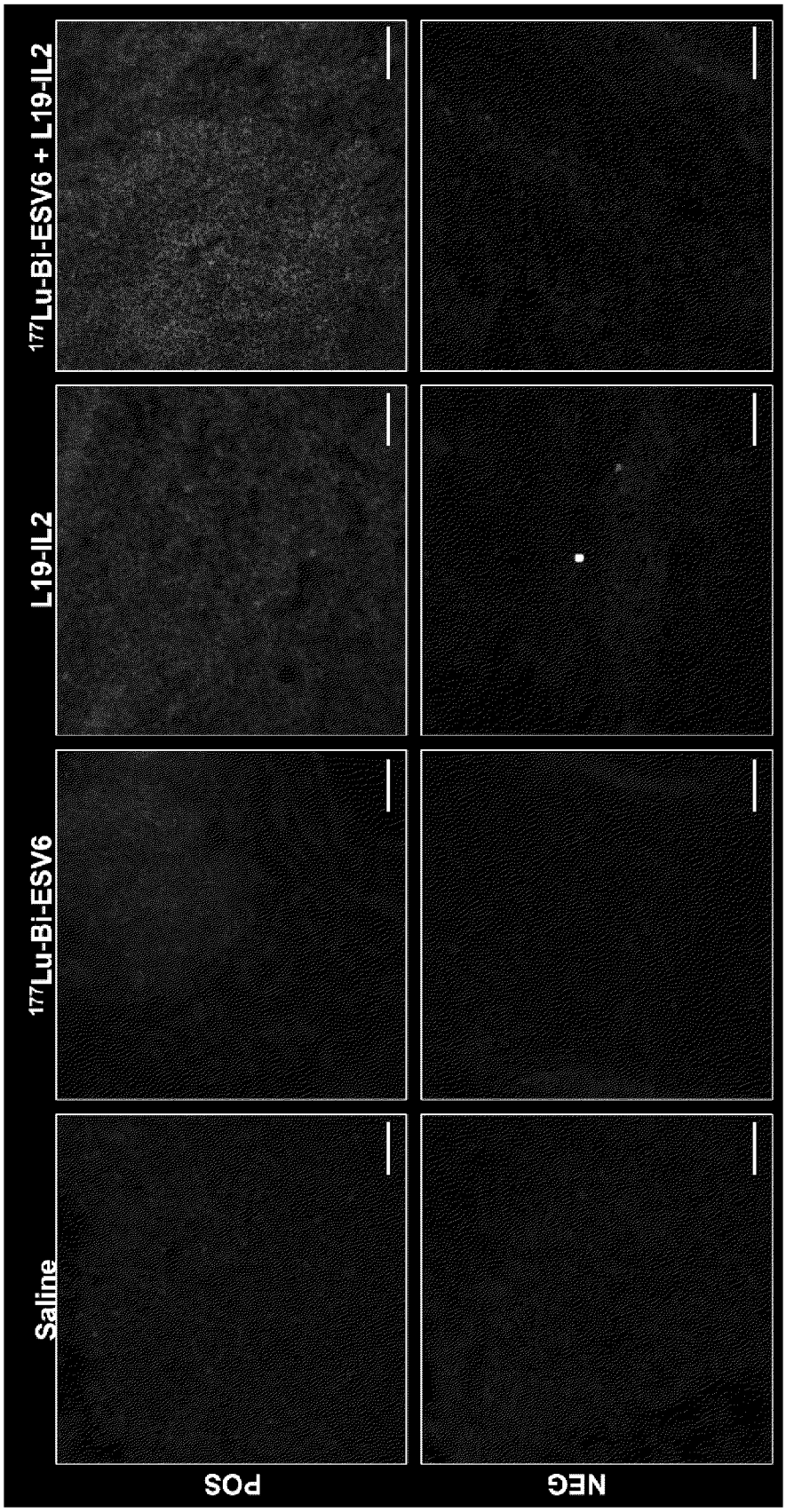


Fig. 6 ctd'

C

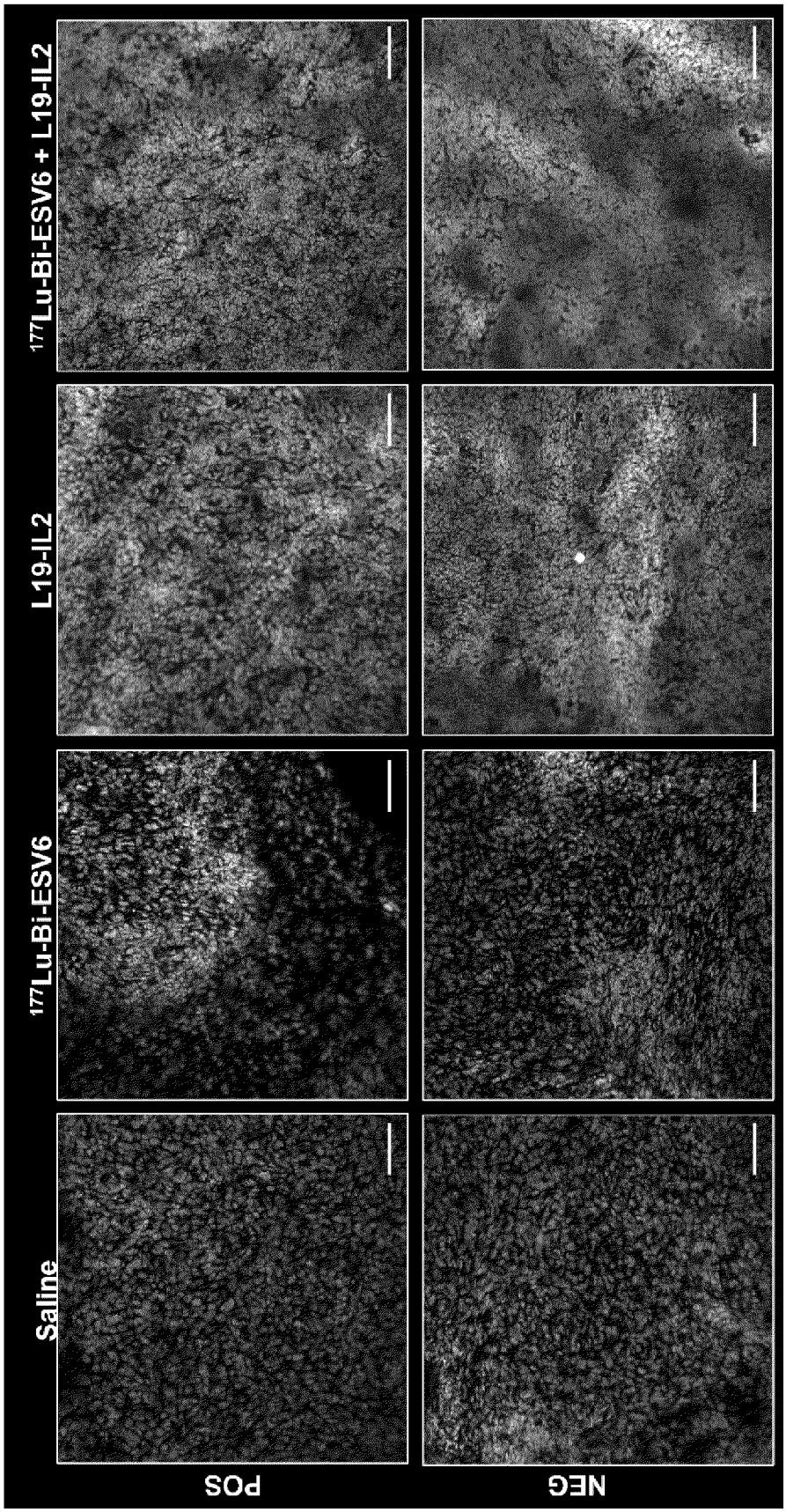


Fig. 6 ctd'

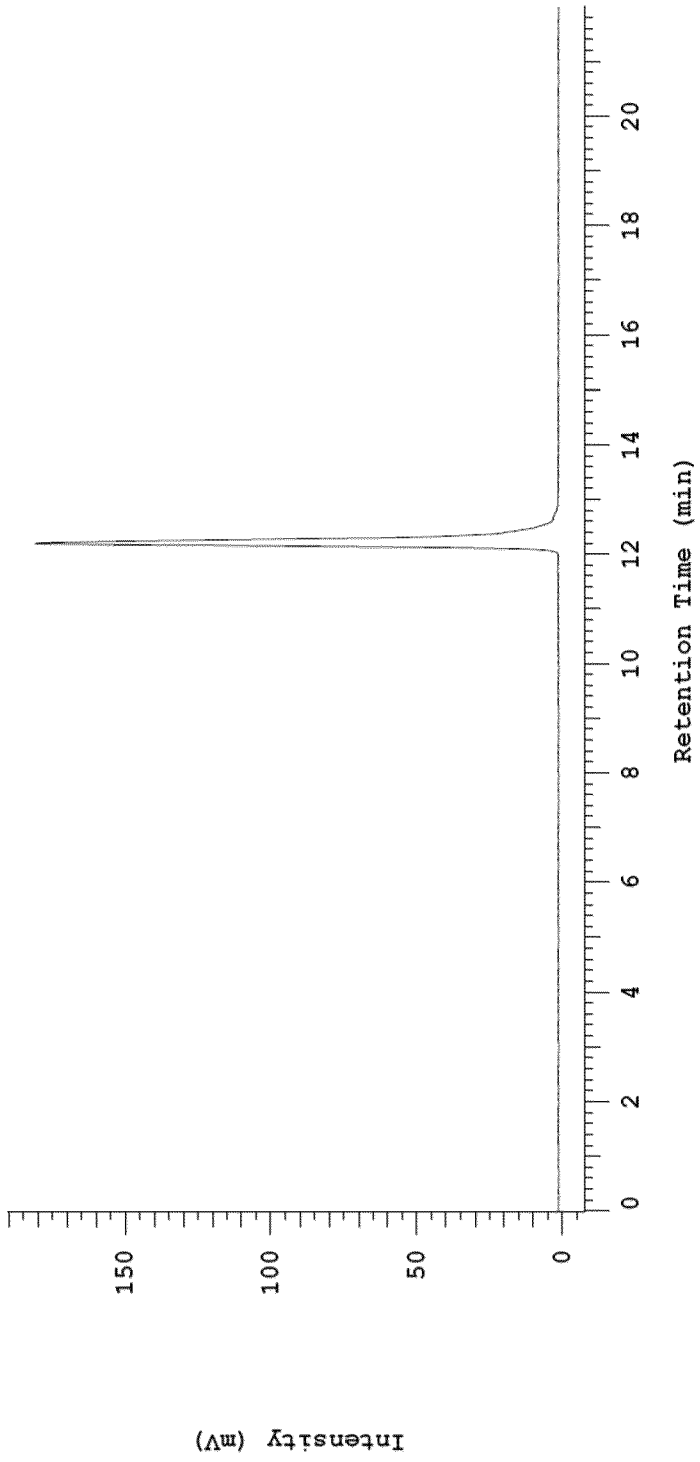

Intensity (mV)
150
100
50
0
0
2
4
6
8
10
12
14
16
18
20
Retention Time (min)

Fig. 7

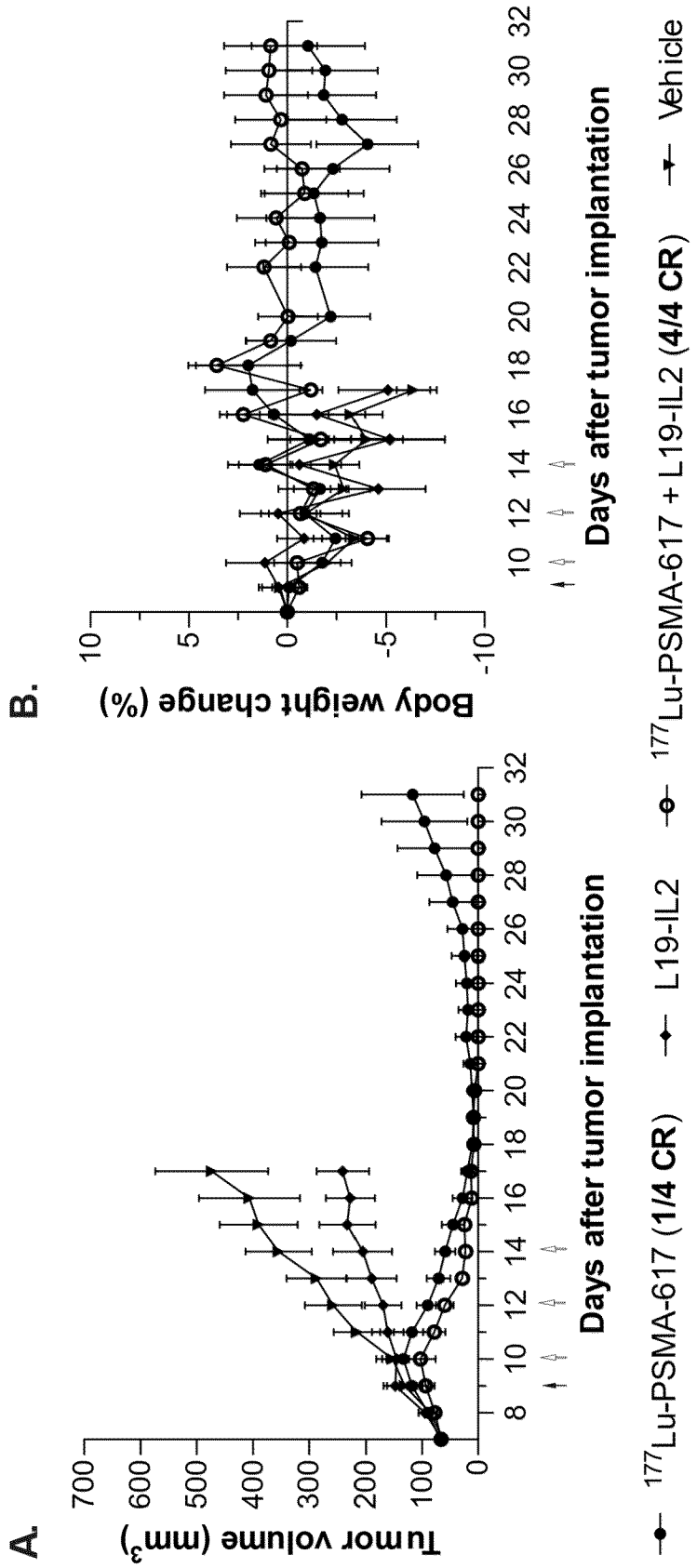

A.
Tumor volume (mm³)
700
600
500
400
300
200
100
0
8 10 12 14 16 18 20 22 24 26 28 30 32
Days after tumor implantation
177Lu-PSMA-617 (1/4 CR)
L19-IL2
177Lu-PSMA-617 + L19-IL2 (4/4 CR)
Vehicle
B.
Body weight change (%)
10
5
0
-5
-10
10 12 14 16 18 20 22 24 26 28 30 32
Days after tumor implantation

Fig. 8

A

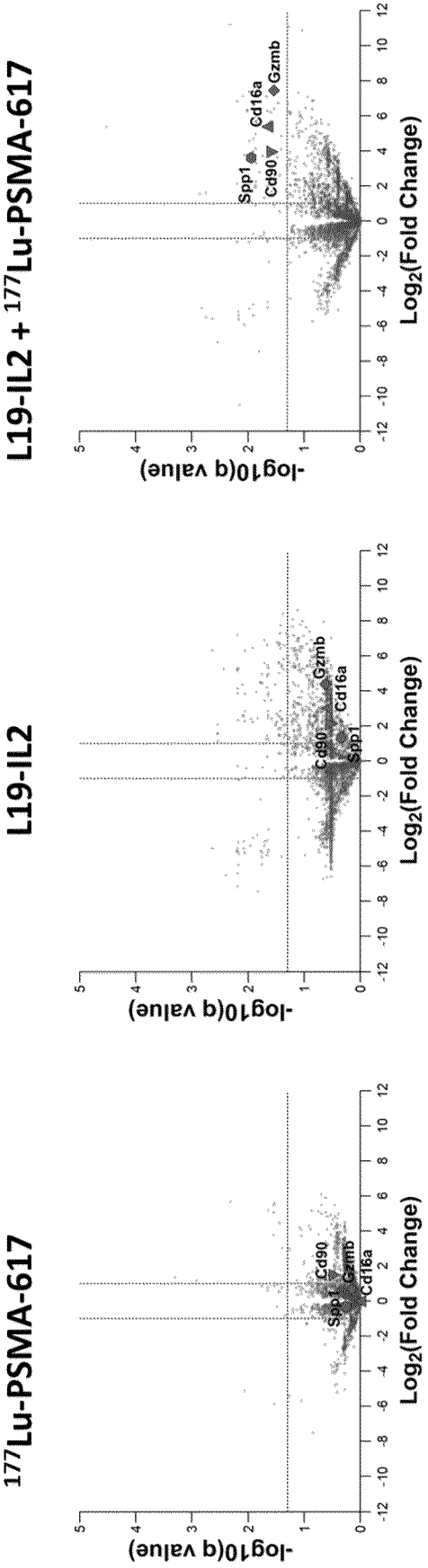

L19-IL2 + 177Lu-PSMA-617
L19-IL2
177Lu-PSMA-617
-log10(q value)
Log2(Fold Change)
Spp1
Cd90
Cd16a
Gzmb

Fig. 9

B

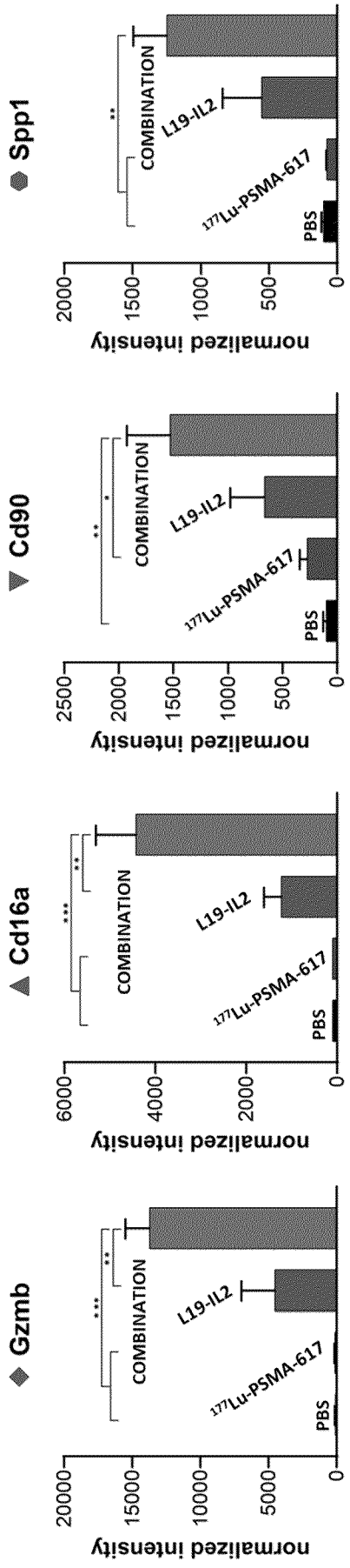

Spp1
normalized intensity
2000
1500
1000
500
0
**
COMBINATION
L19-IL2
177Lu-PSMA-617
PBS
Cd90
normalized intensity
2500
2000
1500
1000
500
0
**
*
COMBINATION
L19-IL2
177Lu-PSMA-617
PBS
Cd16a
normalized intensity
6000
4000
2000
0
***
**
COMBINATION
L19-IL2
177Lu-PSMA-617
PBS
Gzmb
normalized intensity
20000
15000
10000
5000
0
***
**
COMBINATION
L19-IL2
177Lu-PSMA-617
PBS

Fig. 9 ctd'

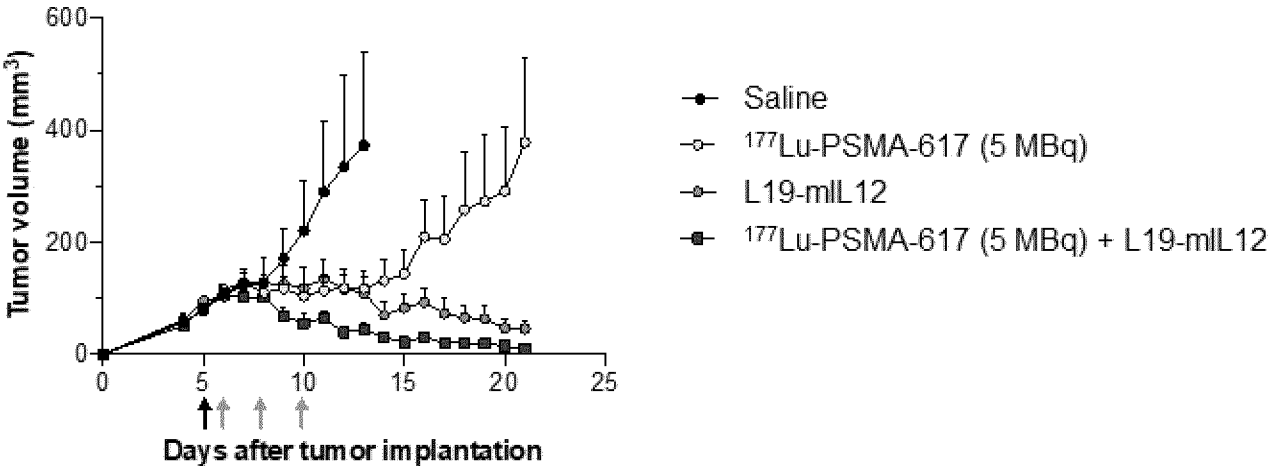

600
400
200
0
Tumor volume (mm3)
0
5
10
15
20
25
Days after tumor implantation
Saline
177Lu-PSMA-617 (5 MBq)
L19-mIL12
177Lu-PSMA-617 (5 MBq) + L19-mIL12

Fig. 10

A

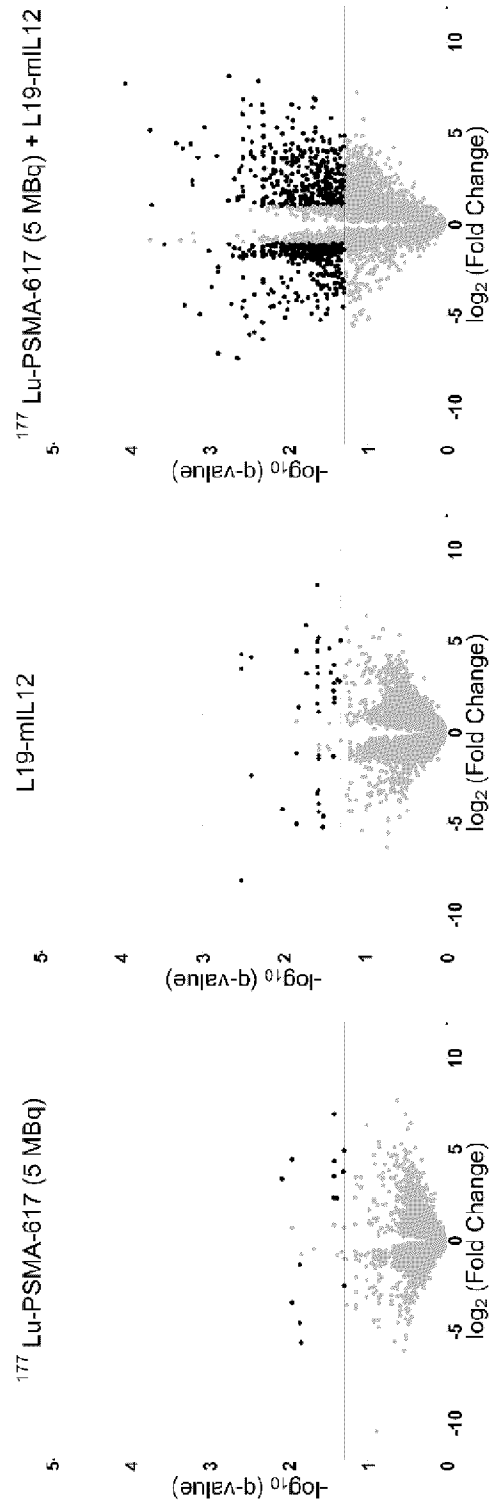

^{177}Lu-PSMA-617 (5 MBq) + L19-mIL12
L19-mIL12
^{177}Lu-PSMA-617 (5 MBq)
-log10 (q-value)
log2 (Fold Change)

Fig. 11

B

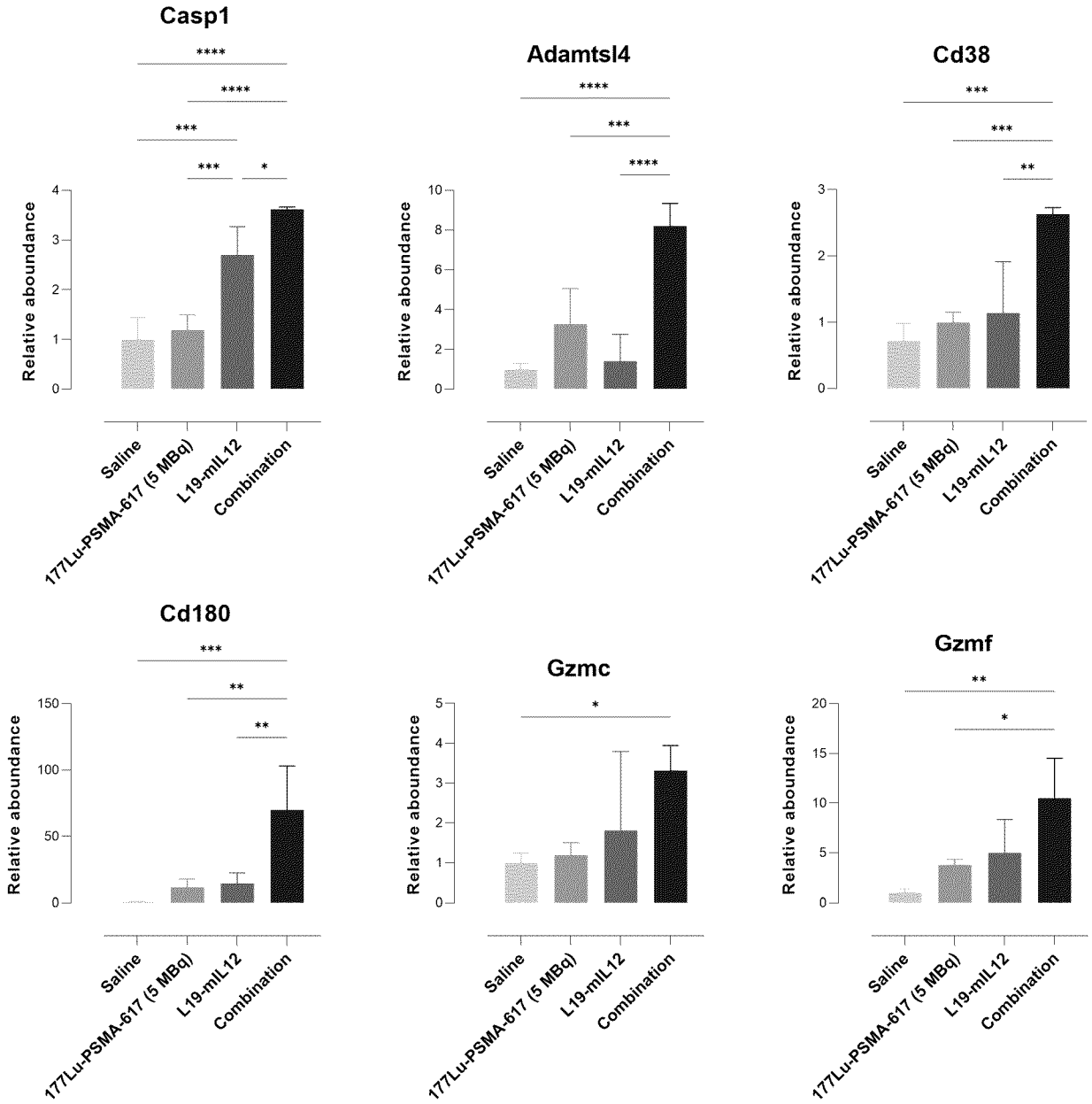

Casp1
Adamtsl4
Cd38
Cd180
Gzmc
Gzmf
Relative abundance
Saline
177Lu-PSMA-617 (5 MBq)
L19-mIL12
Combination

Fig. 11 ctd'

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/076164

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K47/68 A61K51/04 A61K103/30 A61P35/00 C07K14/55
C07K16/28 C07K14/54
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P C07K

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MILLUL JACOPO ET AL: "An ultra-high-affinity small organic ligand of fibroblast activation protein for tumor-targeting applications", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES (PNAS), [Online] vol. 118, no. 16, 20 April 2021 (2021-04-20), XP055893845, ISSN: 0027-8424, DOI: 10.1073/pnas.2101852118 Retrieved from the Internet: URL:https://www.pnas.org/content/pnas/118/16/e2101852118.full.pdf> abstract page 2, column 1, paragraph 2 - paragraph 3 page 2, column 2, paragraph 4 compound 4 page 4, column 1, paragraph 2 -/--	1-14

[x] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 November 2024	05/12/2024

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	Monami, Amélie

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	page 4, column 2, paragraph 3 - page 5, column 1, paragraph 1	
A	REKERS NICOLLE H. ET AL: "Combination of radiotherapy with the immunocytokine L19-IL2: Additive effect in a NK cell dependent tumour model", RADIOTHERAPY AND ONCOLOGY , vol. 116, no. 3 29 June 2015 (2015-06-29), pages 438-442, XP093183425, Ireland ISSN: 0167-8140, DOI: 10.1016/j.radonc.2015.06.019 Retrieved from the Internet: URL:https://pdf.sciencedirectassets.com/271320/1-s2.0-S0167814015X00120/1-s2.0-S0167814015003126/main.pdf?X-Amz-Security-Token=IQoJb3JpZ2luX2VjEE8aCXVzLWVhc3QtMSJHMEUCIQCKSCJNcUKhwB7/4iGfEi/oHe6V0vlPaCTpE8Jmd1cc9wIgQUOZGEKgpEQ4rryH/+cBDiz10eN9dyxnPsGUtahSq0kqswUIGBAFGgwwNTkwMDM1NDY4NjUiDF2m1hHuWBtWYVwfR abstract page 440, column 1, paragraph 1 - page 442, column 1, paragraph 2	1-14
A	EMANUELE PUCA ET AL: "The antibody-based delivery of interleukin-12 to solid tumors boosts NK and CD8+ T cell activity and synergizes with immune checkpoint inhibitors", INTERNATIONAL JOURNAL OF CANCER, JOHN WILEY & SONS, INC, US, vol. 146, no. 9, 28 August 2019 (2019-08-28), pages 2518-2530, XP071291880, ISSN: 0020-7136, DOI: 10.1002/IJC.32603 abstract page 2521, column 1, paragraph 2 - paragraph 4 page 2529, column 1, paragraph 4 - column 2, paragraph 1	1-14

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	M. Matasci: "Abstract 5553: A novel immunocytokine for the treatment of cancer", Cancer Research, 1 July 2018 (2018-07-01), XP055720611, DOI: 10.1158/1538-7445.AM2018-5553 Retrieved from the Internet: URL:https://cancerres.aacrjournals.org/content/78/13_Supplement/5553 [retrieved on 2020-08-06] the whole document -----	1-14
A	Chakravarty Rubel ET AL: "A review of advances in the last decade on targeted cancer therapy using 177Lu: focusing on 177Lu produced by the direct neutron activation route", Am J Nucl Med Mol Imaging, 1 January 2021 (2021-01-01), pages 443-475, XP093022510, Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8727880/pdf/ajnmmi0011-0443.pdf [retrieved on 2023-02-09] figure 7 page 453, column 1, paragraph 2 - page 455, column 1, paragraph 1 -----	1-14
X,P	GEORGIEV TONY ET AL: "Targeted interleukin-2 enhances the in vivo anti-cancer activity of Pluvicto(TM)", EUROPEAN JOURNAL OF NUCLEAR MEDICINE AND MOLECULAR IMAGING , vol. 51, no. 8 2 April 2024 (2024-04-02), pages 2332-2337, XP093183282, Berlin/Heidelberg ISSN: 1619-7070, DOI: 10.1007/s00259-024-06705-x Retrieved from the Internet: URL:https://link.springer.com/article/10.1007/s00259-024-06705-x/fulltext.html abstract figure 1C page 2333, column 1, paragraph 3 page 2336, column 1, paragraph 1 - column 2, paragraph 1 -----	1-14

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/076164

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	GALBIATI ANDREA ET AL: "Tumor-Targeted Interleukin 2 Boosts the Anticancer Activity of FAP-Directed Radioligand Therapeutics", THE JOURNAL OF NUCLEAR MEDICINE , vol. 64, no. 12 1 December 2023 (2023-12-01), pages 1934-1940, XP093183431, US ISSN: 0161-5505, DOI: 10.2967/jnumed.123.266007 Retrieved from the Internet: URL:https://jnm.snmjournals.org/content/jnumed/64/12/1934.full.pdf abstract page 1938, column 2, paragraph 1 - page 1939, column 1, paragraph 4 -----	1-14

INTERNATIONAL SEARCH REPORT

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PCT/EP2024/076164

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.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments: