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(54) Title: COLD KITS BASED ON PSMA LIGANDS FOR THE PREPARATION OF RADIOPHARMACEUTICALS

(57) Abstract: Object of the present invention is a novel formulation, based on *Prostate-Specific Membrane Antigen* (PSMA) ligands, for use as radiopharmaceutical precursors (cold kits). The formulation of the invention is designed to allow the radiopharmaceutical to be prepared instantaneously and in a single step by direct addition of radioactive isotopes, to be used both for diagnostic and therapeutic purposes.

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"COLD KITS BASED ON PSMA LIGANDS FOR THE PREPARATION OF RADIOPHARMACEUTICALS"

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Summary of the invention

Object of the present invention is a novel formulation, based on *Prostate-Specific Membrane Antigen* (PSMA) ligands, for use as radiopharmaceutical precursors (cold kits). The formulation of the invention is designed to allow the radiopharmaceutical to be prepared instantaneously and in a single step by direct addition of radioactive isotopes, to be used both for diagnostic and therapeutic purposes.

Background art

In the recent years, as a direct consequence of the increase in the incidence of prostate cancer, the worldwide demand for diagnostic, therapeutic and theranostic radiopharmaceuticals with which to intervene has also intensified.

In particular, one of the greatest challenges in the prostate cancer is the possibility of identifying the biochemical marker that characterizes it at an early stage, so far identified in an increase in PSA (*Prostate Specific Antigen*) levels. However, PSA in the serum is present in small amounts and increases, sometimes very slightly, only in pathological cases, such as in the case of an enlarged prostate (benign prostatic hypertrophy) or, as mentioned, in the case of the prostate cancer. The current *gold standard* used for PET/CT imaging diagnosis of the prostate cancer is currently the [^{18}F]Choline drug which, however, shows reduced sensitivity and specificity, especially in cases where PSA levels are very low.

The PSMA (*Prostate-Specific Membrane Antigen*) protein is a zinc membrane protein overexpressed in the prostate cancer cells. For this reason it could be a very interesting molecule both for obtaining good PET images of the prostate cancer and for its treatment. In fact, recent studies have shown that the radiopharmaceutical [^{68}Ga]PSMA (*Prostate-Specific Membrane Antigen*) may represent a promising alternative in the early diagnosis and, for this reason, some methods have been developed that allow the radio-labeling of said specific antigen with radioisotopes, both for diagnostic and therapeutic purposes.

One of the disadvantages associated with the use of the [^{68}Ga] isotope is its short

half-life ($t_{1/2}$ = 68 minutes), which implies the need to set up the radio-labeled compound very quickly, preferably instantaneously.

WO2016030103 describes a kit for radio-labeling several possible Gallium-68-based ligands comprising, in addition to the agent to be radio-labeled (functionalized with a radioactive-Gallium chelating agent), also an acetate salt or buffer and a co-chelating agent capable of inactivating any other metals present in the solution eluted by the Gallium-68 generator. The radio-labeling reaction takes place at room temperature, pH between 3 and 5 and following an incubation time of about 10 minutes with a yield ≥85% which varies depending on the type of ligand and chelator considered.

EP3064224 describes a method for radio-labeling a PSMA ligand with radioactive isotopes for the diagnosis and/or therapy of the prostate cancer. In particular, the method uses a sodium-based buffering agent and provides for the possible further correction of the pH by addition of NaOH to a value of 4-8. The incubation of the reaction is maintained for 5-10 minutes.

To date, there is still a need for a radioisotope labeling kit for PSMA that is easy to use, e.g., provides for the direct addition of the labeled isotope in any sterile acid solution, is applicable to different radionuclides, does not require pH adjustment or any incubation time, and allows for complexation yields and radiochemical purity greater than 95%.

Purposes of the invention

A purpose of the present invention is to provide a novel formulation based on *Prostate-Specific Membrane Antigen* (PSMA) ligands, for use as radiopharmaceutical precursors (cold kits), designed to allow the radiopharmaceutical to be prepared in a single step by direct addition of radioactive isotopes.

Another purpose of the present invention is to provide a formulation, based on PSMA ligands, which can be used for the preparation of radiopharmaceuticals to be used for diagnostic and/or therapeutic purposes following direct addition of the most appropriate radioisotope, without the need for pH adjustments of the reaction solution.

Another purpose of the present invention is to provide a process for manufacturing

the PSMA ligand-based formulation (cold kit) object of the present invention.

A further purpose of the present invention is to provide a method for preparing a radiopharmaceutical based on PSMA ligands in a single step, by direct addition of the appropriate radioactive isotope, without the need for pH adjustments of the reaction solution and with yields and radiochemical purity greater than 95%.

These and other purposes are achieved by the object of the present invention which provides a novel composition based on the PSMA ligands to be used as a kit for radiopharmaceutical preparations.

Brief description of the Figures

Figure 1 shows the image of the TLC plate on which the radiopharmaceutical preparation of Example 2.1 was run according to the conditions described in Example 2.2.1.

Figure 2 shows the chromatogram resulting from the HPLC analysis of the radiopharmaceutical preparation of Example 2.1, carried out according to the conditions described in Example 2.2.2.

Description of the invention

An object of the present invention is a *Prostate-Specific Membrane Antigen* (PSMA) ligand-based formulation for use as a kit for radiopharmaceutical preparations. Said formulation, also called "cold kit", is contained in a single vial containing the PSMA molecule functionalized with a chelating group of the radiopharmaceutical, in a quantity in the range between 10 and 30 µg, together with appropriate pharmaceutical excipients.

In the present invention, the PSMA molecule is present in a form characterized by the presence of a functionalization that allows its binding to the radionuclide; in the text it may therefore be referred to as "functionalized PSMA" for brevity. The skilled person in the art is fully aware of the different functionalized forms currently used of this membrane protein and will select the most appropriate one, depending on the type of radiopharmaceutical he intends to use.

In a preferred embodiment, the present invention involves the use of PSMA-11 having a HBED-CC chelating group capable of coordinating radioactive ions of interest, in particular Ga-68, Lu-177 and Ac-225, even at room temperature.

The formulation of the invention is, as mentioned, contained in a single vial/ampoule and is in the form of a white, sterile, apyrogenic lyophilic product.

This vial/ampoule is also the vessel in which the complexation and radio-labeling reaction takes place, without the need for the addition of other reagents or the need for any incubation or heating time.

In particular, in addition to the functionalized PSMA molecule, the formulation contains buffering excipients which, following the addition of concentrated hydrochloric acid in which the radioisotope is normally carried, are able to maintain the pH of the solution that is formed in a range between 3 and 6, more preferably between 5 and 6, considered optimal for the radio-labeling reaction.

In a preferred embodiment of the invention, excipients capable of protecting the functionalized PSMA molecule from degradation, by increasing its stability in the formulation itself and reducing the formation of peroxides and free radicals, as well as agents capable of assisting/coordinating chelation, are also present inside the vial/ampoule. In a preferred aspect said further excipients are multifunctional excipients capable of simultaneously performing more than one of the actions listed above.

In the present text the words "radio-labels", "radioisotopes" and "radionuclides" shall be considered synonymous.

The labeling of the functionalized PSMA molecule can be performed with different types of radioisotopes, particularly preferred are Ga-68, Lu-177 and Ac-225. Said radioisotopes are added directly to the reaction vial/ampoule containing the functionalized PSMA molecule and the other excipients. For example, Ga-68 is added in the form of sterile acid eluate coming from the radionuclide generator. By "eluate" is meant here to refer to the strongly acid solution produced by the machinery generating the radioisotopes and in which the radioisotopes are carried.

Said sterile acid eluate may have an HCl concentration of, for example, 0.05 N, 0.1 N or 0.6 N and be directly introduced into the reaction vial in different volumes, for example from 1 mL to 5 mL.

As previously mentioned, in the present invention the buffering systems forming part of the formulation contained inside the reaction vial/ampoule allow the pH to be

stabilized within values in the desired range, *i.e.* between 3 and 6, preferably between 5 and 6, even following the introduction of the sterile acid eluate resulting from the radioisotope generator.

According to a preferred aspect of the present invention, said buffering systems are preferably characterized by at least one, preferably more than one, of the following aspects:

- they are suitable for the use in injectable pharmaceutical preparations, they are non-toxic and can be administered intravenously in humans;
- they possess a buffering capability able of achieving and maintaining a pH between 3 and 6 inside the reaction vial/ampoule, even after the addition of different volumes (in a range between 1 mL and 5 mL) of a concentrated HCl solution, for example a 0.05 N, 0.1 N or 0.6 N solution, in the presence of functionalized PSMA and a radioisotope, preferably selected from Ga-68, Lu-177 and Ac-225;
- they are able to tolerate and compensate for small variations in volume and/or degree of acidity of the acid solution containing radionuclides (*i.e.* the eluate coming from the generator) caused, for example, by elutions and additions to the reaction vial/ampoule performed manually by the operator and not automatically by a machinery;
- they are able to determine high yields, over 95%, in the complexation reaction which occurs instantaneously and without the need for heating.

In the present invention, by the term "buffering system" is meant to denote the excipients allowing to bring and maintain the pH of the solution in the range of values most suitable for the complexation reaction between the functionalized PSMA molecule and the radionuclide, both when present as a single acid/base pair and when said pair is accompanied by other excipients assisting the buffering activity, the chelation reaction and/or having stabilizing activity for the formulation itself.

In particular, the present invention uses as a basic buffering system the boric acid/sodium borate pair obtained *in situ* in aqueous solution by using sodium tetraborate in the anhydrous or hydrated form, preferably in the decahydrated form. Other inorganic or organic salts of the tetraborate may be used. Alternatively, the boric acid/sodium borate pair can also be obtained *in situ* from any other suitable

analogous precursor. The boric acid compound has also already been used in formulations for intravenous drug administration in humans.

Sodium tetraborate decahydrate may be used in the present invention in an amount range between 80 and 100 mg, preferably between 85 and 88.5 mg. However, the amounts of boric acid/borate currently authorized for human use by EMA are capable of determining the buffering activity inside the formulation of the invention.

To said acid/base pair capable of buffering the solution of the reaction environment other excipients can be added, as previously mentioned, which will implement, on the one hand, the buffering capability of the acid/base pair, and on the other hand improve the coordination capability between the functionalized PSMA molecule and the radio-labels, that is, assisting the chelation and increasing the yield and radiochemical purity of the final compound.

In one of its configurations, the formulation of the invention comprises, for example, in addition to the boric acid/sodium borate pair, the addition of at least one sugar or mixture of sugars, the sugar preferably being a monosaccharide or disaccharide. Non-limiting examples of suitable sugars include glucose, sucrose, fructose, trehalose, mannitol and sorbitol. In some preferred embodiments, the sugar is a reduced sugar, more preferably mannitol or sorbitol. Preferably, the mannitol or sorbitol have the D-configuration, although the L-configuration may also be used.

In another of its configurations, the formulation of the invention comprises, for example, in addition to the boric acid/sodium borate pair, the addition of at least one compound having at least two hydroxyl groups separated by at least two atoms connected to each other in a chain or ring, said chain or ring containing carbon atoms, and optionally a heteroatom or heteroatoms such as N, S, or O. Said compounds having at least two hydroxyl groups may be, for example but not limited to, pinanediol, pinacol, ethylene glycol, diethylene glycol, catechol, 1,2-cyclohexanediol, 1,3-propanediol, 2,3-butanediol, 1,2-butanediol, glycerol and diethanolamine. For the purposes of the present invention, the compound having two hydroxyl groups is preferably a pharmaceutically acceptable compound and is preferably soluble or miscible with water.

In a preferred aspect of the invention said compound having the two hydroxyl groups

is a sugar, as described above, preferably a monosaccharide or a disaccharide, more preferably a reduced sugar, and even more preferably mannitol or sorbitol. In some particularly preferred embodiments, said compound is mannitol, more preferably D-mannitol.

Said compound having two hydroxyl groups, or a mixture of several compounds, preferably two compounds, having two hydroxyl groups, and the sodium tetraborate decahydrate may be introduced into the formulation in a mixture in a molar ratio with the sodium tetraborate decahydrate in a range from about 0.5:1 to 100:1, more preferably between 0.5:1 and 50:1. In the preferred embodiments, the compound having the two hydroxyl groups and the sodium tetraborate decahydrate are present in a ratio between 1:1 and 10:1, preferably between 1:1 and 5:1, even more preferably between 1:1 and 4:1.

The introduction, in the formulation of the invention, of at least one compound having at least two hydroxyl groups may be desired and/or advantageous since their concomitant presence with boric acid leads to the formation of esters thereof. The boric acid ester is an acid stronger than the corresponding unesterified acid (characterized by a lower acid/base equilibrium constant pKa) and thus a better buffering capability of the solution is obtained, i.e. it makes it easier to reach the desired pH values for the solution contained in the reaction vial once the sterile acid eluate from the radioisotope generator is added.

If desired, pyruvic acid may be added to the sodium tetraborate decahydrate of the formulation of the invention, either alone or together with the sugar or compound having two hydroxyl groups, as an acid species or in a salified form thereof, preferably sodium pyruvate, in an amount between about 1 mg and about 50 mg, preferably between 10 mg and 50 mg. The pyruvic acid is an α-keto acid naturally present in living organisms, including humans, and involved in several cellular processes, the safety of which via intravenous route is proven in the literature. In solution with the other components of the invention, the pyruvic acid/pyruvate pair will tend to be created, which will contribute to the buffering activity of the buffering system of which it is part, by bringing and maintaining the pH of the solution in the range of desired values between 3 and 6, preferably between 5 and 6.

The pyruvic acid also has antioxidant properties thanks to its ability to neutralize the peroxides; said molecules could form during the preparation and storage of the lyophilized product as well as during the radio-labeling step. Thus, this compound is able to act not only as an adjuvant in maintaining the pH within the predetermined range but also as a stabilizer of the formulation thus preventing the degradation of the functionalized PSMA molecule by the peroxides.

Alternatively, pyruvic acid may be replaced by another α-keto acid analogue, or salt thereof, which is capable of performing the same action.

A further aspect of the present invention provides for the presence of sodium hydroxide, which allows the buffer pair to respond more efficiently to the possible variations/differences in acidity and/or volume of the acid solution containing the radionuclide, while maintaining the pH of the radio-labeling solution within the desired range. Said sodium hydroxide may be present in an amount in the range between 0 mg and 50 mg, preferably between 1 mg and 30 mg, more preferably between about 1 and 15 mg.

In a preferred embodiment of the invention, the formulation comprises sodium tetraborate decahydrate, D-mannitol, sodium pyruvate and sodium hydroxide in relative ratios expressed as a weight/weight percentage of about 28%, 52%, 16%, 4% respectively, preferably 27.7%, 52.3%, 15.8%, 4.2% respectively.

The formulation of the invention, contained, as mentioned above, inside a single vial/ampoule, is prepared by subjecting to a lyophilization process between 2 and 5 mL of a solution containing the functionalized PSMA molecule in an amount between 10 and 30 μg, the sodium tetraborate decahydrate in an amount between 80 and 100 mg and possibly other desired or necessary excipients. Said lyophilization process may take place, for example, in a LIO5Pascal lyophilizer, for a time of 48 hours, at a temperature of -50°C and a pressure of 0.06 mbar.

The present invention describes the formulation for a "cold kit" for radio-labeling PSMA ligands containing a specific buffering system capable of maintaining the pH of the solution, in which the radio-labeling takes place, in a range between 3 and 6 following the direct addition of the acid eluate resulting from the radioisotope generator.

Said buffering system included in the formulation of the invention is constituted by an appropriate acid/base pair, in particular the boric acid/borate pair, and possibly other pharmaceutical excipients that contribute to improve the buffering capability of the acid/base pair as well as to assist the chelation reaction and stabilize/protect the functionalized PSMA molecule from possible degradation caused, for example, by the possible presence of peroxides.

The formulation of the invention causes that the radio-labeling reaction to take place instantaneously, without the need for heating, with a yield between 95 and 99% and a radiochemical purity between 95 and 99%.

The present invention further describes a process for radio-labeling the PSMA molecule with Ga-68, Lu-177 or Ac-225, which provides for the use of the "cold kit" for the diagnosis and therapy of the prostate cancer.

The invention will now be exemplified, in a non-limiting manner, by the following experimental section.

Experimental section

Example 1

Preparation of a lyophilized formulation containing the functionalized PSMA molecule, sodium tetraborate decahydrate, D-mannitol, sodium pyruvate and sodium hydroxide

Inside a single vial/ampoule, 2 mL of a solution containing 30 µg of the functionalized PSMA molecule, 88 mg of sodium tetraborate decahydrate, 166 mg of D-mannitol, 50 mg of sodium pyruvate and 325 µL of a 1 M NaOH solution are transferred. Such solution is stored in a chill room at -20°C until completely frozen. The vial/ampoule was then placed in a lyophilizer at a temperature between about -40°C and -50°C and pressure of 0.06 mbar, for a time of about 42 hours, until the ice completely disappeared. In a second step of the lyophilization cycle, the temperature of the vial/ampoule was gradually increased from about -40°C to about 25°C over a period of about 2 hours. The secondary drying process of the sample contained in the vial/ampoule was carried out for about 4 hours at a temperature of 25°C. The sample was subsequently sealed with rubber stopper under nitrogen stream and removed from the lyophilizer.

Example 2

Radiopharmaceutical preparation ^{68}Ga-PSMA-11

2.1 - SETTING UP THE RADIOPHARMACEUTICAL PREPARATION ^{68}Ga-PSMA-11

Inside the single vial/ampoule containing the lyophilized formulation derived from Example 1, 5 mL of $^{68}GaCl_3$ eluate coming from the ^{68}Ge/^{68}Ga generator eluted with a 0.1N HCl solution is transferred.

The solution is stirred by turning upside down the vial several times until the lyophilic product is completely solubilized.

The necessary quantities are taken immediately from the prepared radiopharmaceutical preparation to verify the radiochemical purity by analytical methods in TLC and HPLC.

2.2 - QUALITY CONTROLS ON THE RADIOPHARMACEUTICAL PREPARATION ^{68}Ga-PSMA-11

2.2.1 TLC method:

- Stationary phase: chromatographic paper iTLC SG (10 cm)
- Mobile phase: Ammonium acetate 77 g/L in water / Methanol (50:50 volume/volume)
- Sample to be tested: 1.5 µL
- Retention factors:
  - [^{68}Ga]gallium in colloidal form: 0.0 – 0.1
  - [^{68}Ga]PSMA-11: 0.8 – 1.0.

As can be observed from the numerical results in Table 1, which lists the data obtained by means of the TLC plate shown in Figure 1, the Gallium-68 present in the radiopharmaceutical preparation is almost exclusively in the form bound to the functionalized PSMA molecule (higher retention times, evident spot at the top of the plate) compared to a much lower percentage of Gallium-68 in colloidal form (much smaller spot at the base of the plate).

Table 1: Numerical results deriving from the TLC analysis of the radiopharmaceutical preparation ^{68}Ga-PSMA-11

	Percentage of total radioactivity due to Gallium-68
Gallium-68 in colloidal form	**3.2 %**
[^{68}Ga]-PSMA-11	**96.8 %**

2.2.2 HPLC method

- Stationary phase: Deactivated C18 silica gel column
- Mobile phase: [A] Trifluoroacetic Acid/Water 0.1:99.9 (volume/volume); [B] Trifluoroacetic Acid / Acetonitrile 0.1:99.9 (volume/volume)
- Sample to be tested: 5 µL
- Retention times:
  - [^{68}Ga]PSMA-11: between 6.5 and 7.5 minutes
  - Relative retention time free [^{68}Ga]Gallium(III) ion: 0.3 minutes

As can be observed from the numerical data listed in Table 2, derived from the analysis of the chromatogram shown in Figure 2, in the sample analyzed there is no presence of free Gallium-68 ions but only of the form bound to the functionalized PSMA molecule, in the two respective stereoisomeric forms.

Table 2: Numerical results from HPLC analysis of the radiopharmaceutical preparation ^{68}Ga-PSMA-11

	Percentage of total chromatogram area (Figure 2)
Free [^{68}Ga]Gallium (III) ion	**0 %**
[^{68}Ga]-PSMA-11 stereoisomer 1	**60.98 %**
[^{68}Ga]-PSMA-11 stereoisomer 2	**39.02 %**

Claims

1. A pharmaceutical formulation based on functionalized PSMA for the preparation of radiopharmaceutical precursors (cold kits), comprising a buffering system, said buffering system comprising the boric acid/borate pair.

2. The pharmaceutical formulation according to claim 1 characterized in that said buffering system is generated from anhydrous or hydrated, preferably decahydrate, sodium tetraborate.

3. The pharmaceutical formulation according to claim 1 or 2 characterized in that said buffering system further comprises at least one sugar or at least one compound containing at least two hydroxyl groups separated by two atoms connected to each other in a chain or ring, or a mixture thereof.

4. The pharmaceutical formulation according to any one of the preceding claims characterized in that said buffering system comprises an α-keto acid, preferably pyruvic acid, or a salt thereof.

5. The pharmaceutical formulation according to any one of the preceding claims characterized in that said buffering system comprises sodium hydroxide.

6. The pharmaceutical formulation according to any one of the preceding claims characterized in that said buffering system comprises sodium tetraborate decahydrate, D-mannitol, sodium pyruvate and sodium hydroxide in a relative w/w % ratio of about 28%, 52%, 16%, 4% respectively, preferably 27.7%, 52.3%, 15.8%, 4.2% respectively.

7. The pharmaceutical formulation according to any one of the preceding claims characterized in that said functionalized PSMA is a PSMA-11 comprising a HBED-CC chelating group.

8. Use of the pharmaceutical formulation according to any one of the preceding claims for obtaining a radio-labeled form of the PSMA molecule functionalized with a radionuclide, said radionuclide preferably being selected from Ga-68, Lu-177, Ac-225.

9. A method for the preparation of a radio-labeled form of the functionalized PSMA molecule, comprising directly adding a sterile acid solution containing a radionuclide to the pharmaceutical formulation according to any one of claims 1-7,

said radionuclide preferably being selected from Ga-68, Lu-177, Ac-225.

10. A radio-labeled form of the functionalized PSMA molecule according to the method of claim 9 for its use in the diagnosis or therapy of prostate cancer.

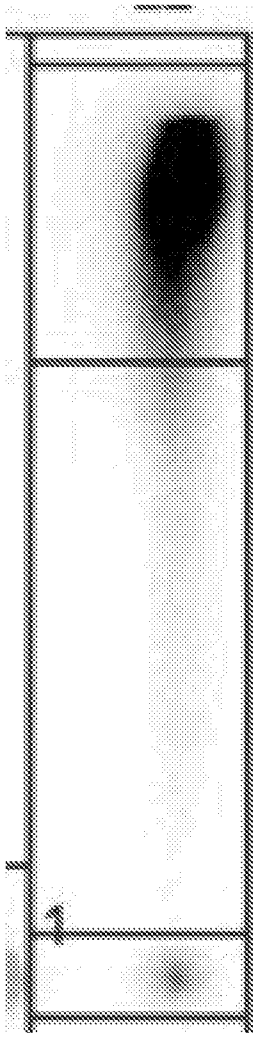

Figura 1

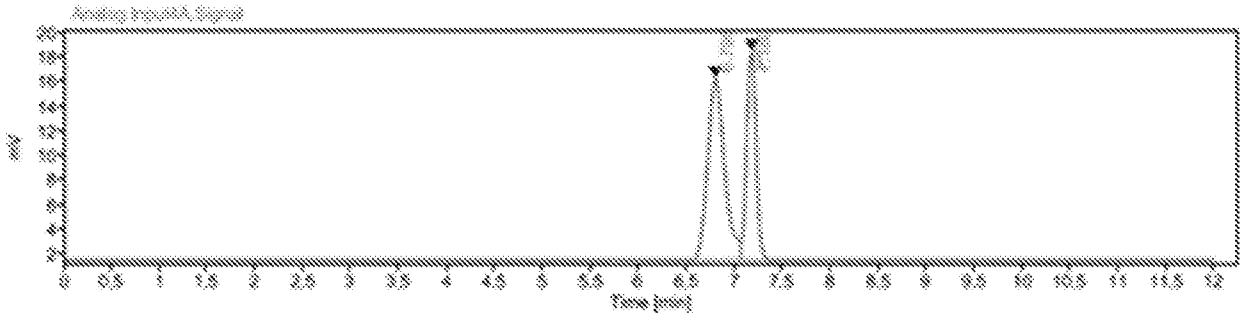

Figura 2

INTERNATIONAL SEARCH REPORT

International application No
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A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K51/04 A61K103/00 A61P35/00
ADD.

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

B. FIELDS SEARCHED

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

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 3 375 787 A1 (CELLBION CO LTD [KR]) 19 September 2018 (2018-09-19) claims 1,8-10 -----	1-6
X	WO 2016/142702 A1 (THERAGNOSTICS LTD [GB]) 15 September 2016 (2016-09-15) claims 1,2,11,13,21,27,29 ----- -/--	1-6,8

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

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Date of the actual completion of the international search	Date of mailing of the international search report
11 April 2022	22/04/2022

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2021/062376

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CALDERONI LETIZIA ET AL: "Evaluation of an Automated Module Synthesis and a Sterile Cold Kit-Based Preparation of 68 Ga-PSMA-11 in Patients with Prostate Cancer", THE JOURNAL OF NUCLEAR MEDICINE, vol. 61, no. 5, 1 May 2020 (2020-05-01), pages 716-722, XP055838503, US ISSN: 0161-5505, DOI: 10.2967/jnumed.119.231605 abstract	10
A	US 2018/267050 A1 (WOUTERS LUDOVIC [BE] ET AL) 20 September 2018 (2018-09-20) examples 3,4	1-10
A	EP 3 064 224 A1 (ISOTOPIA MOLECULAR IMAGING LTD [IL]) 7 September 2016 (2016-09-07) cited in the application example 3	1-10

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2021/062376

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 3375787 A1	19-09-2018	AU 2016352491 A1	14-06-2018
		CA 3004696 A1	18-05-2017
		CN 108290924 A	17-07-2018
		EP 3375787 A1	19-09-2018
		ES 2736134 T3	26-12-2019
		JP 6661010 B2	11-03-2020
		JP 2018537452 A	20-12-2018
		KR 101639599 B1	14-07-2016
		US 2018339071 A1	29-11-2018
		WO 2017082620 A1	18-05-2017
WO 2016142702 A1	15-09-2016	AU 2016230890 A1	31-08-2017
		CA 2979127 A1	15-09-2016
		CN 107438615 A	05-12-2017
		EP 3268337 A1	17-01-2018
		ES 2794581 T3	18-11-2020
		JP 6889665 B2	18-06-2021
		JP 2018517761 A	05-07-2018
		JP 2021130675 A	09-09-2021
		PL 3268337 T3	13-07-2020
		US 2018092996 A1	05-04-2018
		US 2021138094 A1	13-05-2021
		WO 2016142702 A1	15-09-2016
US 2018267050 A1	20-09-2018	US 2018267050 A1	20-09-2018
		US 2019361030 A1	28-11-2019
EP 3064224 A1	07-09-2016	EP 3064224 A1	07-09-2016
		EP 3763394 A1	13-01-2021
		IL 237525 A	29-05-2017
		US 2016256579 A1	08-09-2016
		US 2020138985 A1	07-05-2020