Abstract:
The invention relates to a radionuclide-labeled form of erlotinib comprising the chemical formula: as well as methods for in vivo imaging of cells to detect the overexpression of epidermal growth factor receptor tyrosine kinase (EGFR-TK) in a subject suspected of having a cancerous condition by imaging the subject using positron emission tomography after sufficient time has been provided for the radionuclide-labeled form of erlotinib to bind to EGFR-TK sites in the body.
RADIONUCLIDE-LABELED ERLOTINIB

FIELD OF THE INVENTION

The present invention generally relates to the pharmaceutical erlotinib, and more particularly, to radionuclide-labeled forms of erlotinib.

BACKGROUND OF THE INVENTION

The drug Tarceva (erlotinib, OSI-774) is currently approved in the United States and over 80 countries worldwide for the treatment of refractory locally advanced or metastatic non-small cell lung cancer. Erlotinib, also known according to the IUPAC name N-3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)quinazolin-4-amine, has the following chemical structure:

![Chemical structure of erlotinib](image)

The drug is also useful in treating pancreatic and several other forms of cancer. The drug functions by reversibly binding to and inhibiting epidermal growth factor receptor tyrosine kinase (EGFR-TK), a cell-surface protein involved in the proliferation of normal and malignant cells and which is overexpressed in the majority of cancer types.

Positron emission tomography (PET) is a well-known in vivo imaging technique used for producing a three-dimensional image or map of functional processes in the body. The technique involves the administration to a subject of a positron-emitting radionuclide tracer followed by detection of the positron emission (annihilation) events in the body. The radionuclide tracer is typically composed of a targeting molecule having incorporated therein one or more types of positron-emitting radionuclides. Some examples of positron-emitting radionuclides include
oxygen-15, nitrogen-13, carbon-11, and fluorine-18. These radionuclides have half-lives of about 2, 10, 20, and 110 minutes, respectively. In the case where the radionuclide tracer is a radionuclide-labeled drug, PET has been used for imaging of a drug binding to a target in the body. The imaging of a radiopharmaceutical in the body can have many purposes, including to determine receptivity of the drug by a patient, diagnose the type or extent of a disease in a subject, ascertain physiological or biochemical mechanisms occurring in diseased tissue, or to better understand the pharmacological mechanisms of the drug.

As erlotinib gains approval as a highly effective drug against various types of cancer, there would be a significant advantage in producing a radionuclide-labeled form of the drug. Radiolabeled erlotinib would allow, inter alia, for the improved treatment of patients by careful selection of those patients found to benefit from erlotinib as determined from pre-treatment studies using radiolabeled erlotinib. Such pretreatment studies using radiolabeled erlotinib could also provide improved diagnoses of patients suffering from cancer (e.g., in determining the degree of expression of EGFR-TK and visualization of tumors not detected by other means), as well as improve current knowledge of the drug’s pharmacological behavior and mechanism in the body.

SUMMARY OF THE INVENTION

These and other objectives, as will be apparent to those having ordinary skill in the art, have been achieved by providing a radionuclide-labeled form of erlotinib wherein the radionuclide is imageable by a positron emission imaging technique, such as PET.

In a preferred embodiment, the radionuclide-labeled form of erlotinib is according to the chemical formula:
In the above formula, erlotinib has been labeled with a carbon-11 radionuclide at the 6-position of the quinazoline ring system. The invention also contemplates erlotinib labeled with a carbon-11 radionuclide at the 7-position of the quinazoline ring system. The radionuclide-labeled form of erlotinib includes the neutral, acid salt, hydrous, and anhydrous forms of the drug.

The invention advantageously provides a radionuclide-labeled form of erlotinib that is readily imageable by a PET technique after administration of the drug to a subject. PET imaging of radiolabeled erlotinib in the body can provide a wealth of diagnostic information, most notably an assessment of the degree of efficacy of the drug in a patient before subjecting the patient to a full dosage regimen. The method can also provide information on the degree of expression of EGFR-TK in bodily tissues suspected of being cancerous, and accordingly, useful for determining the malignancy and stage of cancerous tissue that may be present in a subject. The radiolabeled erlotinib described herein can also be used for the radiotherapeutic treatment of cancer.
BRIEF DESCRIPTION OF THE DRAWINGS

The features and aspects of the present invention will be better understood by reference to the following figures.

FIG. 1. (A) Inhibition of proliferation by erlotinib in lung cancer cells. Cells were treated with erlotinib at the indicated concentrations for 48 hours. Viable cells were measured and inhibition of proliferation calculated as the percentage of the control cultures that were only treated with vehicle. Error bars represent standard deviation calculated from five replicate wells. (B) Expression of phosphorylated and total EGFR in lung cancer cell lines by western blotting. Actin is shown as a loading control.

FIG 2. [11C] erlotinib micro-PET imaging of lung cancer xenografts. Coronal micro-PET images of anesthetized athymic nude mice xenografted with (A) A549, (B) NCI358 and (C) HCC827 lung cancer cells at the left shoulder. Mice were injected with 10-15MBq of [11C] erlotinib via the lateral tail vein and dynamic scanning was performed for 90 minutes. White arrows point to the tumors. The hot spot in 3A (red arrow) was found in all mice; however it is not possible to show it in all mice as the tumors are located in different planes. Liver is seen with very high activity with spillover to the surroundings (arrowheads) (D-F) Time Activity Curve (TAC), showing activity in tumors compared to the surrounding tissue. Regions of interest (ROI) were manually drawn by creating a volume of interest in the central area of the tumor and in surrounding tissue. Blue and red lines represent surrounding tissue and tumor respectively.

FIG. 3. In Vivo biodistribution of [11C] erlotinib in lung cancer xenografts. Data are shown as % DPG (percentage of injected dose per gram) of [11C] erlotinib in athymic nude mice bearing s.c. A549 (n=3), NCI358 (n=3) and HCC827 (n=2) lung cancer cells.

DETAILED DESCRIPTION OF THE INVENTION

The invention is directed to radionuclide-labeled (i.e., radiolabeled) forms of erlotinib wherein the radionuclide incorporated into the erlotinib molecule is imageable by a positron emission technique such as PET. Preferably, the
radionuclide incorporated into erlotinib is carbon-11. There may be one or more carbon-11 isotopes incorporated into the erlotinib molecule. The carbon-11 isotope can be preferably located at the 6-position, the 7-position and/or the 6,7 position of the molecule. The invention further contemplates the carbon-11 isotope attached at any position in the molecule that permits imaging by e.g. PET.

In a preferred embodiment, the radionuclide-labeled form of erlotinib is within the general chemical formula:

\[
\begin{align*}
R^1 \quad \text{o} \quad \text{o} \quad \text{o} \quad \text{o} \quad \text{o} \\
R^2 \quad \text{o} \quad \text{o} \quad \text{o} \quad \text{o} \\
\quad \text{HN} \\
\quad \text{N} \\
\quad \text{alk} \\
\end{align*}
\]

In formula 1 above, \(R^1\) and \(R^2\) are independently either -\(^{11}\text{CH}_3\) or -\(\text{CH}_3\), provided that at least one of \(R^1\) and \(R^3\) is -\(^{11}\text{CH}_3\), wherein \(^{14}\text{C}\) designates the carbon-11 isotope of carbon in contrast to natural carbon (i.e., C) containing the natural abundances of isotopes. The carbon-11 labeled form of erlotinib shown above also includes its neutral (as shown), acid salt, hydrous, anhydrous, and known polymorphic forms as, for example, described in U.S. Patent No. 6,900,221, the entire contents of which are incorporated herein by reference. Some examples of acid salt forms include the hydrochloride (HCl) and mesylate (\(\text{CH}_3\text{SO}_3\text{H}\)) forms.

In one embodiment, the radionuclide-labeled form of erlotinib comprises the chemical formula:
The above structure corresponds to the chemical name $N$-(3-ethynylphenyl)-6-(2-[^11]C)methoxyethoxy)-7-(2-methoxyethoxy)-4-quinazolinamine.

In another embodiment, the radionuclide-labeled form of erlotinib comprises the chemical formula:

The above structure corresponds to the chemical name $N$-(3-ethynylphenyl)-6-(2-methoxyethoxy)-7-(2-[^11]C)methoxyethoxy)-4-quinazolinamine.

In another embodiment, the radionuclide-labeled form of erlotinib comprises the chemical formula:

The above structure corresponds to the chemical name $N$-(3-ethynylphenyl)-bis-6,7-(2-[^11]C)methoxyethoxy)-4-quinazolinamine.
Combinations of any two or more of the above compounds are also contemplated. For example, the radionuclide-labeled form of erlotinib can be a mixture of compounds according to formulas 2 and 3, or formulas 2 and 4, or formulas 3 and 4, or formulas 2, 3, and 4.

The radiolabeled erlotinib can be synthesized by any suitable means. For the case of the 6-[\textsuperscript{11}C] form of erlotinib (formula 2), the radiolabeled erlotinib is preferably produced by reaction of the 6-desmethyl form of erlotinib (OSI-420) with carbon-1 1 methyl iodide (i.e., \textsuperscript{11}CH\textsubscript{3}I) under conditions suitable for promoting a methylation reaction. For example, the 6-desmethyl form of erlotinib can be reacted with \textsuperscript{11}CH\textsubscript{3}I in the presence of sodium hydride (NaH) under suitable conditions, as follows:

\[
\text{6-desmethyl erlotinib} \xrightarrow{\text{\textsuperscript{11}CH\textsubscript{3}I, NaH}} \text{6.-\textsuperscript{11}C-erlotinib}
\]

The \textsuperscript{11}CH\textsubscript{3}I reactant can be synthesized by any suitable method. Preferably, \textsuperscript{11}CH\textsubscript{3}I is synthesized by reduction of \textsuperscript{11}CO\textsubscript{2} with hydrogen gas and suitable catalyst (e.g., nickel), and this followed by reaction with iodine (I\textsubscript{2}). See, for example Elsinga PH., Methods. 2002 Jul;27(3):208-17.
The $^{11}\text{CO}_2$ starting material can in turn be synthesized in a cyclotron reactor by proton bombardment of nitrogen gas admixed with a minor amount of oxygen gas (for example, 1% oxygen). See, for example, Elsinga P. H., *Methods*. 2002 Jul;27(3):208-17.

The 7-$[^{1}]C$ and 6,7-$[^{1}]C$ forms of erlotinib can be synthesized similarly to the synthetic procedure shown above for the 6-$[^{11}]C$ form of erlotinib.

The invention is also directed to a pharmaceutical composition of the radiolabeled erlotinib. The pharmaceutical composition includes the radiolabeled erlotinib compound described above in a pharmaceutically-acceptable carrier (i.e., vehicle or excipient).

Any of the excipients known in the art can be suitable herein depending on the mode of administration. A preferred vehicle for intravenous injection is a sterile (and pyrogen free) solution in up to 10% ethanol (EtOH) in normal saline solution, typically about 0.9% w/v of NaCl. For enteral administration, suitable carriers include gelatin, fatty acids (e.g., stearic acid) and salts thereof, talc, vegetable fats or oils, gums, glycols, starches, dextrans, and the like.

The pharmaceutical composition can also include one or more stabilizers, surfactants, salts, buffering agents, additives, or a combination thereof. The stabilizer can be, for example, an oligosaccharide (e.g., sucrose, trehalose, lactose, or a dextran), a sugar alcohol (e.g., mannitol), or a combination thereof. The surfactant can be any suitable surfactant including, for example, those containing polyalkylene oxide units (e.g., Tween 20, Tween 80, Pluronic F-68), which are typically included in amounts of from about 0.001% (w/v) to about 10% (w/v). The salt or buffering agent can be any suitable salt or buffering agent, such as, for example, sodium chloride, or sodium or potassium phosphate (e.g., Na$_2$HPO$_4$, 7OmM), respectively. Some examples of additives include, for example, glycerol, benzyl alcohol, and 1,1,1-trichloro-2-methyl-2-propanol (e.g., chloretone or chlorobutanol). If required, the pH of the solutions can be suitably adjusted and buffered.

The invention is also directed to using the above radiolabeled erlotinib in methods for the in vivo imaging of cells to which the radiolabeled erlotinib has
bound. The methods find particular importance in detecting the overexpression of epidermal growth factor receptor tyrosine kinase (EGFR-TK) in a subject. The overexpression of EGFR-TK is an indicator for the presence of cancerous tissue. By "overexpression" is typically meant at least a 1-fold increase, and more typically, a 1- to 3-fold functional increase in EGFR-TK activity compared to surrounding tissue. Even more typically, an overexpression is indicated by a 1.5- to 2-fold functional increase in EGFR-TK activity compared to surrounding tissue. Accordingly, the methods described herein are capable of detecting cancerous tissue, as well as useful in ascertaining the aggressiveness and stage of the cancer. Such information can, in turn, provide useful information on a proper course of action, e.g., on whether drug treatment, chemotherapy, surgery, radiotherapy, or a combination thereof, are most appropriate.

The method begins with administration of the above-described radiolabeled erlotinib ("the drug" or "radiolabeled drug") into a subject suspected or known to be suffering from a cancerous condition or any other systemic disease involving the EGF receptor. The cancer can be any type of cancer, but is more typically lung cancer (particularly non-small cell lung cancers) or pancreatic cancer. The subject can be any mammal (e.g., a cat, dog, horse, or ape), but the method is more typically directed to human subjects. The drug can be administered to the subject in any suitable manner that can allow the drug to enter the bloodstream. For example, the drug can be administered orally (i.e., enterally). Human pharmacokinetic and pharmacodynamic studies have shown, that when administered orally, erlotinib slowly undergoes metabolism, the main metabolite being the desmethylated form of erlotinib (OSI-420, as shown above). The drug can also be administered parentally (i.e., by infusion through the skin), topically (i.e., on the skin), or by injection (e.g., intravenously or intramuscularly). For oral administration, liquid or solid oral formulations can be given. These include, for example, tablets, capsules, pills, troches, elixirs, suspensions, and syrups.

Following administration of the radiolabeled drug, the subject is scanned using a PET technique, as widely practiced in the art, after sufficient time has been provided for the radiolabeled erlotinib to bind to EGFR-TK in bodily tissue. A
sufficient amount of time for the radiolabeled erlotinib to bind to EGFR-TK is variable, but is typically within about 10-60 minutes and more typically within about 20-40 minutes. The PET scan can be augmented or combined with any related imaging technique known in the art, such as, for example, x-ray computed tomography (CT), magnetic resonance imaging (MRI), functional magnetic resonance imaging (fMRI), ultrasound, and single photon emission computed tomography (SPECT).

The radiolabeled drug is administered in a diagnostic-effective amount. A diagnostic-effective amount is an amount which allows a sufficient amount of the drug to bind to a biological target (such as EGFR-TK) in a subject such that the drug, when bound, can be observed by imaging a subject using a positron emission imaging technique such as PET. The amount of radiolabeled drug administered depends on several factors, including the weight of the subject. Preferably, the diagnostic-effective amount corresponds to a radioactive dosage in the range of about 50 to about 500 MBq, more preferably 100 to about 500 MBq (wherein "Bq" indicates the becquerel unit of radioactivity). In a preferred embodiment, the diagnostic-effective dose is 500 MBq, which corresponds to a radiation dose of 1.7 mSv.

In a preferred embodiment, the result of the PET imaging procedure functions as a pre-trial run (i.e., preclinical trial) of the drug for a patient. The pre-trial run is used typically to optimize future treatment of a patient, establish or refine a diagnosis, or for patient selection. Typically, for this purpose, the radiolabeled drug is administered at a non-therapeutically effective dose. A non-therapeutically effective dose is a dosage level lower than the dosage level typically administered for the corresponding non-radiolabeled version of the drug. This lower dosage level, also known as a "sub-pharmacological dose," can serve, by PET imaging, to provide information on the activity or efficacy of the drug for a particular patient. The resulting information is useful for identifying responders or non-responders, or to predict side effects, or to obtain a biodistribution profile of the drug. The information gathered can also be useful in determining whether the drug can sufficiently distinguish target cells from non-target cells for a particular patient. The
specificity and efficacy of the drug is dependent on several factors, including the
type of cancer and the unique biochemical receptivity to the drug, which differs
from patient to patient.

Accordingly, the information garnered from an initial PET scan of the
radiolabeled drug at a sub-pharmacological dose can be used for determining a
minimum effective and therapeutically effective dose of the drug for a particular
patient, the dose being effective for treatment while keeping within a dosage that
minimizes side effects and toxicity. In this way, a customized treatment plan using
erlotinib can be provided for each patient wherein the customization is based on
results found by performing a PET scan of a subject who has been administered
radiolabeled erlotinib at a sub-pharmacological dose. A significant advantage in
using the pre-trial run is that it prevents a patient who is not responsive to, or
otherwise would not benefit by regular dosage treatment of erlotinib, from
unnecessarily receiving a regular dosage treatment. In so doing, such a patient is
safeguarded from the possible dangers of receiving a regular dosage treatment when
such a treatment would either be too hazardous, ineffective, or unnecessary for the
patient.

The results of the pre-trial run described above can be used to determine an
effective course of treatment of a cancerous condition. For example, it may be found
from a pre-trial run conducted using a non-therapeutic dose of the radiolabeled
erlotinib that an effective course of treatment is to administer a therapeutic dosage
treatment of non-radiolabeled erlotinib. Thus, a pre-trial run using a non-therapeutic
dose of radiolabeled erlotinib may be followed by the administration of non-
radiolabeled erlotinib at a therapeutic dosage level. The therapeutic dosage level of
non-radiolabeled erlotinib can also be adjusted according to the findings in the pre-
trial run. For example, it may be found from the pre-trial run that the patient suffers
from a particularly malignant form of cancer and that the drug appears to function
efficaciously with minimal toxic effects. In such a case, the patient can be provided
a high therapeutic dosage of non-radiolabeled erlotinib of, for example, about 100
mg/kg, 110 mg/kg, 120 mg/kg, 130 mg/kg, 140 mg/kg, 150 mg/kg, 160 mg/kg, 170
mg/kg, 180 mg/kg, 190 mg/kg or 200 mg/kg, or alternatively, a regimen of dosages
kept within a range between any of the foregoing dosages (e.g., within 100-120 mg/kg, or 130-160 mg/kg, or other range). In a preferred embodiment, a high therapeutic dosage is about 150 mg/kg. In contrast, it may be found from the pre-trial run that the patient is highly receptive to the drug, and thereby, a smaller effective dosage of non-radiolabeled erlotinib is sufficient, such as 10 mg/kg, 20 mg/kg, 30 mg/kg, 40 mg/kg, 50 mg/kg, 60 mg/kg, 70 mg/kg and 80 mg/kg, or alternatively, a regimen of dosages kept within a range between any of the foregoing dosages (e.g., 10-40 mg/kg, or 30-70 mg/kg, or other range). In a preferred embodiment, a lower effective dosage of non-radiolabeled erlotinib is 50 mg/kg.

The course of treatment can be combined with any other forms of treatment for cancer. For example, the treatment may also include chemotherapy, the administration of other cancer treatment drugs, surgery, or radiotherapy, as appropriate. Some examples of other cancer treatment drugs include vinblastine, cisplatin, 5-fluorouracil, tamoxifen, and several others. The cancer treatment may also include administration of an anti-angiogenesis agent. Some examples of such drugs include MMP-2 (matrix-metalloproteinase 2) inhibitors, MMP-9 (matrix-metalloproteinase 9) inhibitors, and COX-II (cyclooxygenase II) inhibitors. The cancer treatment may also include drugs other than erlotinib that function as signal transduction inhibitors (e.g., EGFR, VEGF, and ErbB2 inhibitors). Drags that do not directly treat cancer but that have an adjuvant effect can also be included. Adjuvants include compounds that promote a cancer treatment mechanism or that ameliorate side effects.

The radiolabeled drug can be administered once in a single dosage before observation of the subject by a PET technique. Alternatively, the radiolabeled drug can be administered in separate doses before one or more observations. The radiolabeled drag can also be administered in separate doses or in a continuous mode during observation such that the moment of binding of the radiolabeled drag to bodily tissue can be observed, and/or the progress of binding, biodistribution, and release monitored. In addition, the subject can be administered a formulation which releases the radiolabeled drug into the subject in a controlled manner over time (i.e., as a controlled release formulation).
In another embodiment, the method includes administering the radiolabeled erlotinib in non-therapeutic amounts to subjects not suffering from cancer in order to establish a control (i.e., baseline) indicative of the level of a normal level of expression of EGFR-TK. The level of expression of EGFR-TK found in the control can be compared to the level of expression of EGFR-TK found in a patient suspected of suffering from cancer in order to determine if the level of EGFR-TK in the patient is overexpressed, and hence, indicative of a cancerous condition.

Examples have been set forth below for the purpose of illustration and to describe the best mode of the invention at the present time. However, the scope of this invention is not to be in any way limited by the examples set forth herein.
Materials and Methods

Cell lines and reagents

Human lung cancer cell lines A549, NCI 358 and HCC 827 were obtained from the American Type Culture collection (ATCC, Rockville, Md., USA). The HCC827 has a high expression of the EGFR and harbors an in frame deletion mutation (delE746-A750) in exon 19 (Chang, J. W., Chou, C. L., Huang, S. F., Wang, H. M., Hsieh, J. J., Hsu, T. and Cheung, Y. C., Lung Cancer, 58: 414-417, 2007). Cells were grown in DMEM cell culture media supplemented with 10% fetal bovine serum at 37°C in a humidified atmosphere of 95% air and 5% CO₂ (v/v).

Erlotinib hydrochloride and its precursor were provided by OSI pharmaceuticals.

Western Blotting

Western blotting was performed as described previously (Memon, A. A., Sorensen, B. S. and Nexo, E., Scandinavian Journal of Clinical & Laboratory Investigation, 66: 623-630, 2006). Briefly, lung cancer cells were cultured and harvested at -80-90% confluence, the cell pellet was disrupted on ice for 30 minutes in RIPA buffer and cleared by centrifugation as described previously (Schooler, K. and Wiley, H. S., Analytical Biochemistry, 277: 135-142, 2000). Protein concentration was determined with BCA reagent (Pierce Chemical). Equal amounts of protein (25 µg) were resolved by SDS-PAGE. The resolved proteins were transferred onto PVDF membrane, and blocked with 5% (w/v) non-fat dry milk in TBS-T solution. The blots were incubated with specific primary and secondary antibodies according to the data sheet provided by the manufacturers. Immunoreactive bands were detected by ECL reagents (Amersham Biosciences).

Antibody used for western blotting analysis were, p-EGFR (Tyr1173, sc-12351 Santa Cruz), total EGFR (sc-03 Santa Cruz) and Actin (Sigma, Denmark).
Growth inhibition assay

Growth inhibition was assessed by CellTiter 96 Non-Radioactive Cell Proliferation Assay Kit (Promega), a colorimetric method for determining the number of viable cells. Experiments were performed according to the instructions of the manufacture. Briefly, cells were cultured in 96 well plates provided with the kit. The number of cells required for each cell line to obtain an optical density of 1.3-2.2 (linear range of the assay) at a wavelength of 490 nm after 48 hours was determined empirically. The number of cells for each cell lines was: A549, 3000 cells; NCI358 and HCC827, 5000 cells. Twenty-four hours after seeding, cells were treated with 0.5-6 µM erlotinib or with vehicle for 48 hours.

EXAMPLE 1

Preparation of 6-[14C] Erlotinib

14CO2 was produced by the 14N(p, <)14C nuclear reaction (the bombardment of nitrogen gas with protons) using the PET-trace cyclotron at Arhus Sygehus PET centre. In order to form 14CO2 as the synthetic starting material, 1 % of O2 was mixed with the nitrogen target material.

14CO2 was converted to 14^-methyl iodide by a standard procedure using a GE Medical Systems Mel-Box. Briefly, 14CO2 was trapped on molecular sieves, reduced to 14CH4 by H2 on Ni-catalyst, and subsequently reacted with Iodine (I2) in the gas phase to form 14CH3I. The 14CH3I was collected in a reaction vessel in a stream of helium gas. [O-methyl-14C] erlotinib was prepared by methylation of 6-O-desmethyl erlotinib (OSI-420) with 14C methyl iodide in dimethyl formamide (DMF) with sodium hydride as supporting base.

After reacting at 120°C for 5 minutes, the crude product was purified by reversed phase HPLC of the reaction mixture on a Waters Xterra 5µ C-18 ODB (150 x 19 mm) HPLC column using 46:54 sterile ethanol:sterile 70 mM aqueous NaH2PO4 as mobile phase. The fraction corresponding to [O-methyl-14C] erlotinib was collected and transferred to a rotary evaporator where it was evaporated to near-dryness (100 °C under vacuum). The product was then reformulated in sterile saline.
(9 ml) followed by sterile 70 % ethanol (1 ml) and subsequently passed over a sterile 0.22 µm filter into a sterile vial. The activity of the final product was measured in a dose calibrator and ca. 0.5 ml of the formulation was withdrawn for QC using sterile needle and syringe. The vial containing the [O-methyl- 11C] erlotinib remained in quarantine until the product had satisfied all quality control tests and was subsequently released to the PET scanning suite. The shelf life of the product should not exceed 1 hour.

EXAMPLE 2

Test Results For Three Batches of Preparation of 6-[11C] Erlotinib

<table>
<thead>
<tr>
<th>Batch nr.</th>
<th>TAR-070702-T2</th>
<th>TAR-070719-T1</th>
<th>TAR-070719-T2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bombardment time</td>
<td>31 min</td>
<td>44 min</td>
<td>15 min</td>
</tr>
<tr>
<td>Time at End of Bombardment (EOB)</td>
<td>14.52</td>
<td>11.29</td>
<td>13.45</td>
</tr>
<tr>
<td>Time at End of Synthesis (EOS)</td>
<td>15.28</td>
<td>12.06</td>
<td>14.16</td>
</tr>
<tr>
<td>Activity at EOS</td>
<td>0.5 GBq</td>
<td>1.24 GBq</td>
<td>0.35 GBq</td>
</tr>
<tr>
<td>Product:</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Volume (10 ± 1 ml)</td>
<td>10 ml</td>
<td>10 ml</td>
<td>10 ml</td>
</tr>
<tr>
<td>Sterile filter integrity</td>
<td>Intact</td>
<td>Intact</td>
<td>Intact</td>
</tr>
<tr>
<td>Colorless and no particles</td>
<td>Ok</td>
<td>ok</td>
<td>ok</td>
</tr>
<tr>
<td>pH (5-8)</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Radio chemical purity (&gt;95%)</td>
<td>96.4%</td>
<td>95.7%</td>
<td>100%</td>
</tr>
<tr>
<td>Desmethyl tarceva (&lt;1.0 µg/ml)</td>
<td>0.125 µg/ml</td>
<td>0.08 µg/ml</td>
<td>0.13 µg/ml</td>
</tr>
<tr>
<td>Tarceva content (&lt;5.0 µg/ml)</td>
<td>0.12 µg/ml</td>
<td>0.097 µg/ml</td>
<td>0.17 µg/ml</td>
</tr>
<tr>
<td>DMF content (&lt;880 ppm)</td>
<td>20 ppm</td>
<td>13 ppm</td>
<td>710 ppm</td>
</tr>
<tr>
<td>Half-life (20.4 ± 2.0 min)</td>
<td>20.2 min</td>
<td>20.18 min</td>
<td>20.18 min</td>
</tr>
<tr>
<td>©-spectrum (only 511 keV and</td>
<td>Ok</td>
<td>ok</td>
<td>ok</td>
</tr>
<tr>
<td>LAL (&lt;175/Volume, EU/ml)</td>
<td>&lt;2 EU/ml</td>
<td>&lt;2 EU/ml</td>
<td>&lt;1 EU/ml</td>
</tr>
<tr>
<td>Sterility</td>
<td>Sterile</td>
<td>Sterile</td>
<td>Sterile</td>
</tr>
<tr>
<td>Stability after:</td>
<td>1 hour</td>
<td>1 hour</td>
<td>1 hour</td>
</tr>
<tr>
<td>Radiochemical purity</td>
<td>96.6 %</td>
<td>&gt; 99 %</td>
<td>100 %</td>
</tr>
<tr>
<td>pH</td>
<td>6</td>
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<tr>
<td>Colorless and no particles</td>
<td>Ok</td>
<td>ok</td>
<td>ok</td>
</tr>
</tbody>
</table>

Radiochemical purity is designated as the percentage of [O-methyl- 11C] erlotinib radioactivity in relation to the sum of all radioactive components detected.

Specification: [O-methyl- 11C] erlotinib > 95%
Identity of [O-methyl-\(^{11}\text{C}\)] erlotinib is confirmed by coinjection with an authentic reference sample of non-radioactive erlotinib onto a Phenomenex Synergi FUSION 4 RP-80 (250 x 4.6 mm) HPLC column using radio and UV detection (280 nm).

1. injection: pure product or diluted with water if the radioactivity concentration is too high. (For the determination of radiochemical purity).

2. injection: coinjection of product and reference solution (50 mg/ml). (For the confirmation of product identity).

EXAMPLE 3

Pharmaceutical Formulation(s) of 6-[\(^{11}\text{C}\)] Erlotinib

The IV product was formulated in sterile saline (9 ml) followed by sterile 70 % ethanol (1 ml) and subsequently passed over a sterile 0.22 \(\mu\)m filter into a sterile vial. The activity of the final product was measured in a dose calibrator and ca. 0.5 ml of the formulation was withdrawn for QC using sterile needle and syringe. The vial containing the [O-methyl-\(^{11}\text{C}\)] erlotinib remained in quarantine until the product had satisfied all quality control tests and was subsequently released to the PET scanning suite. See also Example 2.

EXAMPLE 4

Lung Cancer Xenografts and in vivo Biodistribution Studies Using 6-[\(^{11}\text{C}\)] Erlotinib

The animal experiments were approved by the Danish Animal Experiments Inspectorate. Female BALB/cA-nude mice (C.Cg/AnBoniTac-Foxnu, Taconic Ltd, Ry, Denmark), approximately 8 weeks old, were used for the study. The mice were housed in plastic cages (Tecniplast, Buguggiate, Italy) under pathogen-free conditions with a 12-hours light/12-hours dark schedule and fed standard chow (Altromin #1324, Lage, Germany) and water ad libitum.

Lung cancer cells were harvested in log phase and were inoculated in the left shoulder subcutaneously. 20 million cells from each cell line in 200 \(\mu\)l ice cold PBS
were injected/site/animal using a 27 gauge 1/2 cc syringe. By 4-5 weeks tumors were growing exponentially and were approximately 1 cm in diameter. Xenografts were randomly selected for biodistribution and 15-20 MBq of [11C] erlotinib were injected in each animal model (A549, n=3, NCI 358, n=3 and HCC 827, n=2) via the lateral tail vein. One hour after injection, animals were sacrificed and the major organs, tumors and blood were weighed and analyzed by a Packard Cobra II γ-counter (Canberra) and the percentage of injected dose per organ/gram (gm) (% DPG) were calculated from the tissue count.

**EXAMPLE 5**

Micro-PET Imaging with 6-[11C] Erlotinib in Lung Cancer Xenografts

Dynamic micro-PET imaging was performed on lung cancer xenografts for 90 minutes using a Concorde R4 micro-PET scanner (Concorde Microsystems, Knoxville, TN). Mice were anesthetized with isoflurane and placed in a polyvinyl chloride tube cut in half longitudinally. Mouse and holder were then positioned in the cavity of the micro-PET scanner. Mice were injected with 10-15 MBq of [11C] erlotinib via the lateral tail vein. Immediately after injection mice underwent scanning for 90 minutes while kept sedated with 1.5-2% isoflurane. Body temperature was maintained by a feedback-regulated light bulb connected to a rectal thermo-probe. The frame duration was defined as 8x15s, 4x30s, 2x60s, 2x120s, 4x300s and 6x600s frames. Images were reconstructed from raw data by Fourier rebinning and two-dimensional filtered back projection, resulting in 63 transverse and 128 coronal and sagittal sections with a thickness of 1.2 mm, 0.85 mm and 0.85 mm, respectively. The PET images were analyzed with the Acquisition Sinogram and Image Processing (ASIP) software that accompanies the Concorde micro-PET. Regions of interest (ROIs) were manually drawn by creating a volume of interest in the central area of the tumor and in its surrounding tissue. Time activity curve was plotted for tumor and area surrounding the tumor.
EXAMPLE 6

Erlotinib Sensitivity to Lung Cancer Cell Lines Related to the Expression of EGFR

Three lung cancer cell lines were analyzed for their sensitivity to erlotinib. The MTT proliferation assay showed a significant decrease in proliferation of HCC827 cells by erlotinib treatment in a dose dependent manner. In contrast, the A549 and NCI358 cells were resistant to erlotinib treatment as concentrations of erlotinib up to 6 µM did not result in a significant decrease in proliferation (Fig. IA). All cell lines were cultured in replicates of five wells and experiments were repeated at least three times. The data is presented as percentage of control cells (cells treated with vehicle).

Analysis of EGFR expression by western blotting showed a high expression of active (phosphorylated) and total EGFR in the sensitive HCC827 cells as compared to the resistant A549 and NCI358 cells (Fig. IB).

EXAMPLE 7

Micro-PET Imaging with [11C] Erlotinib in Lung Cancer Xenografts

Dynamic micro-PET imaging was performed on each tumor model for 90 minutes after tracer injection (Fig. 2). Tumor is marked by an arrow and a high level of uptake was seen in HCC827 xenografts (Fig. 2C), whereas no significant uptake was observed in the A549 and NCI358 xenografts (Fig 2A and 2B, respectively). Furthermore, time activity curves for tumor and the surrounding tissue showed that only in the HCC827 xenograft was the activity higher in the tumor than in the surrounding tissue throughout the measurement period of 90 minutes (Fig. 2F). The activity was also sustained for a longer time in the HCC827 tumors as compared to the A549 and NCI358 tumors (Figs. 2D and 2E, respectively).

EXAMPLE 8

In Vivo Biodistribution of [11C] Erlotinib in Xenografts
Biodistribution studies were performed one hour after injection in xenografts bearing A549 (n=3), NCI358 (n=3) and HCC827 (n=2) lung cancer cells. In all xenograft models the liver took up the highest amount of the activity. In mice xenografted with HCC827 cells the activity in the tumor was higher than in the other organs measured (apart from the liver), whereas this was not observed when the mice were xenografted with A549 and NCI 358 cells (FIG. 3). Tumor accumulation was calculated as 1.6% (± 0.5%), 0.7% (± 0.1%) and 3.7% (± 0.1%) of the injected dose per gram in A549, NCI358 and HCC827 cells, respectively.

The data presented in this study show for the first time the feasibility of labeling erlotinib and using the [11C] erlotinib as a radiotracer. The results show that [11C] erlotinib can be used to identify erlotinib-sensitive tumors.

Three human lung cancer cell lines expressing low (A549 and NCI358) and high (HCC827) EGFR were selected for this study. A549 and HCC827 have been characterized as resistant and sensitive cell lines, respectively, to treatment with gefitinib, which is another EGFR inhibitor with a similar mechanism of action as erlotinib (Mukohara, T., et al., J. Natl. Cancer Inst., 97: 1185-1194, 2005). For the results obtained herein, a significant decrease in proliferation of HCC827 cells was observed in a dose dependent manner, whereas A549 was resistant to erlotinib treatment and so was the NCI358 cell line.

To evaluate the efficiency of [11C] erlotinib as a radiotracer, micro-PET scanning on xenografts bearing A549, NCI358 and HCC827 lung cancer cells was performed. This demonstrated that xenografts from the erlotinib-sensitive HCC827 cells could be visualized by micro-PET scanning, whereas xenografts from the erlotinib-resistant A549 and NCI358 cells could not. This result was confirmed by biodistribution analysis that demonstrated a higher uptake of [11C] erlotinib in HCC827 cells as compared to the A549 and NCI358 cells. It is important to note that HCC827 cells both have a high expression of EGFR and also harbor an in frame deletion mutation (delE746-A750) in exon 19. The presence of this mutation is believed to further increase the sensitivity of the HCC827 cells to erlotinib treatment (Chang, J. W., et al, Ibid.), although the exact mechanism of this increased sensitivity is not fully known. Interestingly, the results obtained from the time
activity curves showed a sustained activity in HCC827 tumors compared to surrounding tissue. It is therefore possible that the presence of this sensitizing mutation might increase the binding of the drug with its target and thereby more efficiently inhibits signaling through EGFR. However, it is not known whether it is the elevated expression or the mutation that causes the accumulation of erlotinib in the tumor. Nevertheless, the data show that $[^{11}C]$ erlotinib accumulates in the tumor that responds to erlotinib treatment.

For biodistribution studies, activity was analyzed in the major organs one hour after $[^{11}C]$ erlotinib injection. As expected, the liver showed the highest uptake of the drug as it is the major organ of erlotinib metabolism (Ling, J., et al., Drug. Metab. Dispos., 34: 420-426, 2006). Interestingly, very little or no activity was measured in the brain, bone and muscle. It has been reported that erlotinib is effective in brain tumors (Gounant, V., et al., Lung Cancer, 58: 425-428, 2007; and Sarkaria, J. N., et al., Mol. Cancer Ther., 6: 1167-1174, 2007). Low uptake of $[^{11}C]$ erlotinib in the brain and other organs shows utility for this radiotracer in identifying primary or metastatic tumors expressing the EGFR in organs with minimal EGFR background in the normal tissue.

In conclusion, there has been shown herein a method for radiosynthesis of $[^{11}C]$ erlotinib and its utility as a new radiotracer for identification of tumors sensitive to erlotinib treatment. The results above demonstrate that $[^{11}C]$ erlotinib can be used for the non-invasive and rapid identification of erlotinib-responding tumors.

While there have been shown and described what are presently believed to be the preferred embodiments of the present invention, those skilled in the art will realize that other and further embodiments can be made without departing from the spirit and scope of the invention described in this application, and this application includes all such modifications that are within the intended scope of the claims set forth herein.
WHAT IS CLAIMED IS:

1. A radionuclide-labeled form of erlotinib comprising the chemical formula:

\[
\begin{align*}
R^1 & \quad O \quad O \\
R^2 & \quad O \quad O \\
\end{align*}
\]

and its neutral, acid salt, hydrous, and anhydrous forms, wherein \( R^1 \) and \( R^2 \) are independently either \(-^{11}\text{CH}_3\) or \(-\text{CH}_3\), provided that at least one of \( R^1 \) and \( R^2 \) is \(-^{11}\text{CH}_3\).

2. A compound according to claim 1 comprising the chemical formula:

\[
\begin{align*}
\text{H}_3^{11}\text{C} & \quad O \quad O \\
\end{align*}
\]

and its neutral, acid salt, hydrous, and anhydrous forms.

3. A pharmaceutical composition comprising a compound according to claim 1 and a pharmaceutically-acceptable carrier.

4. A method for in vivo imaging of cells to detect the overexpression of epidermal growth factor receptor tyrosine kinase in a subject suspected of having a cancerous condition, the method comprising:

   (i) administering to the subject a diagnostic-effective amount of a composition comprising a radionuclide-labeled form of erlotinib comprising the chemical formula:
and its neutral, acid salt, hydrous, and anhydrous forms, wherein R\textsuperscript{1} and R\textsuperscript{2} are independently either -CH\textsubscript{3} or -CH\textsubscript{3}, provided that at least one of R\textsuperscript{1} and R\textsuperscript{2} is -\textsuperscript{11}CH\textsubscript{3}; and

(ii) imaging the subject using positron emission tomography after sufficient time has been provided for the radionuclide-labeled form of erlotinib to bind to EGFR-TK sites in the body.

5. The method according to claim 4, wherein the radionuclide-labeled form of erlotinib comprises the chemical formula:

and its neutral, acid salt, hydrous, and anhydrous forms.

6. The method according to claim 4, wherein the subject is suspected or known to suffer from lung or pancreatic cancer.
7. The method according to claim 4, wherein the subject is suspected or known to suffer from non-small cell lung cancer.

8. The method according to claim 4, further comprising administering the radionuclide-labeled erlotinib composition to subjects not suffering from cancer in order to establish a control indicative of a normal level of expression of EGFR-TK by which a level of expression of EGFR-TK in subjects suspected of having cancer can be compared against in order to determine if the level of EGFR-TK is overexpressed and indicative of a cancerous condition.

9. The method according to claim 4, wherein the erlotinib composition is administered intravenously.

10. The method according to claim 4, wherein the diagnostic-effective amount of the radiolabeled erlotinib is an amount that corresponds to a non-therapeutically effective dose of non-radiolabeled erlotinib.

11. The method according to claim 4, wherein the diagnostic-effective amount of radiolabeled erlotinib corresponds to a radioactive dosage in the range of about 50 to about 500 MBq depending on body weight of the subject.
FIG. 1