Molecular imaging techniques are increasingly being used as valuable tools in the drug development process. Radionuclide-based imaging modalities such as single-photon emission computed tomography (SPECT) and positron emission tomography (PET) have proven to be useful in phases ranging from preclinical development to the initial stages of clinical testing. The high sensitivity of these imaging modalities makes them particularly suited for exploratory investigational new drug (IND) studies as they have the potential to characterize in vivo pharmacokinetics and biodistribution of the compounds using only a fraction of the intended therapeutic dose (microdosing). This information obtained at an early stage of clinical testing results in a better selection among promising drug candidates, thereby increasing the success rate of agents entering clinical trials and the overall efficiency of the process. In this article, we will review the potential applications of SPECT imaging in the drug development process with an emphasis on its applications in exploratory IND studies.

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1. Introduction

Every year thousands of new chemical entities with therapeutic potential enter preclinical research. From these, about 5–10% progress beyond the earliest stage of development and only one in five that enters clinical trials obtains a marketing approval [1]. The length of the whole process, from the discovery of the compound to its
regulatory approval by the US Food and Drug Administration (FDA) takes an average of 12 to 15 years and the total expected costs are estimated at US$802 million [1]. The low predictability of toxicity and efficacy based on traditional preclinical studies are the leading causes of the high failure rate of new drug candidates that occurs during clinical trials and for the prolonged timeline and associated high costs [2,3]. The situation is rather common with the development of the new so-called molecularly targeted agents for cancer treatment, whereas the availability of validated biomarker signals that correlate with clinical endpoints could predict which new drugs will have clinical efficacy before moving to human testing [4]. Therefore, it becomes urgent the adoption of new strategies that are able to identify earlier the promising candidates to move forward clinical trials and to discard the ones that are unlikely to be successful before moving into larger trials. In order to accomplish this goal the FDA introduced in 2006, a guide on exploratory Investigational New Drug (IND) studies to accelerate the development process of new pharmaceutical agents and to increase the success rate of agents entering clinical trials [5]. The exploratory IND studies, also called Phase 0 studies are intended to provide clinical information for a new drug candidate in an earlier phase of drug development. These clinical trials take place early before the typical Phase I trial and involve a limited human exposure to the drug candidate and have no therapeutic intent [6]. Therefore the preclinical pharmacology and toxicology testing required before initiating a Phase 0 trial is less extensive which enables these tests to be initiated sooner compared to a traditional IND [5]. The main endpoints of clinical studies performed under the guidance of an exploratory IND trial might include selection of the lead agent from a group of compounds, drug pharmacokinetics or pharmacodynamic evaluation, drug–target binding affinity, measurement of drug effect and patient selection for subsequent studies [6].

Advances in molecular imaging technology and imaging probes have extended the application of non-invasive imaging approaches into drug discovery and development programs. Radionuclide-based molecular imaging such as positron emission tomography (PET) and single-photon emission computed tomography (SPECT) allow the non-invasive visualization, characterization and quantification of biological processes occurring at cellular and subcellular levels in intact living subjects [7,8]. Such techniques have proven to be useful throughout the drug discovery and initial stages of clinical testing, and are considered as a decision-making tool in the drug development process. In early clinical development, nuclear imaging techniques can be used on validation of potential drug targets, drug pharmacokinetics and biodistribution evaluation and assessment of drug–target interaction [7,8]. Moreover, they can provide insights into the mechanistic aspects linked to the therapeutic intervention (proof-of-principle) and on the proof-of-concept testing through the use of imaging-based biomarkers [9]. The proof-of-concept is demonstrated when the drug-induced biological changes provide a clinical benefit. A considerable number of potential imaging biomarkers labeled with positron and γ-emitting radionuclides have been developed and are currently in preclinical or clinical evaluation [9,10]. Some of them are likely to have a major impact on drug development as they provide relevant readouts of drug–target interaction and of drug efficacy in early clinical studies [11]. This article outlines the potential applications of SPECT imaging in exploratory IND studies and how this approach can facilitate and accelerate new drug approvals.

2. Basic principles of SPECT imaging

Single-photon emission computed tomography (SPECT) is a sensitive nuclear imaging technique that provides a 3D spatial distribution of single-photon emitting radionuclides within the body. The detection of γ-rays is achieved through the use of a gamma-camera, which consists of a scintillation crystal, optically coupled to an array of photomultiplier tubes, which converts the γ-rays into electric signals. Multiple 2D images, also called projections, are acquired from multiple angles around the patient and subsequently reconstructed using reconstruction imaging methods to generate cross-sectional images of the internal distribution of the injected molecules [12,13].

Because of the isotropic emission of γ-rays, a geometric collimation is needed to restrict the travelling direction of the emitted γ-rays from the body, through the use of lead collimators. In clinical systems, collimators typically have many parallel holes producing no magnification. Photons that travel in other directions than those specified by the aperture of collimator are absorbed and do not contribute to the image, which reduces the detection efficiency and sensitivity of SPECT as compared with PET [13]. SPECT detects γ-rays directly at site of tracer accumulation, thereby gaining a theoretical advantage in spatial resolution over PET systems due to the lack of positron range before annihilation, which has been viewed as resolution-limiting. Recently, modern SPECT systems can detect radiotracers within the whole-body, at nano to picomolar levels, conferring the sensitivity required for in vivo tracking of radiolabeled drugs without inducing pharmacological effects or toxicity [12].

SPECT imaging uses γ-ray emitting radionuclides with energies in the range of 30 to 300 keV. Compared to the rapidly decaying positron-emitting isotopes, γ-radionuclides have longer half-lives varying between hours to several days and are commercially available, what makes them relatively cheap and easy to handle (Table 1). The radionuclide technetium-99 m ($^{99m}$Tc) is so far the most commonly used radionuclide in nuclear medicine due to its favorable physical properties ($t_{1/2} = 6$ h, $E_{γ} = 140$ keV) for diagnostic imaging and its widespread availability as a column eluate from commercially $^{99m}$Tc/$^{99}$Mo generators. Apart from $^{99m}$Tc, the other commonly used γ-emitting radionuclides are gallium-67 ($^{67}$Ga), indium-111 ($^{111}$In) and iodine-123 ($^{123}$I). Since γ-radiotracers have their own spectra, SPECT imaging has the unique capability of imaging multiple probes labeled with different isotopes allowing the simultaneous study of multiple cellular or molecular events.

SPECT systems were initially developed for human use, but recently has been scaled down to provide high resolution imaging of small animals being considered a well-established tool for screening of potential drugs in early stages of development. Advances in small-animal SPECT instrumentation have addressed challenges related with camera sensitivity, spatial resolution and image reconstruction and quantification. Nowadays, systems relying on pinhole collimation and using tomographic reconstruction methods offer images of high quality with a millimeter or submillimeter spatial resolution and better quantitative accuracy [14]. Presently, multi-modality imaging combining SPECT with computed tomography (CT) in a dual-modality system (SPECT/CT) allows the simultaneous acquisition of functional and detailed anatomical information providing an accurate localization and quantification of the radiolabeled imaging probe [15]. This is of particular interest when applied in basic medical sciences and preclinical research using small animals. As this technique can be applied in both animal and humans studies it fulfills an important criterion for translational research from the bench to the bedside, which in a perspective of drug development might accelerate

<table>
<thead>
<tr>
<th>Radionuclide</th>
<th>Physical half-life</th>
<th>Energy (keV)</th>
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</thead>
<tbody>
<tr>
<td>$^{99m}$Tc</td>
<td>6.02 h</td>
<td>140</td>
</tr>
<tr>
<td>$^{111}$In</td>
<td>2.8 days</td>
<td>171, 245</td>
</tr>
<tr>
<td>$^{111}$I</td>
<td>8.02 days</td>
<td>364</td>
</tr>
<tr>
<td>$^{123}$I</td>
<td>13.2 h</td>
<td>159</td>
</tr>
<tr>
<td>$^{67}$Ga</td>
<td>78.2 h</td>
<td>93</td>
</tr>
</tbody>
</table>

the process and reduce the costs of bringing new drugs into the market.

3. Applications of SPECT in exploratory IND studies

The exploratory investigational new drug (IND) studies aim to provide crucial information for a new drug candidate at an early stage of clinical drug development. These studies take place early on Phase I prior to the traditional dose escalation, safety and tolerance studies that ordinarily initiate a clinical drug development program [6]. It involves a very limited human exposure, and has no therapeutic or diagnostic intent. An exploratory IND study can provide valuable information of a candidate drug regarding the pharmacokinetic properties and bioavailability, whole-body biodistribution and targeting properties [5,6,16]. This information together with preclinical data enables the selection of the most promising compounds to move forward into the clinical development process.

Nuclear Medicine techniques such as PET and SPECT can be used to image in vivo trace amounts of a candidate drug in a radiolabeled form. Due to the high sensitivity of these techniques, an adequate signal can be obtained even when the radiolabeled drug is administered to subjects in the nano to picomolar range. Lowered at these tiny quantities do not produce any pharmacological effects which reduce the risk of serious adverse effects in human volunteers or patients. Depending on the ligands and radioisotopes used, it is possible to generate pharmacokinetic data and to assess the targeting properties of radiolabeled drug molecules in humans using a microdosing imaging approach. This assay represents one of the most useful tools to rapidly evaluate the benefits of a drug under development leading to a faster approval process. Ideally, the radiolabeled molecule should retain the same physicochemical properties of the parent molecule. This can be achieved via direct radiolabeling by isotope substitution, in which a stable atom of the molecule is replaced by a radioactive isotope, such as $^{13}$C for $^{12}$C for PET ligands. A very few novel small drug molecules contain atoms that have $\gamma$-emitting radioisotopes suited for SPECT imaging applications. In all other cases, a foreign element or a foreign moiety containing the SPECT radionuclide will be attached to the drug molecule. It is important to assure that the attached isotope or the linking group does not influence the biochemical and pharmacological properties of the drug and consequently its biodistribution and pharmacokinetic/dynamic characteristics. The most common $\gamma$-emitting radionuclides available for SPECT imaging studies are the technetium-99m ($^{99m}$Tc), Indium-111 ($^{111}$In) and some iodine radioisotopes ($^{123}$I and $^{131}$I). In general, it is not common to isotopically label a drug with these radionuclides, since new drug molecules having these atoms in their chemical structure are quite few. Instead, they are particularly useful for labeling large biomolecules such as therapeutic antibodies, peptides and proteins, because of the minor influence of the attached radioisotope on the biokinetics of the original molecule considering its larger size. Moreover, as these radionuclides have relatively longer half-lives compared with positron emitters, they are especially suited for radiolabeled peptides and proteins, because of the minor influence on the optimization of engineered antibodies for radioimmunodetection and targeted therapy.

3.1. Imaging drug pharmacokinetics and biodistribution/microdosing

The concept of microdosing was introduced to facilitate the process of gathering human in vivo pharmacological data for candidate new drugs without compromising the safety of volunteers. A microdose study in humans aims to provide valuable data about a candidate compound such as its pharmacokinetic/dynamic properties, biodistribution and targeting properties, before testing the drug’s safety and efficacy in classical clinical trials. Presently, up to 40% of drugs entering clinical trials fail because of inappropriate drug pharmacokinetics and metabolism despite the extensive preclinical studies [17]. A microdosing study employs subpharmacological doses of the test compound which is defined as less than 1/100th of the dose that yields a pharmacological effect established from animal studies, with a maximum a 100 $\mu$g.

Nuclear imaging modalities such as PET and SPECT have an important and evolving role in this aspect. The very low doses of drugs that are recommended for microdosing studies (in the low microgram range) are easily measured in vivo with radionuclide-based imaging modalities. Due to the high sensitivity of such techniques it is possible to predict the behavior of the drugs at pharmacological dose levels through the administration of subpharmacological amounts of the candidate drugs in the radiolabeled form to healthy human volunteers or patients. Microdosing studies can then be used to assess the pharmacokinetic profile of a novel candidate drug using imaging techniques. The investigational new drug must be radiolabeled with a suitable radionuclide and then administered in humans. After administration, real-time disposition data are collected in order to gain essential pharmacokinetic data, such as the rate and extent of drug absorption, the time residence in target and normal organs as well as routes and rates of drug clearance [18]. Based on this information, drugs with an unappropiated pharmacokinetics can be deselected prior to committing large resources to a full-scale phase I study. Although PET imaging has proved to be a useful tool in this context, mainly because of the availability of positron-emitting isotopes of organic elements such as $^{11}$C, $^{13}$N, $^{15}$O and $^{18}$F, their short half-lives make them unsuitable for studies with slow kinetics that require longer follow up times.

In this context, SPECT microdosing imaging is specially suited for pharmacokinetic analysis of antibodies, since the relatively long half-lives of $\gamma$-emitting radionuclides match with their in vivo kinetics. Nevertheless long-lived PET radionuclides such as heavy-metal isotopes $^{64}$Cu ($t_{1/2} = 12.7$ h) or $^{68}$Ga ($t_{1/2} = 11.3$ h) and halogens $^{124}$I ($t_{1/2} = 4.2$ days) and $^{76}$Br ($t_{1/2} = 16$ h) are now becoming available and could also be used to target process with slow kinetics or that require a long follow-up.

Several radiolabeled antibodies have been approved by FDA as suitable for both therapeutic and diagnostic applications. A spectrum of antibodies and derivatives (diabodies and minibodies) are now available and can be labeled with $^{111}$In and iodine radioisotopes ($^{131}$I or $^{123}$I) without inducing significant changes on their biokinetic behaviors [19]. The resulting imaging agents should retain the same affinity and activity as the therapeutic agent. In this field the $^{131}$I-labeled antibodies tositumomab, humanized anti-CEA and anti-tenascin and $^{111}$In-labeled trastuzumab are illustrative examples [20–22]. This approach is currently being applied in preclinical studies on the optimization of engineered antibodies for radioimmunodetection and targeted therapy.

The value of SPECT imaging in the characterization of the immunotargeting properties and pharmacokinetics of an Aminopeptidase P (APP) specific recombinant antibody 833c was demonstrated in a recent study [23]. SPECT/CT images of the $^{125}$I-radiolabeled antibody (125I-833c) in rats were used to demonstrate the temporal and spatial distribution pattern of the antibody. The imaging study revealed the specific targeting of 833c to the thoracic cavity and co-localization with a lung perfusion marker. The pharmacokinetic analysis of biodistribution data confirmed the lung-specific uptake of 833c, which declined by first-order kinetics ($t_{1/2} = 110$ h).

In another study, the targeting properties of 3 recombinant nanobodies with specificity for the colon carcinoma marker carcinoembryonic antigen (CEA) were tested by labeling with $^{99m}$Tc for imaging of xenografted tumors using pinhole SPECT and micro-CT imaging. The results confirmed the specificity of tumor targeting and rapid renal clearance for all nanobodies [24].

A similar approach can be carried out in Phase 0 studies for assessing the pharmacokinetics and targeting properties of antibodies or peptides and to find out about the optimal dosage and dosimetry.
Another application of microdosing imaging studies is to evaluate the brain entry of drug candidates to the central nervous system (CNS) [25]. The inability of drug molecules to cross the BBB is the main reason for the low success rate of CNS drug candidates. It is estimated that 98% of candidate drug molecules that are developed for CNS do not cross the BBB effectively resulting in an inadequate brain exposure for the intended targets [26]. To achieve therapeutic efficacy a candidate CNS has to cross the BBB and accumulate into the brain interstitial fluid at pharmacologically active levels. Phase 0 microdosing studies have been recommended as a safe and efficient approach for assessing whether CNS drug candidates can effectively penetrate the BBB and potentially reach their targets [27]. Two different strategies can be used to assess the biodistribution of candidates CNS drugs. The direct approach in which the candidate drugs are radiolabeled (ideally isotopically to avoid pharmacological changes) provides a general distribution of the compounds in the brain and also some of its washout characteristics. This approach is somewhat limited to PET microdosing studies, because of the radiochemistry involved. The second approach is based on the in vivo measurement of drug receptor occupancy using established receptor specific tracers, in which the candidate drug and the radioligand used to measure occupancy will compete for the available receptors in the brain, thereby proving access of the candidate drug to its specific compartment [28]. Because of the availability of SPECT ligands for neuroreceptor systems, in particular dopaminergic and serotonergic ligands, SPECT imaging can be particularly useful during the development of antipsychotic and antidepressant drugs [29,30].

3.2. Imaging receptor-targeting therapies in oncology and neurology

Receptor-based studies are amongst the most researched and well-succeeded molecular imaging studies with radiopharmaceuticals. In fact, from all SPECT agents listed in the Molecular Imaging and Contrast Agent Database (MICAD) roughly 60% are receptor-targeting probes [31]. Imaging tracers labeled with single-photon emitters can be used in early clinical phases to determine a variety of critical kinetic parameters and relate them to the values obtained in the preclinical stages. Both direct and indirect approaches can be used as described above. Direct approaches, although theoretically attractive, are often very difficult to implement. Besides the technical difficulty associated with the development of a radiolabeling procedure there is a lack of suitable nuclides. A much more practical approach is the use of an established tracer with affinity for a certain receptor (indirect method) that is challenged by the unlabeled test drug that displaces it from the active site. Two studies are usually required, one before (control) and another after the addition of the unlabeled drug [7]. This kind of approach can be very valuable in confirming mechanism of action but also in obtaining important parameters such as receptor density, occupancy and optimal dosing schemes [27,32].

A variety of SPECT tracers exist for many important targets in oncology and neurology. An overview of the most important is given below.

3.2.1. Somatostatin receptors

The detection of high levels of somatostatin receptors in many neuroendocrine tumor cell lines has prompted the development in the past 30 years of many radiotracers for this molecular target [33]. Since somatostatin itself cannot be used due to its short half-life in blood most radiotracers for this system are based on the synthetic octapeptide octreotide. Somatostatin receptors can be divided in 5 different subtypes SSTR1 to SSTR5 and its analogs and their subtype 1 has predominantly high affinities towards subtypes 2 and 5. The first commercially available molecular imaging probe for the somatostatin receptor was Indium-111 labeled Pentetreotide, launched in June 1994 [34]. Severely other 111In based tracers were developed for the SSTR receptors but the high cost of this nuclide, and the relatively poor image quality led to the development of tracers based on the widely available and with better image quality 99mTc. This includes 99mTc vaperotide (RC-160) and 99mTc depreotide (commercial name: NeoTect) both with a higher affinity for receptor subtypes 2 and 5 and others still under development such as lanreotide [35].

3.2.2. Other peptide receptors

The success obtained in imaging somatostatin receptors has encouraged the radiolabeling of additional peptides that could be used to image other important molecular targets in oncology. Those include the cholecystokinin (CCK) receptor with a similar distribution to somatostatin mainly in neuroendocrine tumors [33], the melanocyte-stimulating hormone (α MSH) in melanoma [36], 99mTc-labeled bombesin for breast cancer and adenosarcoma in general and the vasoactive intestinal peptide (VIP) and its analogs labeled with [123I] for gastrointestinal tumors [37].

3.2.3. Antibodies

Radiolabeled antibodies have been used extensively since the 1970’s both as imaging probes (immunoconjugates) as well as radiotherapy agents. The large size of these molecules (typically hundreds of kDa in size) makes it easy for the introduction of a labeling group without significant interference in their in vivo behavior. This makes SPECT imaging a very valuable tool in microdosing studies with candidate therapeutic antibodies. Labeling usually includes the addition of a bifunctional quelling agent such as DOTA, NOTA or TETA that conjugates with a radiometal such as 111In, 67Ga, or 68Cu. A Phase 0 trial with [111In]Trastuzumab was launched by NCI in 2007 for women with primary or metastatic breast cancer [38]. The primary outcome of the study was the correlation of drug uptake with HER2/neu status of the tumors. Secondary outcomes included safety, biodistribution in normal organs and pharmacokinetics. Maximum dose of the drug was 200 μg substantially lower that the expected clinical dose of 2–4 mg/kg [6].

SPECT imaging can also be used to guide the development of iodine-131 radioimmunotherapy agents as this nuclide is both a beta (therapy) as well as gamma (imaging) emitter. This was the case [131I] Tositumomab, approved in the US in 2003 for patients with relapsed, Rituximab-refractory, CD20+, follicular non-Hodgkin’s lymphoma [39]. Other examples include [131I]NP-4 anti-CEA for the therapy of CEA-expressing tumors and [131I]anti-Tenascin monoclonal antibody 81C6 for the treatment of gliomas [21]. In this case, 123I or 125I (for animals) and 123I (for humans) should be used, since 131I is not recommended for imaging to avoid irradiation.

3.2.4. Imaging receptors in neurology/psychiatry

The hypothesized mechanism of action of a candidate therapeutic drug for the CNS can be elucidated in vivo by SPECT imaging, using either established or newly developed radiotracers for a particular target [28]. This approach has proven to be useful in neuroscience drug discovery and development mainly due to the inaccessibility of the human brain. The confirmation that drugs reach their targets is crucial for a successful proof-of-concept testing [27]. There is a growing list of neuroreceptor systems imaged with SPECT (Table 2). Because of the greater availability of dopaminergic and serotonergic ligands, radiotracer imaging studies are particularly useful for development of putative antipsychotic and antidepressant drugs. The action of such drugs arises from their effects on specific receptors and can be revealed by receptor occupancy studies. The direct approach mentioned before has the potential to determine general brain biodistribution of the compound and also some of its kinetic properties, but is somewhat limited to isotopically labeled drugs with positron emitters such as 11C and 18F. Additionally, the relatively short acquisition times possible with these isotopes may underestimate the brain exposure to compounds that cross the blood brain barrier slowly. SPECT imaging studies using the [123I] labeled version of candidate...
drugs have demonstrated to be suitable for estimation of receptor occupancy through spatial localization of molecular activity and is often used to determine the ratio of specific to non-specific binding of slow targeting processes [30]. It should be noted, however, that for these studies to be reliable, the radioligand under study should not be substantially metabolized over the period of the study and that the introduction of iodine into the molecule does not affect its in vivo behavior significantly.

Instead of mapping directly the receptor occupancy, the indirect approach measures the displacement of an established imaging ligand for a specific receptor by the drug under development. Receptor parameters such as receptor density (Bmax), receptor affinity (1/KD) and binding capacity (Bmax/KD) can be estimated. The receptor occupancy of the drug candidate can be estimated quantitatively by comparing the maximum binding capacity of the imaging ligand in the absence and in presence of the drug [30].

4. Molecular imaging biomarkers in early clinical development

In the context of drug development, imaging biomarkers have gained interest as a way to assess the efficacy, the safety and the mechanism of action of drug candidates [40]. A biomarker is by definition any characteristic (anatomical, physiological, biochemical or molecular) that can be objectively measured and evaluated as an indicator of a normal or pathological process and to evaluate pharmacological response to therapeutic intervention [41]. By logical extension, imaging biomarkers may be defined as any anatomical, physiological, biochemical or molecular biomarker detectable by one or more imaging methods. The use of imaging biomarkers for obtaining early indications of the effectiveness and safety of new drugs has gained special attention on the last years. The interest on biomarkers and their integration into drug development programs is a logical consequence of the increasing development of new target specific therapies. The sequencing of the human genome and the considerable advances in molecular cell biology contribute for the increasing identification of potential drug targets. Actually, targeted therapies acting on specific biological pathways have been proved to increase the overall survival or progression-free survival as demonstrated by clinical trials with monoclonal antibodies against ERBB2 receptors (Trastuzumab) in breast cancer or against the vascular endothelial growth factor (VEGF) in several epithelial tumors [42,43]. Also the blockade of tyrosine kinase receptors with imatinib (Gleevec) has been proved to increase the prognosis of patients with gastrointestinal stromal tumors [44].

The anatomical imaging modalities such as CT, MRI or ultrasound are routinely used to assess tumor response to therapy based primarily on changes on tumor size. Although well established, structural readouts are recognized to be poor predictors of therapy response, mainly for molecularly targeted therapies, which many times induce only small changes on tumor size. Neoplastic tissues are characterized for having an excessive proliferation, increased metabolic activity, evasion of apoptosis and sustained angiogenesis. The possibility of visualizing drug-induced changes in such biologic properties could provide early indications of drug efficacy [45].

A main advantage of nuclear techniques over anatomical imaging is their ability to detect functional alterations long before any morphological changes are evident. In the field of oncology, FDG-PET is the most widely used and has been established in clinical routine for initial staging, assessment of therapy response and diagnosis of recurrent disease. Despite FDG's overwhelming success in the clinical setting, many other imaging agents have been labeled with positron- or single-photon emitters for visualizing specific targets or mechanisms (Table 2). Some of them are approved and used in clinical routine others are currently under development. As many of the new therapies under development are targeted against molecular mechanisms, the non-invasive imaging of those events may complement non-imaging endpoints to assess the biological activity of the candidate drug that are likely to confer a clinical benefit and to promote the confidence of therapeutic efficacy.

In the following sections we will present examples of potential applications of SPECT imaging biomarkers during the oncological drug development process.

4.1. Imaging tumor proliferation

Uncontrolled cell proliferation is the hallmark of oncological diseases and an important prognostic factor. The ability of non-invasive imaging methods to assess tumor proliferation could improve the diagnosis, grading and staging of cancer. Additionally, such imaging techniques could be used to evaluate the therapeutic efficacy of drugs with antiproliferative action such as epidermal growth factor receptor (EGFR) inhibitors, cycline dependent kinases and farnesyltransferase inhibitors. Most of the imaging probes available for measuring cellular proliferation are precursors of DNA synthesis labeled with positron emitters. The thymidine analogue 3′-deoxy-3′-fluorothymidine labeled with 18F (18F-FLT) is currently the most widely used radiotracer for imaging tumor cell proliferation. Other agents that are under study as potential proliferation markers are the short lived PET 11C-labeled amino acids, methionine and tyrosine [46] and the 18F-labeled fluorocholine [47]. Some imaging studies have revealed positive correlations between the uptake of these tracers and tumor proliferation, but have the limitation of the short half-life of 11C. Some amino acids have been labeled with longer-lived radionuclides. The most promising for SPECT imaging is the artificial amino acid L-3-iodo-α-methyl-L-tyrosine (IMP) labeled with 123-iodine. This amino acid is avidly taken up in many tumor cells, reflecting the amino acid transport activity, which is an important step in protein metabolism. In two clinical studies 123I-IMP uptake correlated with the Ki-67 proliferation marker and mitotic index in patients with gliomas [48] and soft tissue tumors [49]. These results suggest the usefulness of IMP as a valuable proliferative marker for the in vivo visualization of tumor cells proliferation. Further studies are needed to evaluate the potential of SPECT imaging with IMP for measuring tumor response to chemotherapeutic drugs targeting proliferating cells.

Table 2

<table>
<thead>
<tr>
<th>Biological process</th>
<th>Imaging biomarker</th>
<th>Target</th>
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<tbody>
<tr>
<td>Amyloid deposition</td>
<td>11C-T-IMP</td>
<td>β-amyloid plaques</td>
</tr>
<tr>
<td>Angiogenesis</td>
<td>99mTc-RGD</td>
<td>VEGF receptors</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>99mTc-HYNVC-VEGF</td>
<td>Caspase 3 expression</td>
</tr>
<tr>
<td>Cholinergic function</td>
<td>123I-QNB</td>
<td>Nicotinic receptors</td>
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<tr>
<td>Dopaminergic function</td>
<td>123I-PE2I</td>
<td>Dopamine transporter</td>
</tr>
<tr>
<td>GABAergic function</td>
<td>11C-t-PK11195</td>
<td>Central benzodiazepine sites</td>
</tr>
<tr>
<td>Inflammation</td>
<td>123I-iodobenzamide (HIZM)</td>
<td>Peripheral benzodiazepine sites</td>
</tr>
<tr>
<td>Perfusion</td>
<td>99mTc-HMPAO</td>
<td>Blood flow</td>
</tr>
<tr>
<td>Proliferation</td>
<td>3-(11C)iodo-α-ethyl-L-tyrosine</td>
<td>Aminoacid transport activity</td>
</tr>
<tr>
<td>Serotoninergic function</td>
<td>123I-β-CIT</td>
<td>Serotonin transporter</td>
</tr>
<tr>
<td>Somatostatin receptor expression</td>
<td>111In-octreotide</td>
<td>SSTR2 and SSTR5 receptors</td>
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4.2. Imaging tumor angiogenesis

Angiogenesis i.e., the formation of new blood vessels from preexisting ones plays a critical role in tumor growth and initiation of metastasis [50]. Angiogenic endothelial cells undergo a complex sequence of events including the secretion of metalloproteinases (MMPs) and other matrix-degrading enzymes, endothelial cell division and proliferation and vessel formation under the control of several local and circulating angiogenic factors including vascular endothelial growth factors (VEGF) and their receptors, angiopoietins and activators of integrins [51]. The increased understanding of the molecular mechanisms that regulate angiogenesis has led to the development of angiogenic drugs that block specific steps in this process. Several molecular effectors of angiogenesis, such as VEGF receptors, integrin αvβ3 and MMPs have been used as potential targets for angiogenic therapy as well as for non-invasive imaging of angiogenesis [52]. The VEGF receptors and the integrin αvβ3 have been identified as favorable targets for imaging angiogenesis.

VEGF cell-surface receptors are attractive targets for molecular imaging of tumor neovascularization because they are highly expressed in tumor endothelial cells, being considered an important feature of tumor angiogenesis. Recent studies using 99mTc-labeled VEGF have proved the feasibility of targeted SPECT imaging for VEGF receptor expression in angiogenic vessels of solid tumor xenografts in mice [53,54]. A microSPECT imaging study revealed a prominent uptake of 99mTc-HYNIC-VEGF in mice with 4T1 murine mammary carcinoma [53]. After treatment with low dose metronomic cyclophosphamide that selectively targets tumor endothelial cells, a decrease in tumor uptake of the radiotracer was observed. The ability of 99mTc-HYNIC-VEGF to visualize vivo the VEGF expression should allow for clinical translation of this tracer to image angiogenesis during tumor growth and following antiangiogenic treatment.

The integrin αvβ3 is a cell-surface receptor that mediates the migration of endothelial cells through the basement membrane during the angiogenic process and is highly expressed on activated endothelial cells [55]. This receptor is also involved in other pathological dysfunctions including tumor metastasis, osteoporosis and inflammatory processes. Peptides containing the amino acid sequence Arg-Gly-Asp (RGD) have been proven to be selective antagonists of αvβ3 integrin inducing apoptosis of activated endothelial cells. A variety of radiolabeled RGD peptides for PET and SPECT imaging have been developed for planning and monitoring treatment targeting the αvβ3 integrin [52]. Although the most studied is the glycosylated cyclic pentapeptide 18F-galacto-RGD used in PET, the 99mTc-labeled RGD derivatives are also considered as good candidates for SPECT imaging of αvβ3 integrin receptor expression in tumor-induced angiogenesis [56,57]. Several preclinical studies conducted in experimental models of tumor angiogenesis have demonstrated the abilities of SPECT imaging with 99mTc-RGD-containing peptides for the non-invasive detection of αvβ3 integrin expression. Significant correlations were obtained between the tumor uptake of 99mTc-RGD and the αvβ3 expression levels in tumors of different origins. Decristoforo et al. demonstrated the potential of a 99mTc-labeled RGD peptide derivatized with HYNIC (99mTc-HYNIC-RGD) for targeted imaging of angiogenesis in a murine tumor model with αvβ3-receptor positive and αvβ3-receptor negative tumors using planar γ imaging [58]. The αvβ3-receptor positive tumors were clearly visualized and the radiotracer uptake was of 2.72% after 1 h, whereas receptor-negative tumors showed a significantly lower value (0.85%).

To further improve the retention of αvβ3 ligands, multimeric RGD peptides were recently introduced. Ligands containing multiple RGD sequences represent a powerful approach to increase the binding affinity to the αvβ3 by enhancing the local density of αvβ3 ligands surrounding the molecular target. Multimeric RGD peptides showed increased binding affinities and improved tumor accumulation compared with the monomeric compounds [59,60]. Saney et al. evaluated the potential of a novel tracer containing four cRGD sequences labeled with 99mTc in tumor bearing mice [61]. The whole-body planar imaging showed increased accumulation of 99mTc-RAFT-RGD in tumors with high microvessel density. The Western blot analysis and autoradiographic studies detected the expression of αvβ3 integrin in the tumor neovessels, which confirm the affinity of 99mTc-RAFT-RGD for the angiogenic areas. 99mTc-NC100692, a cyclic peptide containing the RGD motif shows efficient αvβ3-integrin targeting and can be safely administered to patients for detection of αvβ3-positive tumors [62]. Other RGD peptides labeled with 111In and 124I have also been developed for both in vitro and in vivo imaging of tumor angiogenesis, with excellent binding affinity for αvβ3 integrins and favorable pharmacokinetics [63,64]. These findings point out the capabilities of SPECT imaging with radiolabeled RGD derivatives for appropriate selection of patients entering clinical trials of αvβ3-targeting therapies as well as for monitoring the therapeutic efficacy in both preclinical and clinical stages.

4.3. Imaging tumor apoptosis

Most signaling pathways activated by anticancer drugs cause cell death through the induction of apoptosis. Defective apoptotic pathways might occur during malignant transformation or can be acquired after exposure to chemotherapy, limiting the effectiveness of anticancer therapy [65,66]. The non-invasive assessment of apoptosis would be desirable to provide clinicians with information on therapeutic efficacy as well as for the development and testing of new anticancer drugs. Annexin V is a protein that binds with high affinity to phosphatidylinerine (PS), a membrane-associated intracellular phospholipid that is externalized from the inner to the outer leaflet of cell membrane during early apoptosis [67]. Accordingly, Annexin-mediated imaging of PS has been extensively investigated for identifying cells at the early stages of apoptosis. Several Annexin probes radiolabeled with 99mTc, 124I and 131I have been developed for SPECT and PET imaging to evaluate the apoptotic cell status in response to anticancer agents [68,69]. Among these, 99mTc-labeled Annexin V is the best candidate to detect in vivo and non-invasively apoptotic cells in tumors following treatment with apoptosis-inducing agents or radiotherapy [70]. Belhocine et al. in a phase I clinical study observed that patients who had a significant increase in 99mTc-Annexin V uptake after the first course of chemotherapy had either a complete or partial response whereas patients who showed no significant post-treatment tumor uptake has progressive disease [71]. This study was performed in patients with lung cancer and lymphoma. In another clinical study Kartachova et al. found similar results. Complete or partial tumor response was correlated with a marked increase in tumor uptake of 99mTc-Annexin V during early treatment over the baseline value [72]. The same group mapped the treatment-induced 99mTc-Hynic-Annexin V uptake in patients with various malignant tumors (lymphomas, NSCLC, and head and neck carcinomas) using co-registration of SPECT/CT [73]. A precise localization of increased tracer uptake in physiological areas (e.g. salivary glands, bone marrow) and within the tumor was possible by the additional landmarks depicted on the CT images. This is particularly important for non-invasive monitoring of myelosuppressive effect of the treatment regimen. These results raise the possibility of using SPECT imaging with 99mTc-Annexin V to provide evidence of the biological activity of new drug-induced apoptosis and to predict the severity of the normal tissue toxicity.

5. Future challenges

Molecular imaging has become in recent years an attractive tool in drug development with applications ranging from preclinical research to early clinical trials. As discussed above, SPECT imaging provides a relatively simple and minimally invasive approach to obtain
measurable indications of the pharmacological properties and therapeutic efficacy of novel drugs in early clinical drug development using only minimal amounts of the substances to be tested. Therefore, the introduction of imaging biomarkers into the drug development process has the potential to increase the efficiency and effectiveness of the whole process by directing the focus towards molecules with the highest potential to succeed. This approach has proven to be useful in many pharmacokinetic/dynamic studies by validating targets, confirming mechanism of action, assessing kinetic profiles and providing prognostic indicators. However, despite the fact that regulatory agencies in the EU and USA have recognized the usefulness of imaging biomarkers in the early development process, they are not routinely incorporated into clinical trials design. The lack of standardization of image acquisition protocols and data analysis, a deficient validation of the biomarkers and the scarcity of specific imaging probes for some molecular targets are the critical points that need to be addressed. We anticipate that solving these issues would further accelerate and improve the introduction of SPECT imaging and other imaging modalities into the drug development process and ultimately contribute to its success.

References


