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MOLECULAR IMAGING WITH SPECT AND PET IN EXPLORATORY INVESTIGATIONAL NEW DRUG STUDIES

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SUMMARY

The phase 0 microdosing concept was introduced recently by the FDA in order to shorten the timeline for drug development and to reduce the overall cost of the process. The aim of this approach is to provide drug pharmacokinetic and pharmacodynamic data using subpharmacological doses of prospective drug candidates in a small number of human volunteers at the earliest stages of drug development. A microdose is defined as 1/100th of the pharmacological dose or a maximum of 100 μg, which requires ultrasensitive analytical methods for measuring such a small amount of drug. Molecular imaging plays a fundamental role in this process. Radionuclide-based imaging modalities such as single-photon emission computed tomography (SPECT) and positron emission tomography (PET) allow the noninvasive visualization and quantitative assessment of physiological and biochemical processes occurring at cellular and subcellular levels within the human body. Due to the high sensitivity of such techniques it is possible to evaluate in vivo the biodistribution and pharmacokinetics of drug candidates in the nano- to picomolar concentration range. This information obtained in the earlier phases of drug development might have a tremendous impact on the success rate of agents entering clinical trials and on the overall efficiency of the process. In this article we provide an overview of the basic principles of molecular imaging with SPECT and PET and explain how these techniques can be implemented in exploratory IND studies and used to accelerate new drug approvals.

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INTRODUCTION

The exploratory investigational new drug (IND) studies were introduced in 2006 by the U.S. Food and Drug Administration (FDA) in order to increase the success rate of new therapeutic agents entering clinical trials and to accelerate the entire process of drug development (1). Currently, less than 10% of new chemical entities with therapeutic potential move beyond the earliest phase of development, and only one in five that enter clinical trials obtains marketing approval (2). The length of the whole process takes an average of 12-15 years and the total expected costs are estimated at $802 million (2). This condition is somehow related with the low predictability of toxicity and efficacy of the novel molecules provided by traditional preclinical studies, as well as with the lack of validated biomarkers able to assess the drug action earlier in the development process, especially for molecularly targeted agents (3, 4).

The exploratory IND studies, also called microdosing or phase 0 trials, are intended to provide a more rational selection of promising new drug candidates and to eliminate those that are likely to fail, before moving into traditional clinical trials involving a large number of patients. This takes place early in phase I prior to the traditional dose-escalation, safety and tolerance studies that ordinarily initiate the clinical development of a drug and involves limited human exposure to the drug candidate, with no therapeutic intent (5, 6).

The main goal of such phase 0 studies is to acquire in a relatively small number of patients receiving nontoxic drug concentrations information regarding drug pharmacokinetics or pharmacodynamics, drug–target binding affinity, measurement of drug effect and patient selection for subsequent studies (1, 5, 7). As defined in the guidelines issued by the FDA, the exploratory IND studies employ subpharmacological doses of the test compound, which

are generally less than 1/100th of the dose that yields a pharmacological effect established from animal studies, with a maximum of 100 μg (8).

Radionuclide-based molecular imaging techniques, including single-photon emission computed tomography (SPECT) and positron emission tomography (PET), are at the forefront of clinical imaging modalities that can be applied throughout the drug discovery and development process (9, 10). Both techniques provide a noninvasive or minimally invasive way of visualizing, characterizing and quantifying physiological processes at the cellular and subcellular level in intact living subjects (9, 11). Current equipment can detect within the whole body trace amounts of radiolabeled probes, in the nano- to picomolar range. The administration of imaging probes at these minute quantities is not expected to elicit any pharmacological or serious adverse events in healthy human volunteers or patients, which make them especially suitable for microdosing studies. Depending on the type of ligand and radionuclide, SPECT and PET can be used for assessing the biodistribution and pharmacokinetics of candidate drugs in the radiolabeled form, as well as to validate potential drug targets (12).

Additionally, nuclear techniques can also provide key imaging biomarkers in such exploratory studies, which can be used to elucidate mechanistic aspects linked to the therapeutic intervention (proof of principle) and with the drug-induced biological changes that are expected to provide a clinical benefit (proof of concept) (13). This is becoming particularly important in the clinical development of molecularly targeted therapies, especially in oncology, since conventional approaches are frequently inappropriate (14, 15). In fact, because of the growing number of novel molecularly targeted agents, several imaging biomarkers are currently under development to serve as in vivo predictors of therapeutic effects (16). Compared with anatomical imaging modalities, such as computed tomography (CT), magnetic resonance imaging (MRI) or ultrasound, nuclear techniques detect functional alterations long before any morphological changes are evident. Therefore, there is a growing interest to promote the integration of nuclear imaging techniques in exploratory IND studies in order to reduce the costs of and accelerate drug development, since they can be performed in vivo earlier in clinical trials.

This article provides a broad overview of nuclear imaging techniques, SPECT and PET and radiolabeling approaches, and how their implementation in exploratory IND studies could accelerate new drug approvals.

BASIC PRINCIPLES OF NUCLEAR IMAGING TECHNIQUES

Nuclear imaging techniques rely on the use of radiolabeled probes for visualizing physiological and biochemical processes occurring at the cellular and subcellular level in intact living subjects. These techniques are distinguished from the other in vivo imaging modalities by their high sensitivity, and therefore by the ability to detect very low amounts of a specific tracer. Adequate signals can be obtained following the administration of radiolabeled probes in the nano- to picomolar ranges, providing three-dimensional images of the distribution of the compound. Candidate drug molecules can be labeled with gamma ray-emitting nuclides to be imaged with a gamma camera (SPECT) or with positron-emitting nuclides for PET. Although both techniques offer exquisite sensitivity to image in vivo trace amounts of radiolabeled drugs, PET imaging is gaining particular importance in pharmaceutical development, since a great number of drug molecules can be labeled with short-lived organic positron emitters (^{11}C, ^{18}F, ^{13}N, ^{15}O) without inducing significant changes in their physicochemical properties. The higher spatial resolution of PET scanners compared with SPECT systems and the possibility for in vivo quantification of tracer concentration in absolute units make it more appealing for application in drug development. However, for tracking drugs with slow kinetics, such as antibodies and peptides, gamma ray-emitting radionuclides with longer half-lives are preferable. In the following sections we will describe the fundamentals of SPECT and PET, and discuss the most important aspects regarding the choice of the radionuclides and the radiolabeling methods for application in exploratory IND studies.

SPECT

SPECT is a sensitive tomographic imaging technique that provides a three-dimensional spatial distribution of gamma rays emitted by single-photon radionuclides. Planar projection data are acquired at multiple angles around the patient. These individual projections are subsequently reconstructed using reconstruction imaging methods to generate cross-sectional images of the internal distribution of radiopharmaceuticals. Nowadays, the advances in SPECT instrumentation and image-processing algorithms have pushed the limits of this technique into the millimeter range (17). The main problem inherent to SPECT imaging is the accuracy of quantification that is degraded by photon scattering and tissue attenuation. Because of the isotropic emission of gamma rays from the subject, a collimation is needed to restrict the gamma rays of certain predefined directions through the use of lead collimators containing many parallel aligned channels (18). Photons that travel in other orientations are absorbed by the collimator and do not contribute to the image, which reduces the detection efficiency and sensitivity of SPECT (17) (Fig. 1).

The introduction of multimodality imaging combining SPECT with CT in a dual-modality system (SPECT/CT) allows the simultaneous acquisition of functional and detailed anatomic information, which provides an accurate localization and quantification of the radiolabeled imaging probes (17). Moreover the anatomic data obtained from the CT scan can be used to derive a transmission map for object-specific attenuation correction in SPECT images, improving the image quality and the accuracy of quantification (19). This is essential when assessing in vivo the biodistribution and pharmacokinetic profile of new drugs, or the validation of drug targeting and efficacy as well.

Several gamma ray-emitting radionuclides are available for SPECT imaging purposes. The most commonly used emit gamma photons with energies in the range of 80-250 keV and have half-lives varying between a few hours to several days. Since each radionuclide has its own energy spectra, multiple probes can be imaged simultaneously, allowing the study of multiple cellular or molecular events. A list of the most common is given in Table I. The radiometal technetium-99m (^{99m}Tc) is the most widely used in diagnostic nuclear medicine due to its favorable physical proper-

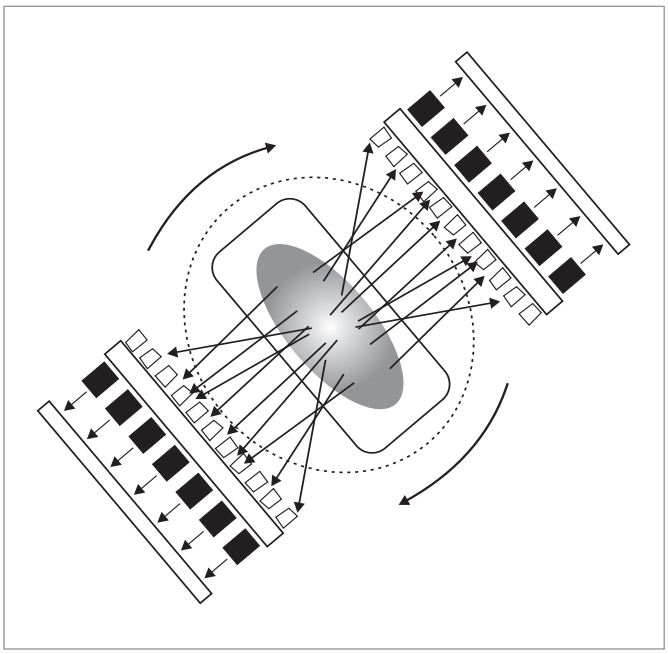

Figure 1. Schematic diagram of a gamma camera used in SPECT imaging.

ties ($t_{1/2}$ = 6 h; Eγ = 140 keV) and its widespread availability as a column eluate from a $^{99}Mo/^{99m}Tc$ generator at low cost. Other clinically relevant gamma emitters are indium-111 (^{111}In), gallium-67 (^{67}Ga) and some radioiodine isotopes (^{123}I and ^{131}I).

PET

PET relies on the use of nuclides that are unstable by β^+ (positron) decay. Positrons are the antiparticles of electrons, and after being emitted, travel a short distance in tissue until they lose most of their kinetic energy and then combine with an electron and annihilate. From this process, two gamma photons are emitted, in opposite direction of each other, with an energy of 511 keV each (Fig. 2).

These gamma photons can be detected by dedicated tomographs that use rings of detectors to detect photons that arrive "in coincidence" (in fact, within a narrow time window of a few nanoseconds) and thus allow us to determine the line of response (LOR) in which the annihilation took place. Some PET tomographs can even determine the relative difference in arrival time between the two coincidence photons (time-of-flight PET), and thus reduce the uncertainty of the annihilation position within the LOR (20). Multiple LORs are collected, organized into projections and used to reconstruct three-dimensional images in a similar fashion to SPECT. Nevertheless, in PET, because there is no need for physical collimation, sensitivity is greatly enhanced, and if appropriate corrections are applied, PET images can be quantitative. Spatial resolution can also be considerably high for a nuclear technique, and nowadays there are scanners under development that reconstruct images in the submillimeter range (21).

Nevertheless, there are physical limits to spatial resolution in PET, as the distance traveled by the positron in tissue before annihilation and small deviations from collinearity between the two emitted photons introduce an uncertainty in the position of the emitting tracer that limits spatial resolution to the range of hundreds of millimeters (22). PET can also benefit substantially from the simultaneous acquisition of a CT image (PET/CT) for anatomical localization and attenuation correction, and in fact, all clinical scanners currently available for PET are hybrid scanners.

SPECT AND PET IN EXPLORATORY IND STUDIES

The high sensitivity of nuclear imaging techniques allows us to image in vivo, noninvasively and quantitatively the pharmacodynamic and pharmacokinetic parameters of investigational new drugs (IND). In particular, pharmacokinetic parameters are crucial for the success of a new drug and are a frequent cause of failure in latter stages of drug development. SPECT and PET imaging can provide quantitative in vivo biodistribution data in normal and diseased tissue, as well as renal and hepatobiliary excretion (23). It can also be used to analyze the biodistribution and patterns of deposition of drugs administered by different routes of administration. As an example, PET imaging was used to determine the tumor and normal tissue pharmacokinetics of an antineoplastic agent labeled with ^{11}C

Table I. *Commonly useful radionuclides for SPECT and PET.*

Radionuclide	Type of emission	Physical $t_{1/2}$	Energy (keV)	Source
^{99m}Tc	γ	6.02 h	140 (89%)	Generator
^{111}In	γ	2.8 days	171 (88%), 245 (94%)	Cyclotron
^{131}I	β^-,γ	8.04 days	284 (6%), 364 (81%)	Nuclear fission
^{123}I	γ	13.2 h	159 (83%)	Cyclotron
^{67}Ga	γ	78.2 h	93 (40%)	Cyclotron
^{18}F	β^+	110 min	634	Cyclotron
^{11}C	β^+	20.4 min	960	Cyclotron
^{15}O	β^+	2.07 min	1720	Cyclotron
^{13}N	β^+	9.96 min	490	Cyclotron
^{68}Ga	β^+	1.13 h	1899	Cyclotron
^{64}Cu	β^+	12.7 h	653	Cyclotron
^{76}Br	β^+	16 h	3980	Cyclotron
^{124}I	β^+	4.2 days	1532 (11%) 2135 (12%)	Cyclotron

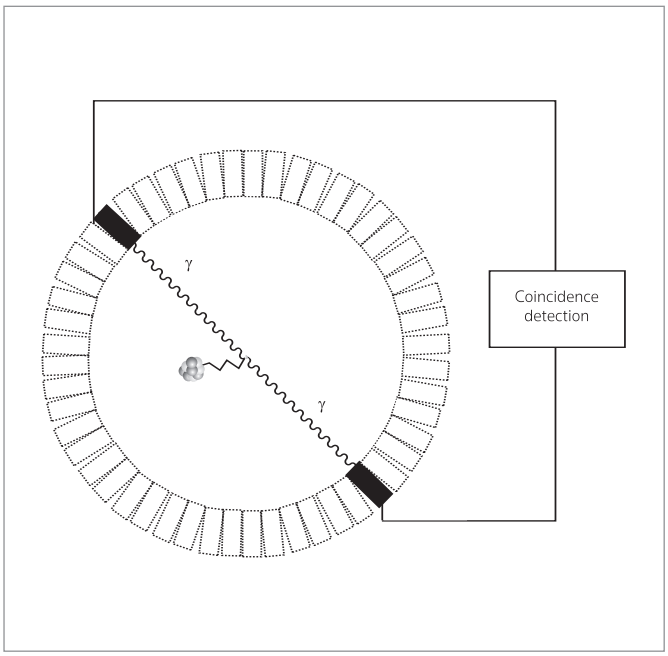

Figure 2. Positron emission and annihilation lead to the production of two 511 keV photons traveling in opposite directions. Rings of detectors placed around the subject detect photons that arrive simultaneously (coincidence detection).

([^{11}C]-DACA) at tracer concentrations before conventional phase I trials (24). Additionally, the effects of pharmacological doses of DACA were evaluated on radiotracer kinetics in patients undergoing phase I clinical trials (24). In another study, a PET microdosing approach was performed to obtain the neuropharmacokinetic properties of an investigational anti-amyloid drug (ST-1859) labeled with ^{11}C in healthy volunteers and in patients with Alzheimer's disease. The results of this study demonstrated that there was a rather uniform distribution of radioactivity in the brain, including both β-amyloid-rich and -poor regions, with slow washout of radioactivity in both healthy volunteers and patients (25).

Furthermore, drug metabolism can be assessed with appropriate blood sampling and analysis, e.g., by radio-HPLC. Additionally, using data modeling techniques available for PET studies, the main pharmacokinetic parameters can be easily extracted. These include: standardized uptake values (SUVs), peak concentration (C_{max}) and time to reach peak concentration (t_{max}), area under the curve (AUC), tissue volume of distribution (V_d), mean residence time (MRT), permeability–surface area product (PS product), influx of drug into tissue (K_1), clearance of drug from tissue (k_2) and selective binding of the compound to the specific molecular targets (k_3).

The assessment of drug–target engagement facilitates proof-of-concept testing and improves the quality of decision-making in early drug development. This could be performed using the radiolabeled form of the candidate drug or through the use of an established tracer with affinity for a certain molecular target. The first approach is often difficult to implement and somewhat limited to PET imaging studies because of the radiochemistry involved. The second approach is especially suited for receptor studies in neurology (26). Instead of directly mapping the receptor occupancy, we can measure the displacement of an established imaging ligand for a specific target by the drug under development. Several parameters, such as receptor density, receptor affinity and binding capacity, can be estimated quantitatively. Because of the greater availability of established radiotracers for dopaminergic and serotonergic receptors (Table II), this technique is particularly useful for the development of putative antipsychotic and antidepressant drugs (27, 28).

RADIOLABELING CONSIDERATIONS

For a successful application of nuclear imaging in drug development, the radiolabeling procedure should not alter the physicochemical properties of the test molecule. Several factors must be taken into account when selecting the radioisotope and the radiolabeling approach. Major considerations should be given to the effects of the radionuclide conjugation on the targeting properties and biodistribution of the molecules, and stability under physiological conditions as well. The resulting imaging agent should retain the same affinity and activity as the investigational therapeutic drug. The direct labeling method by isotopic substitution in which a stable atom of the molecule is replaced by a radioactive isotope is the most suitable.

Novel drug molecules rarely contain atoms that have gamma-emitting radioisotopes suited for SPECT imaging. In such cases, a foreign element or a foreign moiety containing the SPECT radionuclide is attached to the drug molecule. Most of the time, the drug molecule itself does not permit the direct attachment of the radiolabel and an appropriate linker or chelator should be used. This approach does not guarantee that the radiolabeled form retains the same properties as the native drug, especially in small molecules with simple structures, since the radionuclide–chelator complex contributes greatly to the overall size and molecular weight of the molecule. In this regard, gamma-emitting radionuclides are especially suited for radiolabeling of large biomolecules such as therapeutic antibodies and derivatives or small peptides, because of the minor influence of the attached radioisotope on the biokinetics of the original molecule considering its larger size.

Contrary to SPECT, there are positron-emitting isotopes of some of the most prevalent elements in organic molecules. These include carbon-11 ($t_{½}$ = 20.4 min), nitrogen-13 ($t_{½}$ = 9.97 min) and oxygen-15 (^{15}O; $t_{½}$ = 122 s), which allow us to label isotopically virtually any candidate drug (Table I). However, the relatively short half-lives of these nuclides require a cyclotron available on site and limit the complexity of the molecules to be labeled, as well as the range of processes that can be studied in vivo. In contrast, halogen positron emitters present half-lives that are more appropriate for complex labeling procedures and more prolonged studies (^{18}F: $t_{½}$ = 109.8 min; ^{76}Br: $t_{½}$ = 16 h; ^{124}I: $t_{½}$ = 4.2 days) and can be distributed to centers located up to three half-lives of the production facility. Although their presence is not as common as C, N and O, a halogen can sometimes be introduced without significantly disturbing the physicochemical

Table II. *SPECT and PET imaging probes for neurosciences and oncology.*

Field	Imaging probe	Biological process/target
Neurology and psychiatry	^{123}I-Benzovesamicol ^{123}I-QNB ^{123}I-A-85380	Cholinergic function
	^{123}I-FP-CIT ^{123}I-PE2I ^{123}I-Epidepride ^{123}I-IBZM ^{123}I-β-CIT ^{123}I-Altropane ^{99m}Tc-TRODAT-1 ^{11}C-Raclopride ^{11}C-SCH -23390 ^{18}F-Fluorodopa	Dopaminergic function
	^{123}I-Iomazenil	GABAergic function
	^{123}I-β-CIT ^{123}I-ZIENT ^{123}I-ADAM ^{123}I-5-I-R-91150 ^{11}C-Methylspiperone	Serotonergic function
	^{123}I-PK-11195	Inflammation
	^{123}I-IMPY	Amyloid
	[^{15}O]-H_20	Cerebral blood flow
Oncology	^{18}F-FDG	Glucose metabolism
	^{18}F-FLT ^{124}I- IUdR ^{11}C-FMAU ^{11}C-Choline 3-[^{123}I]-Iodo-α-methyl-L-tyrosine	Proliferation
	^{99m}Tc-RGD ^{18}F- Galacto-RGD ^{99m}Tc-HYNYC-VEGF	Angiogenesis
	^{99m}Tc-Annexin ^{18}F-Annexin	Apoptosis
	^{18}F-FMISO	Hypoxia
	^{111}In-Octreotide	sst_2 and sst_5 receptors
	^{99m}Tc-Bombesin	Bombesin, gastrin-releasing peptide
	^{123}I-VIP ^{99m}Tc-TP-3654	Vasoactive intestinal peptide receptor

characteristics of a bioactive compound. This is frequently the case with ^{18}F, which has been used with great success as a substitute for the hydrogen atom, with which it shares a similar van der Waals radius. An example of this approach is the molecule 2-[^{18}F]-fluoro-2-deoxy-D-glucose ([^{18}F]-FDG), a marker of oxidative metabolism and the most widely used radiotracer in PET for diagnosis and staging of many tumors. Other positron emitters of interest include gallium-68 ($t_{½}$ = 68 min), which can be produced in a generator and thus does not require the use of a cyclotron, and copper-64 ($t_{½}$ = 12.7 h), which can either be produced in a cyclotron or a nuclear reactor. The relatively long half-lives of these nuclides make them suitable to target processes with slow kinetics or that require a longer follow-up.

ANTIBODIES AND PEPTIDE-BASED PROBES FOR MOLECULARLY TARGETED THERAPIES

In the past decade, significant progress has been made in the development of monoclonal antibodies (MAbs) and small peptides as targeting molecules for both therapeutic and diagnostic purposes. The characterization of molecular signaling pathways responsible for tumor growth and progression has identified a number of potential targets for new anticancer treatments. Trastuzumab, an MAb targeting the human receptor tyrosine-protein kinase erbB-2 (HER2), is a component of the first-line treatment of HER2-positive metastatic breast cancer patients (29). New molecularly targeted therapies under development include agents targeting epidermal growth factor receptors, the most important growth factor controlling angiogenesis, including tyrosine-protein kinase inhibitors such as gefitinib and erlotinib, and the MAbs cetuximab and panitumumab (30-32).

The development and optimization of new engineered antibodies for targeted therapies can be facilitated by nuclear imaging. Due to the high sensitivity of nuclear imaging techniques, these approaches are currently being applied in preclinical studies on the optimization of engineered antibodies for radioimmunodetection and targeted therapy. The ^{131}I-labeled antibody tositumomab, humanized anti-CEA and anti-tenascin, ^{111}In-trastuzumab and [^{68}Ga]-trastuzumab are illustrative examples (33, 34). The same approach can be carried out in phase 0 studies for assessing the pharmacokinetics and targeting properties of those biomolecules and to determine the optimal dosage and dosimetry.

RADIOLABELING APPROACHES FOR BIOMOLECULES

A wide variety of strategies have been developed in recent years for a convenient and efficient labeling of biomolecules, including MAbs and derivatives (minibodies and diabodies), small peptides and non-peptide receptor ligands originally developed for diagnostic imaging and radiotherapy. The ^{111}In-radiolabeled somatostatin analogue octeotride (OctreoScan®) was the first approved peptide-based radiopharmaceutical and one of the most commonly used in clinical neuroendocrine tumor imaging (35). Other peptides, such as bombesin analogues, vasoactive intestinal peptide (VIP), cholecystokinin and neurotensin, have been intensively evaluated in preclinical and clinical trials as potential imaging agents in nuclear medicine (35-37). SPECT imaging studies using ^{111}In-radiolabeled trastuzumab have shown promising results for assessment of the HER2 tumor status and for staging of HER2-positive breast cancer patients (38).

Generally, two different approaches can be used for radiolabeling biomolecules: direct labeling and the chelate method in which the radionuclide is bound indirectly to the targeting molecules through a bifunctional chelating agent (BFCA) covalently attached to the biomolecule (39). In some cases a linker group is introduced between the BCFA and the biomolecule. Several BFCAs (e.g., DTPA, NOTA, DOTA and HYNIC) have been designed and can potentially be linked to proteins, peptides, antibodies and other biologically relevant molecules (36). The choice of a proper BFCA is very important and depends on the coordination requirements of the specific radiometal and on the kinetic stability of the complex. This requires a well-defined knowledge of the radiometal coordination chemistry and of the structure of BFCA and the types of conjugation groups (e.g., active esters, isothiocyanates, hydrazides, etc.) available for attaching the biomolecules (39, 40). The National Cancer Institute (NCI) initiated in 2007 a phase 0 trial with ^{131}I-trastuzumab for women with primary or metastatic breast cancer expressing *ERBB2* (41). The antibody was first conjugated with DTPA and then labeled with ^{111}In. The resulting imaging agent showed the same affinity and activity as the original therapeutic agent.

In the direct labeling approach, the radionuclide is incorporated directly into the biomolecule using the coordinating groups (electron donor atoms) present in the targeting molecule. This method eliminates the need for conjugation with a bifunctional chelating agent, reducing the likelihood of alterations in the immunospecificity of the biomolecules, but it requires a good knowledge of the number of donor atoms in the biomolecule and of the radionuclide coordination chemistry (42). It has been successfully used in labeling antibodies and their fragments with ^{99m}Tc, since they contain many disulfide bonds acting as metal coordinators. Octeotride can be directly labeled with ^{99m}Tc via reduced disulfide bonds (S-S) in the residue (43). However, small molecules that do not have any disulfide bond or are unable to retain their biological properties after labeling cannot be labeled directly (40). In some cases, the experimental conditions (e.g., pH, temperature, solvents, reducing agent) required for radiolabeling might result in the loss of receptor binding activity.

A variety of engineered antibodies and derivatives have been labeled with some iodine radioisotopes for both therapy and diagnosis. Commonly used radioisotopes of iodine include ^{131}I, used in therapy, ^{123}I for SPECT and ^{124}I as a positron emitter. Because of the well-known chemistry of iodine, radioiodination of engineered antibodies and their fragments is frequently employed for optimizing anticancer antibodies for clinical usefulness. The tumor-targeting properties of an engineered intermediate-molecular-mass ^{123}I-labeled antibody construct directed against CEA (cT84.66 minibody) was demonstrated in patients with colorectal cancer in a pilot trial (44).

Several different labeling procedures have been reported. The most conventional methods employ oxidizing agents such as chloramine-T or Iodogen for the generation of electrophilic radioiodine species to react with functional groups present on the biomolecule, usually the phenolic groups of tyrosine residues (45). This is a practical and very useful technique in preliminary studies, since it provides radiolabeled products with high specific activity. A disadvantage is the fact that, during the labeling, the biomolecules are exposed to oxidizing agents that can damage sensitive biomolecules. Moreover, many antibodies have an elevated number of tyrosine residues in the binding regions and the iodination of these residues may decrease the antibody immunoreactivity. In such cases, alternative indirect methods should be applied, namely engineering an iodine-accepting group on the antibody, or through the conjugation with a prosthetic group previously radioiodinated (45, 46). The main functional groups in the biomolecules that can be used for conjugation reactions with prosthetic groups are amines, sulfhydryls and oxidized sugars. As an example, the Bolton–Hunter method employs *N*-succinimidyl 3-(4-hydroxyphenyl)propanate firstly iodinated using the chloramine-T method and then coupled to the protein (46).

The substitution of a stable iodine atom already incorporated in the molecule by a radioactive iodine atom can be performed easily and

Table III. *Selective list of labeled antibodies and peptides previously tested in humans.*

Probe	Target	Application
^{99m}Tc-Bombesin	Bombesin/gastrin-releasing peptide	Neuroendocrine tumors central neurocytoma, pancreatic tumors, adenocarcinomas
^{111}In-DTPA octreotide	Somatostatin	
^{99m}Tc-TP-3654	Vasoactive intestinal peptide	
^{111}In-ch806	Epidermal growth factor receptor	Several tumors
^{131}I-CC49-deltaCH2	TAG-72	Gastrointestinal adenocarcinomas
^{99m}Tc-Arcitumomab	CEA	Colon carcinoma
^{18}F-[Gluc-Lys]-TOCA	sst	Neuroendocrine tumors
^{18}F-Galacto-RGD	Integrin	Head and neck cancer
^{18}F-AH-111585	Integrin	Metastatic breast cancer

is a suitable method for radioiodination of small organic molecules. However, the poor radiolabeling yields, together with the low specific radioactivity products, limit their usefulness in clinical nuclear medicine (45). Table III lists some labeled antibodies and peptides that have been previously tested in humans in clinical trials.

IMAGING BIOMARKERS

The integration of biomarkers, including imaging biomarkers, in the drug development process is the logical consequence of recent advances of molecularly targeted drugs. An imaging biomarker is by definition any characteristic (anatomical, biochemical or molecular) that can be measured objectively by one or more imaging methods, as an indicator of normal biological processes, pathological processes or pharmacological responses to a therapeutic intervention (47). The efficacy of a drug candidate is measured by a clinical endpoint. Ideally, a biomarker may serve as a substitute for a clinical endpoint. The use of appropriate biomarkers is critical in both preclinical and clinical drug development, as they can provide insight into the mechanistic aspects linked to the therapeutic intervention and on proof-of-concept testing. Opposite to anatomic imaging modalities, nuclear imaging techniques detect functional alterations long before any morphological changes are evident. This is extremely important in the field of oncology, since new molecularly targeted therapies can improve survival without inducing measurable changes in tumor size. A wide range of imaging probes are presently being developed for in vivo characterization of tumor biology and are likely to have a major impact on drug development. For example, PET imaging with [^{18}F]-FDG can be used to assess the tumor metabolic response induced by novel anticancer drugs immediately after treatment. Other potential imaging biomarkers under development address several hallmarks of neoplastic tissues, such as the formation of new blood vessels, excessive proliferation and dysregulation of cellular homeostasis, as well as biological endpoints occurring downstream of the drug target (Table II). Some of them may soon become available for drug development. A variety of radiolabeled RGD peptides for SPECT and PET imaging are considered good candidates for monitoring therapies targeting $\alpha v\beta 3$ integrins in tumor vessels, as well as for the development of antiangiogenic therapies in general (48-50).

SPECT imaging with [^{99m}Tc]-annexin V provides evidence of tumor cells undergoing apoptosis following treatment with apoptosis-inducing agents (51, 52). This approach might serve as a potential biomarker to assess proapoptotic responses to novel drugs in cancer patients. For drugs with strong antiproliferative activity, such as cyclin-dependent kinase or epidermal growth factor receptor inhibitors, PET imaging with [^{18}F]-FLT (thymidine analogue) can be used as a biomarker for tumor proliferation (53, 54). Other imaging agents under development as potential proliferation markers include [^{11}C]-FMAU, [^{18}F]-choline and [^{124}I]-IUdR (iododeoxyuridine). Because of the growing list of radioligands for neurotransmitter systems (Table II), neurology is another field in which imaging biomarkers can be helpful in the early stages of central nervous system drug development (26, 55).

FINAL CONSIDERATIONS

Continuous advances in imaging technology combining functional and anatomical information render molecular imaging highly attractive for drug discovery and development. Among other potential advantages, imaging technology provides a noninvasive way to gather important information related with the pharmacokinetics and the targeting properties of investigational new drugs in early human clinical testing. The interest of imaging biomarkers and their integration into drug development programs have gained special attention in recent years because of the development of new target-specific therapies. Several companies are active in this field, and tremendous progress has been made in recent years. The regulatory agencies have recognized the potential of SPECT and PET and appear to be willing to facilitate the implementation of such imaging approaches into drug development programs. Nevertheless, clinical studies employing such imaging techniques in clinical phase 0 trials are restricted. A closer collaboration between pharmaceutical companies, regulatory authorities and groups working in the field of molecular imaging needs to be encouraged in order to accelerate the implementation of SPECT/PET in exploratory clinical development.

DISCLOSURES

The authors state no conflicts of interest.

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