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Teaser Radioactivity-based techniques strongly facilitate the drug development process; the application of radiotracers in the development of new drugs and molecular imaging are described.

Radionuclides in drug development

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Recently, molecular imaging has gained broad interest for the stratification procedures in personalized therapies. In this regard, the importance of radionuclides for the drug development process shall not be underestimated. The methods range from target identification, pharmacokinetics studies, Phase 0 microdosing studies, endoradiotherapy with low molecular weight drugs to radioimmunotherapeutics such as Zevalin®. This review provides a comprehensive overview of the use of radionuclides in medical sciences from autoradiography over radioimmunoassay, post-labeling and target identification to the determination of the pharmacokinetics and metabolization and molecular imaging techniques. We demonstrate the enormous potential of different radionuclides with respect to specific classes of drugs, the radiolabeling procedures and their limitations, the instrumentation technologies and their implementation in the drug development process.

Introduction

Drug development is a delicate subject. The particular responsibility toward the patients and the commercial potential account for ultimate performance and reliability in the analytical methods applied. Radionuclides are signal sources that can be incorporated into drugs without any change of their characteristics. They emit an exclusive, intense signal – an ideal signal source for the methods applied in the development of drugs. The main benefit of radionuclide-labeled compounds arises from the extremely high sensitivity that can be achieved when detecting and quantifying minimal amounts of radioactive elements. The resulting ability to study molecules and processes *in vivo* or *in vitro* is a valuable tool for a wide range of applications in the field of life sciences, such as drug development and molecular imaging. The distribution of radioactive isotopes can be detected by the radiation resulting from the decay of their nuclei. The most prominent examples are ^{14}C-labeled compounds used to study the pharmacodynamic and

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Walter Mier has studied chemistry at the University of Heidelberg and performed his Ph.D. at the German Cancer Research Center. He holds a professorship at the Institute of Pharmacy and Molecular Biotechnology of the University of Heidelberg, Germany. His main interests are peptide pharmaceuticals and drug targeting in the field of oncology using radiotracer techniques for the preclinical development of peptidic and proteinaceous drugs.

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pharmacokinetic profile of drug candidates [1]. A determining factor of the extent a nuclide might be used for a particular application is the type of radiation emitted. To be useful in imaging techniques, the radionuclides have to emit either gamma or positron radiation. A high specific activity (the activity per mass or molar quantity) is of importance for applications in which the amount of the applied agent can interfere with a binding process studied. A typical example is the analysis of targets expressed at low density such as receptors. If the target site can be saturated by the mass of the tracer, the amount of the radioactive agent has to be kept as low as possible and also a high specific activity is required. ^{125}I is the isotope of choice for laboratory studies but also used for the synthesis of tracers for receptor-binding studies (e.g. neurological studies). Jones *et al.* studied the iodinated α1-adrenergic antagonist HEAT {([2-β(4-hydroxyphenyl)-ethylaminomethyl)-tetralone]; BE 2254}, a high affinity radioligand with specificity for imaging the localization of α1-adrenergic receptors in the brain. They showed that the autoradiographs obtained with HEAT provide a higher signal-to-noise ratio when compared with tritiated ligands [2]. This review gives an overview of the different possibilities involving radiotracers in the development of new drugs as well as in imaging technologies and as the drugs themselves.

Drug development process

Drug development is an increasingly complex and expensive procedure. Only a very small selection of compounds reaches preclinical or clinical studies owing to unfavorable pharmacokinetics and regulatory hurdles. Nowadays only one in ~10 000 compounds accomplish an approval by the FDA [3], which clarifies the immense costs spent for drug development by the pharmaceutical companies. Starting from lead structure screening and synthesis of potential new drug candidates, first screenings are usually arranged by *in vitro* procedures using cell culture assays. Appropriate candidates undergo further screening including studies of stability, biodistribution, toxicity and metabolism. In preclinical trials drug candidates are examined under *in vivo* conditions, predominantly in rodents like mice or rats. Clinical trials include the application of the drug candidate to healthy volunteers as well as to patients. So that the drug development process can be shortened, new methods to accelerate the process generated great interest during past decades. In the 1990s HTS of drug compounds dramatically accelerated the early drug discovery phases by the development of several strategies predominantly based on fluorimetric detection. The methods currently used are capable of 100 000 assays per day, facilitating the extraction of knowledge from millions of data points [4]. Surprisingly, the number of new molecular entities approved by the FDA as yet has not increased (http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugInnovation/ucm381263.htm) and the failure rate of drug candidates associated with inappropriate drug metabolism and pharmacokinetics has also not changed significantly over the past years [5]. One simple explanation for this discrepancy is the fact that neither the increasing of the number of hits nor the selection of candidates with excellent *in vitro* data helps to overcome the major bottleneck of drug ability: pharmacokinetics. Apart from lead structure screening, the monitoring of pharmacokinetics (e.g. acquisition bioavailability, biodistribution data and, of course, the identification of metabolites and their properties) represents a key step in the development of new drugs [6]. Radioactive tracing has been used for decades to analyze dynamic processes in living cells or related systems and, with the development of contemporary imaging methods such as positron emission tomography (PET), single photon emission computed tomography (SPECT) and the microdosing concept to decrease the failure rate of new drug candidates, the use of radiotracers has increased in importance. Fig. 1 schematically represents the individual steps of drug development and the radioactive methods Q2

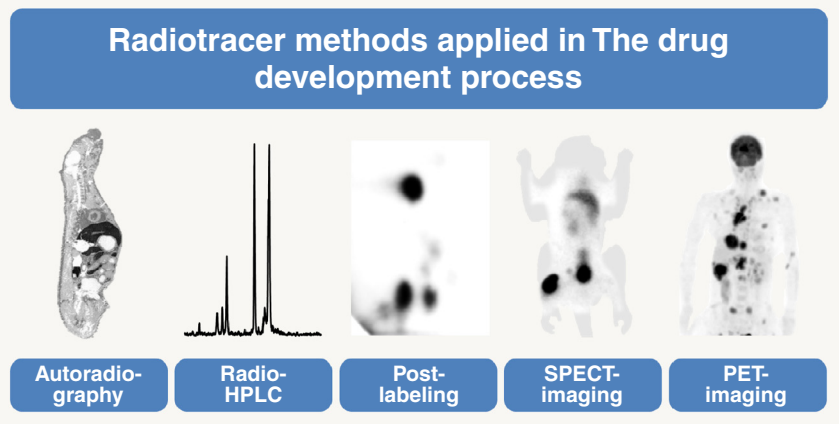

FIGURE 1

Q5 Drug development has become an ever more complex and expensive process. In general, up to 15 years pass before receiving regulatory approval for a new drug because drug development is a multistep process. These methods range from target identification over metabolism, toxicology studies and ADME to clinical trials. Based on the possibility of unambiguously assigning the signals of radiotracers it has become unimaginable to develop drugs without the involvement of radiotracer methods. Many of the pharmacokinetic/pharmacodynamic (PK/PD) techniques applied rely on radiotracer methods as reflected by the radioactive imaging and detecting methods, such as molecular imaging for obtaining data illustrated here. *Abbreviations*: PET, positron emission tomography; SPECT, single photon emission computed tomography.

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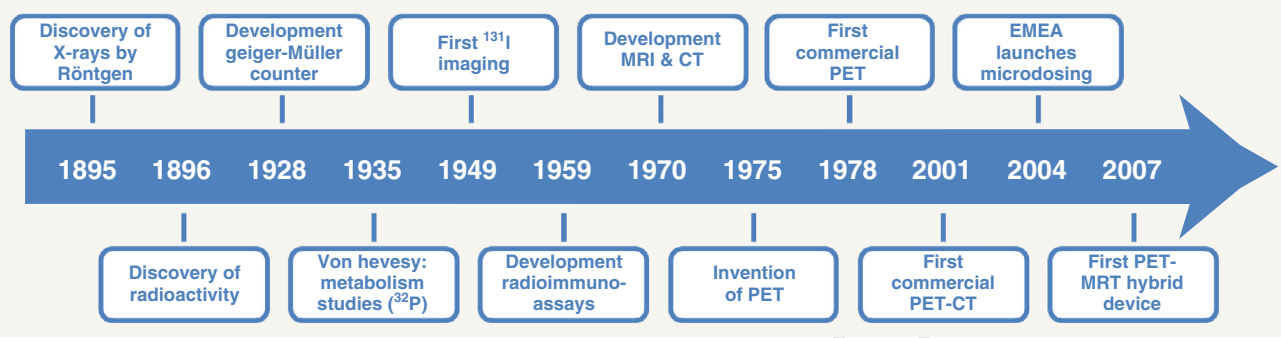

FIGURE 2

This timeline shows the impact of radioactivity on the drug development process since the discovery of X-rays by Röntgen. An important milestone was the development of radioimmunoassays offering a technique to assay minimal amounts of compounds otherwise immiscible or difficult to detect. The development of positron emission tomography (PET) imaging (a 3D, noninvasive imaging method) in the 1970s enabled the visualization of the allocation of radionuclides in living organisms. Offering a high sensitivity with a range of 10^{-11}–10^{-12} mol/l and the independence of the location depth of the reporter probe, PET imaging enables the detection of molecular as well as cellular changes in diseased tissue. Microdosing [launched by the European Medicines Agency (EMEA) in 2004] is a promising method to identify drug candidates with unfavorable pharmacodynamics and pharmacokinetics at the earliest stages possible. *Abbreviations*: CT, computed tomography; MRI, molecular resonance imaging.

used. Personalized medicine promises to enhance the performance of drug therapy significantly. The success of this strategy depends on the availability of accurate diagnostic tests that identify patients who can benefit from targeted therapies [7]. Again, radionuclides play an important part in allowing pretherapy low-dose imaging as well as high-dose therapy in the same patient.

Flashback: the historical development process of radionuclides and radiology

Over the past century, the use of radionuclides has increased in importance owing to their many applications. The most important historical events are pointed out in Fig. 2. The use of radionuclides in the drug development process as well as the contemporary imaging methods computed tomography (CT), magnetic resonance imaging (MRI), PET and ultrasound can be traced back to the German physicist Wilhelm Conrad Röntgen. In 1895 Röntgen recognized that X-rays penetrate solid matter. He further discovered that the attenuation of X-rays depends on characteristics of the penetrated object. Only a few months later, natural radioactivity was discovered by the French physicist Henry Becquerel followed by the discovery of radium as a natural source of γ-rays in 1898 by Marie Curie [8]. Although radium was the only source of γ-rays for the first 20 years, clinical radiotherapy was born and thrived rapidly. The first radiotherapeutical treatment was implemented by Emil Grubbe who treated breast cancer with X-rays in 1896 [9]. The first radiotherapies were applied without protecting healthy tissue. The observation of severe side-effects caused by radiotherapy and the facilitated assessment of radiation after development of the Geiger–Müller counter in 1928 formed the basis for techniques applying a radiation source in or near the tumor. In 1936, Heyman developed the 'packing technique', a method for filling a body cavity with capsules containing radium sources [10]. In the 1950s, new treatment options were accessible through the rise of artificial radionuclides such as ^{60}Co, paving the way for the era of high-energy teletherapy [11]. With the discovery of fractionation of radiation in the 1960s, normal tissue toxicity could be reduced, and the sensitivity for tumor tissue increased at the same time [12]. The introduction of CT in the early 1970s and MRI in the late 1970s enabled the imaging of tumors to determine the size and the location of the tumor [8]. PET provides data about the tumor biology and has consequently taken a key role in clinical research [13] and in the drug development process providing pharmacological data of drug candidates and the possibility of microdosing studies. Cellular radiosensitivity is influenced by intrinsic (e.g. phase of the cell cycle) and extrinsic (e.g. oxygen, nutrients) factors, allowing radiologists nowadays to identify molecular targets that can be manipulated pharmacologically to increase the destruction of tumor cells and to minimize healthy tissue toxicity [8]. This provides a glimpse of hope that personalized medicine can be a powerful tool in the near future.

Radionuclides

The principle of applying radioisotopes for the development of drugs, either measured in samples or detected from the outside of a tissue sample or a patient, relies on the use of adequate radioactive material [14]. The development of powerful radiotracers and radiopharmaceuticals requires careful consideration in the selection of the radionuclide. The decay properties of the attached radionuclide must be balanced with the *in vivo* targeting and clearance properties of the carrier molecule [15]. Table 1 shows the most important radionuclides for the drug development process and molecular imaging alongside their most common fields of application.

The choice of the labeling method also depends on the structure of the molecule of interest. Three different labeling methods are preferred for radionuclide applications in living organisms:

- The radioisotope can be directly used as a substitute for a stable atomic element or a functional group [15] (the most common being the radiohalogens ^{123}I, ^{124}I, ^{125}I and ^{131}I). The isotopes of iodine are highly versatile owing to their different characteristics in combination with their ready labeling procedure of phenolic compounds. They provide properties that are adequate for a number of different applications. In addition, these

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TABLE 1

Most common radionuclides and their applications in drug development and molecular imaging

Radionuclide	Half-life	Application
^{14}C	5730 years	Autoradiography; later drug development phase; ADME
^{18}F	110 min	PET (molecular imaging)
^{68}Ga	68 min	PET (molecular imaging)
^{3}H	12.4 years	Autoradiography; early drug development phase; ADME
^{123}I	13.2 h	Protein labeling; SPECT
^{124}I	4.2 days	Protein labeling; PET
^{125}I	59.4 days	Protein labeling; *in vitro*
^{131}I	8.0 days	Biodistribution studies
^{111}In	67 h	Chelator-modified proteins
^{32}P	14.3 days	Postlabeling
^{99m}Tc	360 min	SPECT

Abbreviations: PET, positron emission tomography; SPECT, single photon emission computed tomography.

radionuclides are easy to handle and readily available from commercial sources [16]. Nucleophilic substitution is a further common type of labeling reaction. It is used for the production of radioligands such as [^{18}F]-FDG (2-deoxy-2-[^{18}F]fluoro-D-glucose), currently the most important radioligand for PET imaging [17].

- The label can also be inserted into the appropriate molecule in a direct isotopic switch as in the use of ^{14}C replacing ^{12}C (also ^{3}H at the place of ^{1}H) in the molecule of interest (e.g. for determining the absolute oral bioavailability of molecules in early drug development by simultaneous oral therapeutic and intravenous ^{14}C microdoses) [18]. A further variation presents the use of a radioactive element from the same group of the periodic table [15].
- The third possibility is the use of molecules of high molar masses which are not significantly influenced by insertion of artificial moieties. In these cases a chelator such as DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) can be attached. After purification, characterization and ensuring of the biological function of this conjugate, a metallic radioisotope such as ^{111}In or ^{177}Lu is bound by complex formation. Besides iodination, complex formation is the most common method for the labeling of peptides and proteins [14]. A prominent example is DOTATOC [(DOTA^0, Tyr^3)octreotide] used for peptide receptor radionuclide targeting [19].

Yet, radiolabeling has two major limitations: on the one hand it must be possible to attach or substitute the emitter into the molecule of interest using one of the three methods mentioned above and, on the other hand, the physiological kinetics must match to the physical half-life of the emitter chosen [14].

Tritium and carbon-14 radiolabeling in the drug development process

As already mentioned, using radionuclides enables one to follow the fate of a molecule and its metabolites. Hydrogen and carbon are the main constituents of organic compounds, their long-lived isotopes ^{3}H ($t_{1/2}$ = 12.4 y) [20] and ^{14}C ($t_{1/2}$ = 5730 y) [21] are widely used throughout the drug development process. Both of them are commonly used in binding studies [22] or in biotransformation studies [23]. Nevertheless, owing to their different characteristics, both radionuclides also show limitations that determine their use in the drug development process. The most important limiting factors are chemical instabilities of the labeled compound, costs and also biological instability of the label. In contrast to ^{14}C, ^{3}H has a higher specific activity and also lower material costs. It is often suitable for more-direct synthetic pathways. However, its main drawback is the biological instability of the carbon–hydrogen bond in many molecules, which is associated with the loss of the ^{3}H label and following formation of tritiated water [1]. The poor labeling-positional selectivity and thus intramolecular nonuniformity of the tritium distribution is another limiting factor. In contrast to ^{3}H, ^{14}C offers more stable labeling modalities because of its integration into the molecular framework or skeleton of the molecule. In general, the drug development process can be subdivided into the discovery and the development phase [1]. The discovery phase includes target selection, lead generation and candidate selection. In this case, the ^{3}H label seems to be more attractive as a result of its rapid and often cheaper synthesis as compared with ^{14}C. There are two main routes to incorporate ^{3}H into the drug candidate. On the one hand, the final compound is tritiated directly and, on the other hand, ^{3}H is incorporated upon the synthesis of a precursor [24]. In such a case, it is necessary to synthesize an appropriate precursor to be reacted with $^{3}H_2$ gas or another source of ^{3}H. The most common route is an aromatic halogenation of a precursor molecule (typically using N-iodosuccinimide and trifluoroacetic acid) followed by reduction with Pd/C and $^{3}H_2$ gas to yield the final ^{3}H-containing compound [25]. The method allowing properly functionalized molecules to incorporate $^{3}H_2$ gas directly into the molecule without any modification is called the 'Ir-catalyzed hydrogen isotope exchange' (HIE) developed by Heys [26] and Hesk *et al.* [27]. The two possibilities of ^{3}H labeling are compared in Fig. 3.

In contrast to the discovery phase, in the development phase, which includes toxicity/animal studies and clinical development, the ^{14}C label is beneficial owing to its high biological labeling stability. In contrast to ^{3}H, the labeling of ^{14}C has to be introduced early in the synthetic route as part of a basic building block and, hence, requires the total synthesis of the drug candidate. Owing to the fact that the formation of C–C bonds is difficult and the required reagents are more expensive than for the ^{3}H synthesis, the synthesis of ^{14}C needs a larger resource commitment. Only a few reagents for ^{14}C synthesis are commercially available. Typical ^{14}C starting materials are carbon dioxide, metal cyanides, malonates, acetic acid and urea [24].

Radioactive detection and imaging methods used during the drug development process

Fig. 4 contains a selection of the main applications of radionuclides in molecular imaging and drug development as exemplified for some prototypical tracers. The different radioactive detecting and imaging methods used during the drug development process are described in detail.

Radioimmunoassay

The radioimmunoassay (RIA), developed by Berson and Yalow in the late 1950s, offers a technique to assay minimal amounts of

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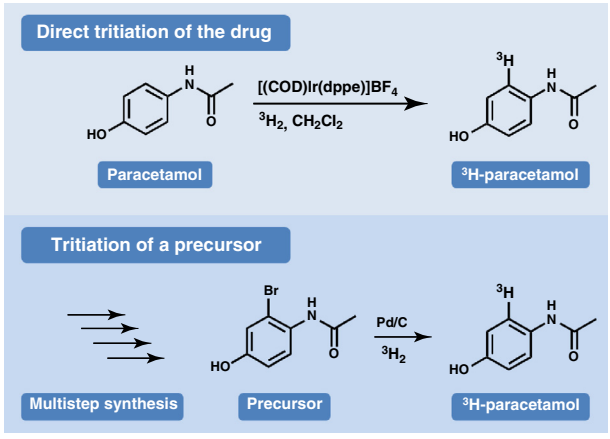

FIGURE 3

Methods for the ^{3}H labeling of drugs enabling the fate of a molecule and its metabolites to be followed. Hydrogen is one of the main constituents of organic compounds, its long-lived isotope ^{3}H ($t_{1/2}$ = 12.4 y) is widely used throughout the drug development process. Two methods of ^{3}H labeling are used nowadays for the early drug development process. Depending on their structure some drugs are amenable to a straightforward labeling procedure. In this procedure the 'Ir-catalyzed hydrogen isotope exchange' (HIE) enables the direct tritiation. Alternatively, tritium halogen exchange can be performed with appropriate precursors obtained by multistep synthesis.

compounds otherwise immiscible or difficult to detect. The competition between radiolabeled and unlabeled antigen for specific antibody sites forming antigen–antibody complexes is the basic principle of this kind of assay [28]. Initially, a defined amount of the radiolabeled antigen and the antibody together with an unknown amount of the unlabeled antigen (sample) with the same binding characteristics are incubated. Afterwards, the antigen–antibody complexes are separated from the free antigens and the radioactivity of either or both phases is measured, determining the ratio of bound:free radiolabeled antigen (B:F ratio). The antigen concentration in unknown samples is determined by comparing the B:F ratio to the B:F ratios obtained by incubating varying amounts of known nonradioactive antigen with the same amount of antibody as in the unknown sample under similar assay conditions [29]. In contrast to other methods, RIA offers various advantages, namely a method to detect peptide hormones and other biologically important substances accompanied by a high sensitivity (10^{-12} mol/l) [28] and the handling is very simple and feasible. The sensitivity of the RIA depends on the production and purification of the radiolabeled antigen and the induction of antibodies with a high specificity and affinity for the antigen [30]. There are also non-immune competitive binding assay systems, which use non-immune binding reagents such as the thyroxine assay. In this case, thyroid-binding globulin (TBG) is used as the binding reagent [31]. However, these assays are only available for selected compounds and lack the versatility of the RIA.

Autoradiography

Autoradiography (ARG) is a high-resolution, quantitative molecular imaging technique that can be used for studying tissue distribution and pharmacokinetics. Autoradiography can be considered as a collection of techniques to localize radioactivity visually by a detector layer such as photographic emulsions and/or films, phosphorimaging plates or direct nuclear imagers or counters. One further option is the use of autoradioluminescence with the aim to obtain a digital image from the scanning of a phosphorimaging plate or a screen that has been exposed to a radioactive source inside or on an object [32]. Receptor microscopic autoradiography, being the latest method of ARG, serves for preserving tissue structure and deposition of noncovalently bound diffusible compounds and to enable microscopic viewing, quantitative analysis and characterization of target sites [33].

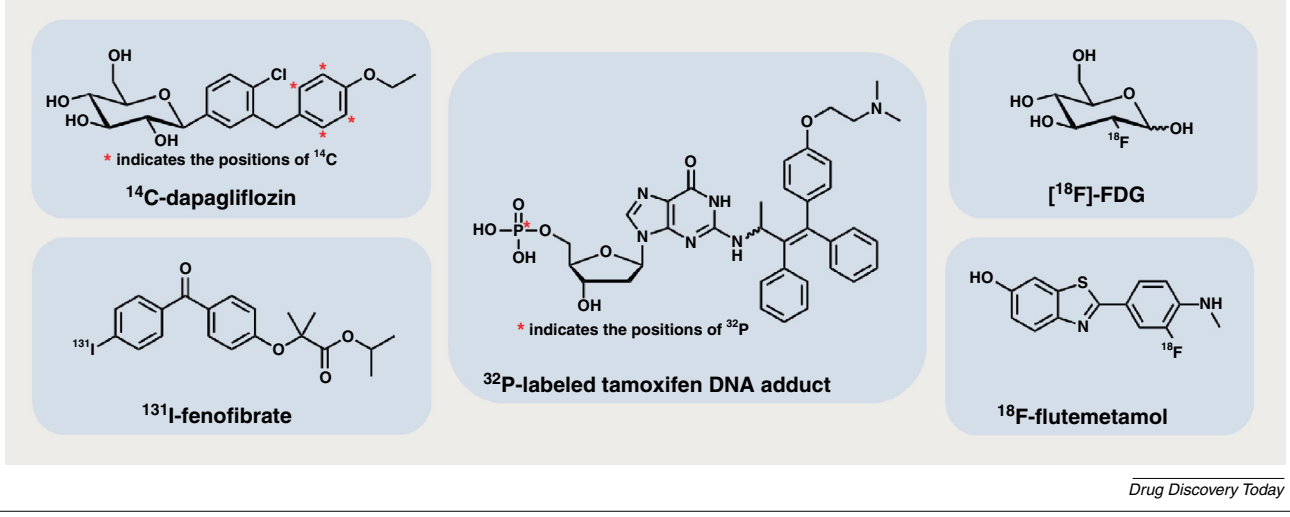

FIGURE 4

A selection of radiolabeled drugs demonstrating the diversity of applications for radionuclides in the drug development process. The incorporation of ^{14}C into the drug is mainly used during the drug development phase to follow the fate of a molecule and its metabolites while [^{18}F]-FDG is used as a radiotracer for positron emission tomography (PET) imaging. ^{131}I, with its relatively long half-life of 8 days, is often used for biodistribution studies and ^{18}F-flutemetamol is one of the latest PET radiotracers used for the detection of beta-amyloid plaques in Alzheimer's disease. ^{32}P postlabeling, with its extremely high sensitivity, can be used to detect covalent DNA adducts, that eventually predict carcinogenic potential of developmental drugs.

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Two variants of ARG can be distinguished: quantitative whole-body autoradiography (QWBA: imaging of organs, organ systems or whole-body sections) and microautoradiography (MARG: imaging at the cellular level in a histological preparation).

The first step using the QWBA method is the administration of a radiolabeled compound (^{14}C, ^{3}H, ^{35}S or ^{125}I being the isotopes of choice) to a laboratory animal followed by euthanasia and quick freeze-drying (e.g. in a hexane–dry-ice bath). Afterwards, the frozen carcasses are embedded in carboxymethylcellulose and cryosectioned at a thickness of 20–50 μm. After dehydrating the whole-body sections, they are generally exposed to phosphorimaging plates along with radioactive calibration standards. Depending on the isotope, the exposure time lasts up to 4–7 days. Finally, the phosphorimaging plates are scanned in a phosphorimaging scanner and after obtaining the digital images the tissue concentrations of radioactivity can be determined by image analysis [32]. In contrast to QWBA, using the MARG method the application of the radiolabeled drug is followed by the exsanguination of the animal. Subsequently, the tissues are dissected and snap-frozen in, for example, liquid nitrogen followed by cryosection of the tissue at −20 °C to obtain sections 5–10 μm thick. These sections are then thaw-mounted under darkroom conditions onto dry glass microscope slides that have been precoated with photographic emulsion. The slides are placed in light-tight boxes to be exposed for a definite amount of time. Finally, the slides are developed and stained using conventional histologic staining methods. Autoradiography can be used for studying the tissue distribution of radiolabeled compounds in biological models, in general in addition to organ homogenates and liquid scintillation counting techniques [32]. To provide qualitative and quantitative tissue concentration data, most QWBA studies are realized using phosphorimagers or by the autoradioluminography technology. But it is important to realize that both methods can only visualize the total amount of radioactivity which can include the parent molecule and its metabolites [34]. Owing to the necessity for *in vivo* localization of drugs in tissues and cells and for preserving tissue structure and deposition of noncovalently bound diffusible compounds, receptor microautoradiography should be used [33].

^{32}P postlabeling

Beyond the methods to follow the pharmacodynamics and pharmacokinetics of drugs, the extremely high sensitivity of radioactive labeling can be used to detect covalent DNA adducts of carcinogenic compounds. Owing to its high sensitivity (a detection limit of one adduct per up to 10^{10} nucleotides can be reached) [35] and its applicability to structurally diverse classes of chemicals, the ^{32}P-postlabeling assay can be used for human biomonitoring. DNA adducts have already been detected in a large variety of biological systems, starting from cell cultures, animals and, finally, humans. These adducts can be detected and quantified even in samples containing only a few micrograms of DNA and without radiolabeling of DNA-reactive chemicals using the ^{32}P-postlabeling procedure. Offering the highest sensitivity for detection of DNA interactions, ^{32}P-postlabeling has a wide range of applications in humans, animals and *in vitro*. The procedure consists of four principal steps: (i) enzymatic digestion of the DNA to nucleoside 3?-monophosphates; (ii) enrichment of the adduct fraction of the DNA digest; (iii) 5?-labeling of the adducts by transfer of ^{32}P-orthophosphate from [γ-^{32}P]ATP mediated by polynucleotide kinase; (iv) chromatographic or electrophoretic separation of the labeled adducts or modified nucleotides and quantitation by measurement of their radioactive decay [36,37]. Separation is performed by multidimensional thin layer chromatography (TLC) or alternatively by high-performance liquid chromatography (HPLC) or mass spectrometry (MS) [38]. Owing to the circumstance that some adducts show only low labeling efficiencies, the performance of the ^{32}P-postlabeling analysis for a study needs to be evaluated on a case-by-case basis, preferentially with synthetic adduct standards [36]. Alternatively to ^{32}P, ^{33}P can be used with advantages and disadvantages. It offers a prolonged half-life (25.3 days versus 14.3 days) and the emission of β-particles of lower energy (0.249 versus 1.710 MeV). This enables a safer use and a higher theoretical limit of detection with the disadvantage that it is considerably more expensive.

Storage phosphorimaging

Storage phosphorimaging (also called radioluminography) is an imaging technique developed in the 1980s that is mainly used in X-ray diagnostics but also applied for research purposes such as pharmacological studies (e.g. for the quantitative analysis of radioligand binding to human brain tissue) [39] or the analysis of electrophoresis gels and chromatography [40]. The use of phosphorimaging provides detailed quantitative data and high-resolution image information. In general, the resolution ranges from 40 to 100 μm [41]. Imaging plates provide a more sensitive method than the use of X-ray films. In addition, they enable minimizing of the exposure times [42], which offers a wider range of applicable radionuclides including those with short half-lives such as ^{123}I, ^{99m}Tc or ^{18}F [43]. Additional benefits are the possibility to reanalyze imaging data analyses and 3D reconstruction, as well as the fact that the plates can be erased and reused [44]. In general, phosphorimaging is based on the absorption and storage of radioactive energy by phosphorescent crystals, which are coated on a plastic plate. The crystals release the stored energy by luminescence light only upon excitation with a laser beam. The emitted light is detected by a photomultiplier tube and the amplified signal is transferred to a computer for the analysis with appropriate software [44]. Being a good alternative for recording and quantifying autoradiographic images, storage phosphorimaging is often used in QWBA to determine the tissue distribution of drug-related radioactivity. In this respect, storage phosphorimaging can be seen as an attractive alternative for biodistribution studies of radiotracers with a short half-life in small animals.

Positron emission tomography

PET is a 3D, noninvasive imaging method used to visualize the allocation of radionuclides in living organisms. Offering a high sensitivity with a range of 10^{-11} to 10^{-12} mol/l and an independence of the location depth of the reporter probe[45], PET imaging enables the detection of molecular as well as cellular changes in diseased or injured tissue. The radioisotopes used in PET are positron emitters, the most typical being ^{18}F ($t_{1/2}$ = 110 min), ^{68}Ga ($t_{1/2}$ = 68 min), ^{11}C ($t_{1/2}$ = 20 min), ^{13}N ($t_{1/2}$ = 10 min) and ^{15}O ($t_{1/2}$ = 2 min) [5]. Owing to their short half-lives, the radiolabeled drug must be manufactured in close proximity to the camera. ^{18}F is the most common nuclide for application owing

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to its relatively long half-life compared with the other radioisotopes. The principle of PET imaging is very simple. First, the collision of a positron with an electron results in their mutual annihilation and the emission of two 511 keV γ-photons at 180° to each other [46]. When two counterpart scintillation detectors are both stimulated they transmit a coincident signal. The photon energy is absorbed by the detectors and causes the emission of visual light which can be detected by photomultiplier tubes. After converting the light signal to an electric current, which is proportional to the incident photon energy, a 3D image of the tissue can be calculated by computer [47]. Monitoring the binding affinity of a drug to a specific receptor is an established application of PET. For this purpose a tracer and an unlabeled drug are applied and then compared to an initial PET scan carried out prior to drug application. The competition between tracer and drug for binding to the receptor leads to different intensities of both scans. Owing to the difference in intensity, the binding affinity of the drug can be estimated. This is particularly useful for drugs designed to treat central nervous system disorders such as binding to dopamine D2 receptors [48]. PET can also be used to obtain pharmacodynamics data in early drug development (see microdosing) [3]. As mentioned above, the radioligand [^{18}F]-FDG is also used as a radiotracer for PET imaging in many fields ranging from oncology, neurology to cardiology [49]. As a glucose derivative, [^{18}F]-FDG is absorbed and then transported like conventional glucose. Subsequent to the entry into the cell, [^{18}F]-FDG is phosphorylated to [^{18}F]-FDG-6-phosphate which cannot be further metabolized in the glycolytic pathway. Because the cell membrane is not permeable to [^{18}F]-FDG-6-phosphate, it is enriched inside the cells (this phenomenon is also called metabolic trapping). As the uptake of glucose is enhanced in tumor cells, [^{18}F]-FDG accumulates in tumors which can then be detected by PET [50]. The sensitivity of PET is significantly reduced in areas of the body with high glucose uptake. This means that, if the tumor is near (or inside) the tissues of which glucose uptake is physiologically high, the signal-to-noise ratio will decrease in [^{18}F]-FDG-PET. Additionally, monitoring response is less likely because many of these tumors have lower metabolic rates and are located close to inflammatory scar tissue [51]. Currently, there is a large range of other tracers in preclinical and clinical trials. Initial clinical studies of RDG tracers (peptides consisting of arginine-glycine-aspartic-acid), for example, show promising tumor-to-background activity, rapid renal clearance and dosing that correspond closely with that of [^{18}F]-FDG [52]. One further tracer, used for apoptosis imaging, is ^{18}F-labeled annexin V [53]. Annexin V, a human protein consisting of 319 amino acids with a molecular weight of 36 kDa, belongs to the family of membrane-binding proteins. The extremely high affinity to bind phosphatidylserine (PS), a phospholipid that is translocated from the inner leaflet of the plasma membrane to the outer leaflet of apoptotic cells, allows the detection of apoptosis by radiolabeled annexin V [54].

Single photon emission computed tomography

The principle of SPECT is similar to PET. Because the photons emitted by γ-decay cannot be localized by coincidence measurement, SPECT requires lead collimators to reach a defined image compared with electronic collimation in the case of PET. The use of collimators always leads to compromises between sensitivity and resolution that can be varied by the size and length of the septa. Therefore PET is at least one log order more sensitive than SPECT [55]. A particularly higher spatial resolution can be obtained by using pinhole-SPECT, a combination of micro-pinhole apertures with ^{125}I [55]. Because the range of α- or β-emitters is too short to permeate the tissue, typically γ-emitters like ^{99m}Tc, ^{123}I, ^{111}In and ^{201}Tl are used. Because typical parallel-hold collimators for SPECT scanners only have sensitivities of approximately 0.02%, merely about 1/100 000th of the photons emitted by the source are detected which limits the resolution [49]. Compared to PET, the advantages of SPECT are the lower costs and a more feasible application as a result of the use of the radionuclides that typically possess longer half-lives. Additionally, by the use of SPECT, it is possible to detect and to distinguish more than one isotope at the same time and therefore multiple molecular events with differing energy γ-rays can be monitored [45]. There are SPECT tracers for virtually every diagnostic imaging application. Typical examples are ^{99m}Tc-mercaptoacetyltriglycine (MAG3) used for the imaging of renal function [56], ^{123}I-alpha-methyl-L-tyrosine (IMT), a tracer for the evaluation of brain tumors [57] and ^{123}I-2-β-carbomethoxy-3-β-(4-iodophenyl)tropane (β-CIT), a tracer for imaging dopamine transporter expression [58]. However, as already mentioned in the context of autoradiography, PET and SPECT only visualize the localization of the radionuclide – disregarding a potential metabolism of the parent molecule.

As described above, a multitude of imaging methods is used during the drug development process. Apart from the crucial radioactive methods, nonradioactive methods also play an important part in the drug development process. Table 2 compares the

TABLE 2

Fields of application for radioactive compared with nonradioactive methods in the drug development process.

Radioactive Autoradiography	Tissue localization of radioactive drugs	Nonradioactive MALDI	Spatial distribution of drugs and their metabolites in tissue
^{32}P postlabeling	Human biomonitoring; toxicology	HPLC-MS	Detection and identification of drugs of particular masses
PET SPECT	Molecular imaging; microdosing Pharmacodynamics	MRI Fluorescence imaging	Diagnosis; therapy response *In vivo* diagnostics
Radio-HPLC/MS	Detection of radiolabeled drugs and metabolites	HPLC-MS	Detection and identification of drugs of particular masses
Radioimmunoassay	Quantification of drugs	ELISA	Colorimetric determination of serum drug concentrations
Scintillation counting	Biodistribution studies; ADME	Accelerator-MS ICP-MS	Microdosing Toxicology studies

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applications of the radioactive and nonradioactive methods used for drug development.

Microdosing

Owing to the increase of regulatory hurdles, drug development is becoming an ever more complex and expensive process leading to a decrease in the number of drugs receiving regulatory approval. To prevent the waste of resources and to facilitate the development process, several authorities, such as the FDA and the European Medicines Agency (EMA), have launched programs to enable the identification of drug candidates with unfavorable pharmacodynamics and pharmacokinetics at the earliest stages possible. In general, the characteristics of new drug candidates in humans is predicted from *in vitro*, *in silico* and animal data and the candidates for Phase I trials are selected on this basis. However, this selection method is not satisfactory because of an attrition rate of up to 40% (the most common reasons being undesirable metabolism and pharmacokinetics) [5]. This illustrates the need for more-precise drug candidate selection strategies, including earlier investigation of potent drugs in humans. Based on this need, a new promising method, called microdosing, has been developed over the past years. The concept of microdosing is based on the administration of subtherapeutic doses of novel drug candidates in humans to obtain essential pharmacodynamic and pharmacokinetic data prior to Phase I and II trials [5]. Two ultrasensitive techniques are involved in this procedure: on the one hand PET to gain pharmacodynamic data and on the other hand accelerator mass spectrometry (AMS) for pharmacokinetic data. According to the EMEA, a microdose is defined as less than 1/100th of the dose calculated to yield a pharmacological effect of the test substance. This calculation is based on primary pharmacodynamic data obtained by *in vitro* and *in vivo* studies (typically the doses are in, or below, the low microgram range and the maximum dose must be less than 100 μg) (http://www.ema.europa.eu/docs/de_DE/document_library/EPAR_-_Product_Information/human/002653/WC500156172.pdf). Prior to the start of a microdose experiment, animal toxicity studies have to be conducted to prevent risks for the volunteers. Fig. 5 compares the microdosing with the common drug selection approach. As mentioned above, AMS microdosing can be used to analyze drug and metabolite concentrations in body fluids to obtain information of the pharmacokinetics. Determining the isotope ratio of ^{12}C:^{14}C, the drug candidate has to be isotopically enriched with ^{14}C before its administration [59]. At defined time points after administration, blood, urine and feces samples are collected. To separate the metabolites from the drug candidate, blood and feces can be analyzed and fractionated by HPLC. Comparative pharmacokinetics between a microdose and therapeutic dose for clarithromycin,

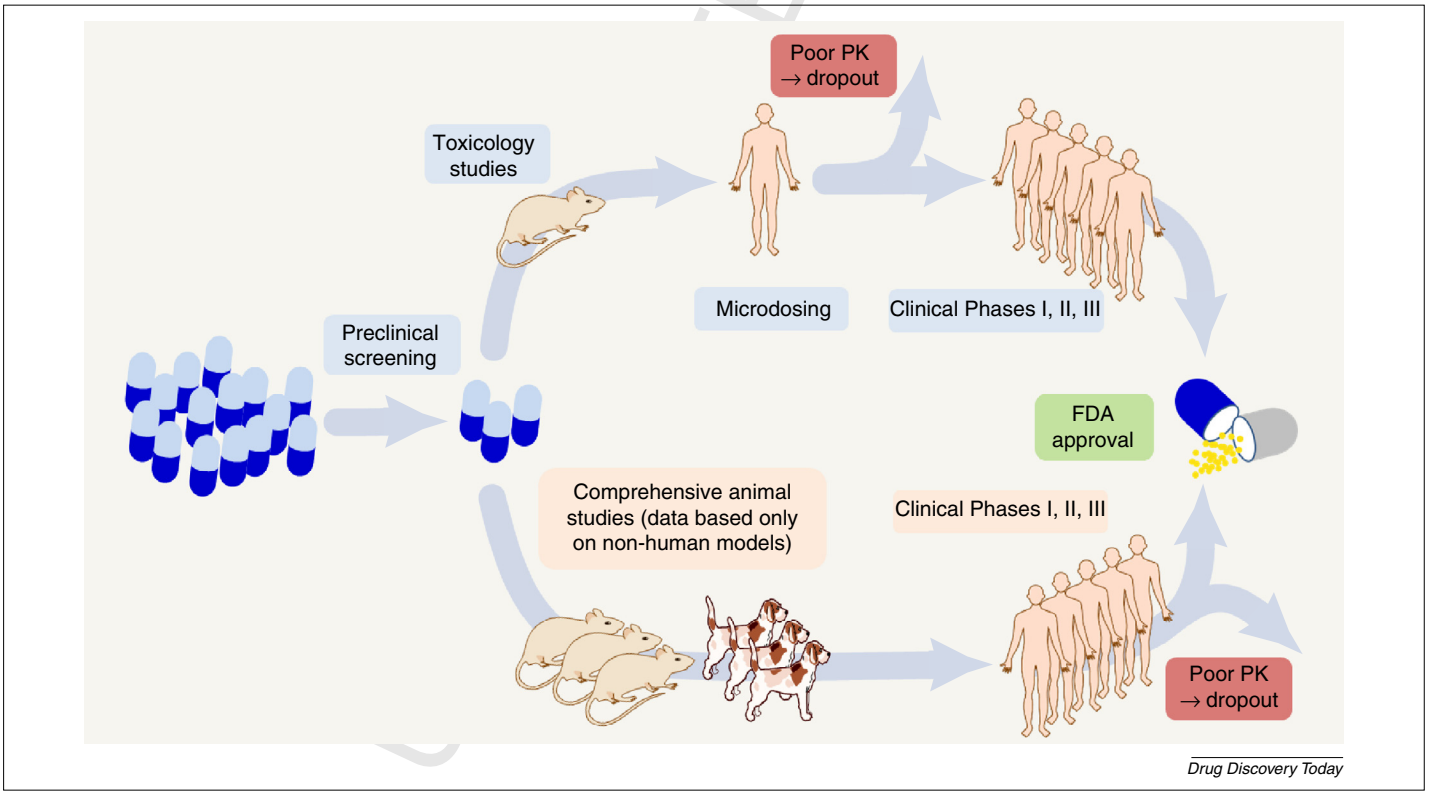

FIGURE 5

Comparison of the conventional and the microdose approach to drug selection. To prevent the waste of resources and to facilitate the drug development process, both the FDA and the European Medicines Agency (EMEA) have launched programs to enable the identification of drug candidates with unfavorable pharmacodynamics and pharmacokinetics (PK) at the earliest stages possible. In the early stages of drug development a multitude of drug candidates exist. After preclinical screening, the promising candidates are commonly introduced into animal studies and the candidates for clinical trials are selected on this basis (human PK data not yet available) leading to an attrition rate of 40%. By contrast, by the use of microdosing, the behavior of the drug candidate is predicted from the microdose study (human PK data already available), only limited animal studies are performed to ensure the safety of the volunteers. Therefore, the attrition rate can be reduced because candidates with poor PK data can be identified earlier.

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sumatriptan, propafenone, paracetamol and phenobarbital have been shown by Lappin *et al.* [60]. By contrast, pharmacodynamics can be received by PET or SPECT by two different ways: first the drug itself can be labeled to study its ability to reach the target tissue; alternatively an isotope-labeled ligand that displaces the drug candidate can be used.

In summary, microdosing speeds up the drug development and also reduces the number of animals required for preclinical toxicology studies. The relatively high costs for the instrumentation (AMS, PET) involved in the microdosing concept are readily amortized, in particular by the gain of time for obtaining the necessary pharmacokinetic/pharmacodynamic data. Radionuclides play an important part in the concept of microdosing owing to their essential use in both analyzing methods (AMS and PET).

There is more to radionuclides than analytical applications: radionuclides as radiopharmaceuticals and in the radioimmunotherapy

Besides the use of radionuclides in the drug development process and for imaging modalities, they can also act as drugs themselves. Two classes of drugs, namely radiopharmaceuticals and radioimmunotherapeutics, can be distinguished. Being β- or α-emitters, the most remarkable characteristics of radiopharmaceuticals is their small emission range protecting surrounding tissue from damage. A straightforward application is the use of radioactive elements that specifically accumulate in diseased tissue, for example ^{131}I can be used for the therapy of differentiated thyroid cancer [61], ^{224}Ra chloride for rheumatic diseases like the Bekhterev syndrome [62] or ^{89}Sr and ^{153}Sm for the pain management of skeletal metastasis, generally caused by carcinomas of the breast, prostate and bronchi [63]. In 2013, the FDA approved Xofigo® (Bayer HealthCare Pharmaceuticals), the latest radiopharmaceutical. Xofigo® contains ^{223}Ra chloride and is used for the treatment of patients with symptomatic late-stage (metastatic) castration-resistant prostate cancer that has spread to bones but not to other organs.

Radioimmunotherapy is a form of targeted radionuclide therapy using a monoclonal antibody labeled with a radionuclide to deliver localized radiation [64]. Although radiopharmaceuticals are well-established therapeutic options for the diseases mentioned above, only a few approved radioimmunotherapeutics are on the market. The most famous representatives are Zevalin® (Spectrum Pharmaceuticals) and Bexxar® (GlaxoSmithKline). Zevalin® (^{90}Y-ibritumomab tiuxetan and unlabeled rituximab) as well as Bexxar® (^{131}I-tositumomab and unlabeled tositumomab) are FDA-approved drugs for the therapy of non-Hodgkin's lymphoma [64]. Unfortunately, Bexxar® was withdrawn from the market in 2014. The application of radioactive drugs is delimited to specialized institutions – this thwarts the outstanding therapeutic potential of radioimmunotherapeutics.

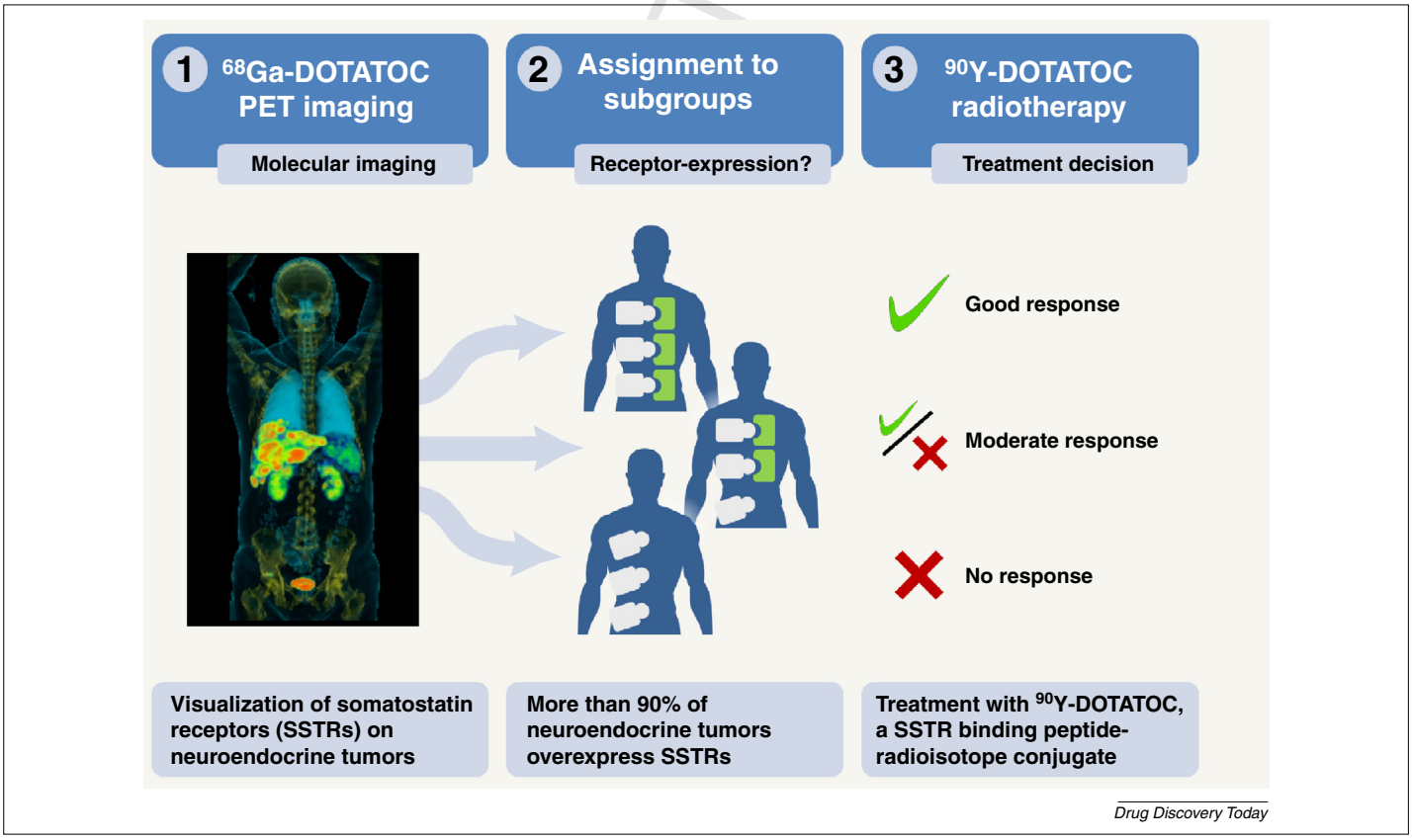

FIGURE 6

Q7 Nowadays the number of drugs designed to target diseased tissue specifically is rapidly increasing. To exploit the potential of drugs specialized for the individual response of single patients, stratification is mandatory. This approach is called personalized medicine. The figure shows an example of personalized medicine: the use of ^{68}Ga-DOTATOC/^{90}Y-DOTATOC in the therapy of neuroendocrine tumors – allowing low-dose imaging as well as high-dose therapy.

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Glance into the future: personalized medicine

Nowadays the amount of macromolecular drugs is rapidly increasing (already four of the top ten drugs by sales in the USA are macromolecules; http://www.fda.gov/Drugs/InformationOnDrugs/default.htm) demonstrating the need of drugs specialized for the individual response of each single patient. This approach, called personalized medicine, is based on three different pillars [65]: specialty drugs such as Kadcycla® (trastuzumab–emtansine; approved in 2013 by the FDA) [66] that targets specific genetic variations but also diagnostic tests (assays) to detect genetic variations and to measure the expression of multiple genes. Molecular imaging methods such as PET are required to stratify the patient on the basis of the pharmacological data of the drugs that are available. For this purpose the use of specific 'theranostic' individual radionuclides or radionuclide pairs showing emissions that allow pretherapy low-dose imaging as well as higher-dose therapy in the same patient are in demand [67]. One example is the use of ^{90}Y-DOTATOC in the therapy of neuroendocrine tumors (Fig. 6) [68]. More than 90% of these tumors overexpress somatostatin receptors that can be detected by ^{68}Ga-DOTATOC-PET imaging. But, ultimately, as a limiting factor, the success of personalized medicine still depends on the availability of accurate diagnostic tests to identify patients that benefit from targeted therapies.

Concluding remarks

The advances in understanding human biology and the development of new imaging technologies enable a deeper understanding of the molecular mechanisms of diseases. This leads to a new era of predictable, targeted research as exemplified by the replacement of natural products, which dominated the available anticancer drugs, with designed drugs. In this respect, reliable and highly sensitive analytical methods are required. Radionuclides ideally support this process, especially owing to their broad use in the latest drug development strategies such as microdosing and personalized medicine. Despite the critical public awareness of radioactivity, radioactive isotopes have always been of invaluable importance for the life sciences and medical progress. Yet, their potential has not been completely exploited.

Conflicts of interest

The authors declare no conflict of interest.

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