The role of molecular imaging in modern drug development

Lídia Cunha1, Krisztián Szigi1, Domokos Mathé3, Luís F. Metello1,4

1 Nuclear Medicine Department, High Institute for Allied Health Technologies, Polytechnic Institute of Porto (ESTSP.IPP), Vila Nova de Gaia 4400-330, Portugal

2 Nanobiotecnology & In Vivo Imaging Center, Semmelweis University, Budapest H-1094, Hungary

3 CROmed Ltd, H-1047 Budapest Baross u. 91-95, Budapest, Hungary

4 IsoPor, SA, Porto, Portugal

Abstract

Drug development represents a highly complex, inefficient and costly process. Over the past decade, the widespread use of nuclear imaging, owing to its functional and molecular nature, has proven to be a determinant in improving the efficiency in selecting the candidate drugs that should either be abandoned or moved forward into clinical trials. This helps not only with the development of safer and effective drugs but also with the shortening of time-to-market. The modern concept and future trends concerning molecular imaging will assumedly be hybrid or multimodality imaging, including combinations between high sensitivity and functional (molecular) modalities with high spatial resolution and morphological techniques.

Introduction

The discovery and development of new drugs is perhaps one of the most challenging activities driven by the scientific community and simultaneously one of the most expensive and time consuming. On average, the cycle of bringing a new drug to market can last between 5 and 10 years (with around 8 years being typical), costing more than US$1 billion. Drug development is a rather inefficient process, because only one in 5000 tested compounds will reach the market 3 and 4. Owing to the dimension of the involved investment, all stakeholders (pharmaceutical companies, physicians and patients) want effective drugs to be developed and become widely available at a reasonable cost. To achieve this, mechanisms of disease need to be well understood and the compounds to be tested need to be well characterized in the early phases of research, those that generally are less costly. In this review we will provide an overview of: (i) the main steps of drug development and its related challenges; as well as (ii) the role and potential of molecular imaging and how it might contribute to improving the overall efficiency of the process.

Stages of drug development
The process of drug development comprises five stages, characterized by specific objectives, using a set of resources (Fig. 1). The decision to undertake the process of developing a new drug is usually triggered by the need to treat a pathological condition presenting an unsatisfactory response to the existing drugs.

**Figure 1. Phases of drug development and the respective main objectives. Solid circles highlight the phases where molecular imaging is most useful.**

**Target identification and validation**

The first step involves target selection and usually requires a solid knowledge of the underlying pathophysiological processes to do it in a timely fashion. The sequencing of the human genome (as well as those of many pathogens) has broadened the number of potential drug targets in the past decade. A drug target can be a membrane or a nuclear receptor, an ion channel, an enzyme, a hormone, DNA or RNA molecules, or an unidentified biological entity. At this phase, molecular imaging has proven to be useful in detecting the presence of drug targets, their spatial and temporal distribution and the functional consequences of drug target interaction. Nuclear imaging modalities and positron emission tomography (PET) in particular are being increasingly widely used for the study of receptor expression and occupancy in central nervous system drug development 7 and 8, as shown in Fig. 2, which illustrates the use of two different brain receptor antagonists (i.e. $^{11}$C-raclopride for dopamine D2 receptors and $^{125}$I-SD7015 for cannabinoid-1 receptor) to obtain qualitative and quantitative data about receptor occupancy over time.
Figure 2. Receptor binding quantification by nuclear imaging modalities. (a) Dynamic positron emission tomography (PET) list mode images obtained (NanoScan, Mediso, Hungary) from a male Wistar rat, after the intravenous injection of 12.0 MBq of $^{11}$C-raclopride, a dopamine D2 receptor ligand. Transverse slices of the rat brain are shown: T2-weighted magnetic resonance (MR) images showing anatomic detailed information and PET images acquired 75–90 min post injection showing receptor D2 receptor binding. (b) After the delineation of three volumes of interest (VOI) in the striata (exhibiting the highest receptor expression, 150 fmol/mg tissue), cortex (moderate receptor expression) and cerebellum (control tissue with no receptor expression), radioactivity in kiloBecquerel per cubic centimeter (kBq/cc) was quantitatively determined in the PET images. Region volumes were determined by MR images. The time–activity curves represent tracer concentration over time. Kinetic D2-receptor binding constants can be then derived from the data. (c) Brain coronal images of a C57BI6 male mouse acquired after the intravenous administration of 1.40 MBq of a central cannabinoid-1 receptor (CB1R) ligand, $^{125}$I-SD7015. On the left, single-photon emission computed tomography and computed tomography (SPECT/CT) image, acquired 3 h after tracer injection (NanoSPECT/CT Silver Upgrade, Mediso, Hungary) is shown. Images were then co-registered with a magnetic resonance imaging (MRI) atlas using nonlinear co-registration software (Fusion, Mediso, Hungary). On the right, the corresponding thin-slice autoradiography coronal section from the brain is depicted, with CB1R-rich regions such as hippocampus, cortical region and the substantia nigra showing increased uptake in the grayscale image.
An alternative to the direct approach of target imaging (often a complex and time-consuming process) would be the use of reporter genes. A variety of reporter genes have been developed for optical, magnetic resonance and nuclear imaging. Reporter genes are useful in the evaluation of the levels of expression of specific genes as well as several intracellular biologic processes, such as signal transduction pathways, nuclear receptor activities and protein–protein interactions. During recent years, reporter gene imaging techniques have emerged as excellent tools, not only for drug target interaction monitoring but also for therapy evaluation.

Lead finding and optimization

After being identified and validated, the drug target is exposed to a large number of compounds and analyzed by biochemical and cellular assays compatible with automated high-throughput screening 4 and 6. In this phase, relevant parameters are evaluated, such as compound purity, integrity, solubility, lipophilicity, safety, dissociation constant, permeability and target affinity. Furthermore, compound analogs can be synthesized and tested to identify those exhibiting better responses in more complex models (leads), which are then optimized and pursued for preclinical testing. Moreover, in vitro optical imaging (OI) can be useful at this stage for compound screening in cell-based assays owing to its high sensitivity, low cost and high-throughput capabilities.

Compound profiling in animal models

In this phase, in vivo imaging techniques using relevant animal models of disease play an important part, providing valuable information concerning drug absorption, distribution, metabolism, elimination (ADME) and efficacy. Usually, to study the pharmacodynamics and biodistribution properties of a candidate drug, the drug itself is labeled with an imaging probe (e.g. a radionuclide, a fluorophore metal with high relaxivity). A great advantage of this approach is that it requires only a minimal number of animals (compliant with the 3Rs policy, see Box 1), which can be imaged repeatedly for longitudinal studies, in direct contrast to the more conventional destructive biodistribution approaches, in which animals are sacrificed at predefined timepoints, thus requiring larger amounts of animals (even if traditional pharmacokinetic methods are not totally replaceable by imaging).

Box 1.
The 3Rs policy

This concept was developed by Russell and Burch in 1959, they introduced humane techniques such as replacement, reduction and refinement (now commonly known as the 3Rs) as the main guideline for the responsible use of animals in research [85]. The use of preclinical imaging allows not only the reduction of the number of animals required for a given experiment but also the refinement of the applied procedures, essentially as a result of their noninvasive and nondestructive nature.
Usually, longitudinal imaging results not only in lower cost but also more-reliable results, because intersubject variability is higher than test–retest intrasubject measurement results. Rodents are the most commonly used living animals for preclinical imaging studies and mice have been particularly important for a number of reasons: the genetic similarities with humans; the availability and diversity of genetically modified strains and disease models; and the lower amounts of compound required for testing compared with higher species. Preclinical imaging establishes a relationship between findings at the molecular level and clinical observations with the application of the same candidate entities. This is vital for the success of the subsequent phases because the role of a molecule in a disease model in vitro might not mimic its role and molecular interactions in vivo. Imaging techniques based on the in vivo biomarker readout concept anticipate decision making regarding compound safety and efficacy, leading to significant cost reductions by shortening the time to market and by eliminating poor candidate drugs earlier in the development process 14 and 15. In fact, through the use of functional imaging, it is possible to determine whether the target can be modulated by an external therapeutic chemical compound and if the concentration of drug reaching the target is sufficient to induce the desired therapeutic effect and does not accumulate in non-target organs, potentially inducing toxicity. If that is not the case, then research will be directed to other promising candidates, potentially leading to consequent financial and time savings. A possible example could be the case of a drug designed to have antiangiogenic properties, cutting-off tumor blood supply. Imaging would lead to evaluating not only the tumor size but also other important aspects such as tumor blood supply, oxygen consumption, tumor metabolism, tumor shrinkage or scaring. In fact, imaging techniques can provide not only qualitative but also quantitative data with rather good precision levels and reproducibility, being of paramount importance in go–no-go decisions. From the ethical perspective, it is indeed important to spare the patient from exposure to experimental drugs with little or no therapeutic effect. However, before final conclusions are drawn, researchers must be aware that, despite genetic similarities, animal models might not represent the full phenotype of human physiology for many reasons, for example differences in membrane transporter proteins. In addition, before clinical testing, the toxicity profile of the drug must be characterized using two or more animal species (usually one rodent and one non-rodent) because the drug might affect species differently. Imaging can also play a part in the determination of the amount of the drug that remains in the circulation, the amount that is taken up by the different organs, how much is broken down and the resultant metabolites and its excretion from the body. In the literature, there are a few examples of toxicity studies where the contribution of imaging was valuable, for example the paper by Zhang et al. that studies the effects of anesthetics in developing brains using biomarkers of apoptosis ([18F]-annexin-V), brain metabolism (2-deoxy-2-[18F]-fluoro-D-glucose, [18F-FDG), neuroinflammation ([18F-(N-(2-(2-fluoroethoxy)benzyl)-N-(4-phenoxypyridin-3-yl)acetamide), [18F-FEPPA] and cell proliferation (3'-deoxy-3'-[18F]-fluoro-L-thymidine, [18F-FLT).

Safety evaluation

Drug safety can be assessed either in vivo or ex vivo, aiming to characterize the toxicological effects on organ function and structure. In the past during this stage in vivo imaging was not widely used, possibly
because toxicological studies must be carried out in compliance with good laboratory practice (GLP) guidelines. Because these conditions are not always common in standard animal imaging laboratories, the use of imaging for toxicological studies might require separate installations or separate groups of animals. Importantly, in the discovery phases, information from animal studies is crucial for the determination of the proper dose to be tested in the clinical trials; thus, in recent years, some pharmaceutical companies have established their own imaging laboratories.

Clinical evaluation

Once the drug candidate safety has been established for human administration, clinical studies can be launched, providing that authorization by a regulatory agency [e.g. FDA or European Medicines Agency (EMA)] has been given. They are divided into four sequential phases (I–IV), although more recently regulatory agencies approved the so-called 'Phase 0' trials, which are held with a very small group of individuals prior to Phase I, aiming to provide pharmacokinetic data and target interaction in humans. In this phase, very low doses (typically 100 times less than the intended therapeutic dose) of drug candidates are used, without any intended therapeutic purposes, to determine whether the compound has appropriate pharmacokinetic and pharmacodynamic profiles in humans. Often, compounds can be labeled with a suitable radionuclide and image acquisition (PET, for instance) can thus be performed. Potentially, this phase can help in eliminating poor candidate drugs before they reach Phase I, once again contributing to time sparing and cost reduction, thus improving the overall efficiency of the drug development process. In Phase I, the drug is tested in healthy volunteers (typically 20–80) to evaluate pharmacokinetics and safe dose range 4 and 6. In the case of some oncology agents, studies can be performed in patients to verify whether pharmacological effects observed in animals are reproduced in humans. Usually, they are critically ill patients or have a terminal disease and have tried the conventional therapeutic options. Because some of these patients might benefit from the new treatment, sometimes they are included in the trials after careful consideration of the risk:benefit ratio. These studies can result in go–no-go decisions based on parameters such as safety, tolerability and pharmacokinetic and pharmacodynamic properties of the candidate drug. Approximately two-thirds of Phase I compounds will be found safe enough to progress to Phase II, which is carried out to provide a well-controlled and detailed assessment of the drug’s safety and therapeutic efficacy in a larger population. Moreover, dose regimens (i.e. dosing interval, method of delivery: e.g. oral and intravenous) to be administered to the target population in Phase III are tested at Phase II. Determining the optimal dose (i.e. the one inducing the maximum therapeutic effect with minimal side effects) is of the utmost importance. Once again, results from Phase II can lead to go–no-go decisions based on criteria such as drug effectiveness, safety and observed side effects. The introduction of biomarkers in Phase II trials has not only improved the understanding of the biology of disease but also the molecular mechanisms of action of the drug candidate, helping in the identification of patients that are more likely to respond to the new treatment. However, to obtain useful information in a timely fashion, the selection of the biomarker is a crucial aspect. In cancer treatment, for instance, there are a few successful examples regarding the
introduction of biomarkers in Phase II trials: trastuzumab for HER-2-positive breast cancer; and imatinib mesylate for BCR-ABL-positive chronic myelogenous leukemia, for details see.

Phase III trials are usually multicenter, placebo-controlled randomized studies, including several hundred to a thousand subjects. At this stage, the aim is to collect more evidence about drug safety and effectiveness and long-term tolerability. These data are crucial for the approval and registration 4, 6 and 24. Phase IV corresponds to post-marketing studies after the drug has been approved aiming to strengthen data concerning long-term effects of the drug on morbidity and mortality.

The use of imaging in clinical studies, whether only in the phase of proof-of-concept (Phase I) or in subsequent clinical studies, will depend not only on the disease but also on the quality of the imaging biomarker as well as the available resources. If imaging represents an added value for disease diagnosis, shorter studies including fewer patients would be feasible, which would potentially lead to considerable savings as a result of a shorter time-to-market. Although efforts have been made to approve more imaging biomarkers, the FDA's critical path initiative might be considered as an example 25, 26 and 27, currently there is still no imaging biomarker accepted as a surrogate endpoint, because validation has been very challenging. With the joint effort of regulatory agencies, academia and industry, perhaps in the future imaging biomarkers might serve as surrogate endpoints in clinical trials. More importantly, there is an urgent need to reduce the gap between clinical practice and regulatory requirements. The utilization of $^{18}$F-FDG is a good example of such a gap, because it is widely used for tumor response evaluation but it is not approved by the regulatory agencies as a surrogate endpoint for clinical studies. More recently, $^{18}$F-FLT has been used to characterize the transport of nucleosides as a measure of cellular proliferation, providing valuable information about drug efficacy 30 and 31.

Imaging modalities in the drug development process

Over the past decade, imaging has been applied at several stages of drug discovery owing to the tremendous advantages arising from its use. Particularly, in vivo functional imaging (Box 2) provides a unique opportunity for studying disease noninvasively and, in many cases, quantitatively at the molecular level, along with the ability to monitor disease progression or response to treatment efficiently and repeatedly. The introduction of functional imaging in drug development at preclinical and clinical stages allows the shortening of the project length, improving the level of confidence in the results while reducing the inherent costs (increasing the revenues and cost-effectiveness). By contrast, the assessment of some important parameters is only possible through the use of functional imaging. Some examples are the time on target as well as the quantitative biodistribution and the inherent kinetic data, which are important for dosage optimization and dose frequency.
At the preclinical stage, the most commonly used imaging modalities have been PET, single-photon emission computed tomography (SPECT), computed tomography (CT), magnetic resonance imaging (MRI), magnetic resonance spectroscopy imaging (MRSI) and ultrasounds. With the exception of optical imaging, where clinical applications are still modest and not routinely used, all the other techniques have the advantage of being used in the diagnosis and follow-up of human diseases, easing the correlation between preclinical and clinical data (bench-to-bedside model).

The perfect imaging modality (i.e. providing simultaneously high sensitivity, specificity, temporal and spatial resolution) does not exist. In many ways, the existing imaging technologies provide complementary information about the biological processes being studied; the reason why multimodality devices have definitely gained a huge popularity over the past years. In fact, nowadays, it is more and more common in daily practice to use two or more imaging modalities to study a (patho) physiological process adequately in the preclinical and the clinical context. The decision to choose one imaging modality over another will depend on the resources available as well as the type of information needed. In this paper, we provide an overview of the main in vivo imaging modalities that have major impacts on drug development, with a particular emphasis on nuclear modalities, owing to their high sensitivity and temporal resolution, and look out for the state-of-the-art hybrid imaging modalities such as PET/MRI, PET/CT and SPECT/CT/MRI combinations.

Imaging biomarkers

The FDA, EMA and the pharmaceutical industry have already recognized that the use of imaging biomarkers in the future could have a key role in accelerating drug development and lowering costs (namely by eliminating poor candidate drugs earlier in the development process). The number of available molecular imaging and contrast agents is increasing. This trend also applies to those approved by the FDA (http://www.ncbi.nlm.nih.gov/books/NBK5330/). The purpose of using imaging biomarkers early in clinical trials is to gather evidence in support of moving to larger and expensive trials, by demonstrating, for instance, that the drug candidate binds to the intended receptor or crosses the blood–brain barrier.
Imaging biomarkers can be useful in a variety of applications such as a diagnostic tool for a symptomatic disease or as a screening tool to identify a disease not yet symptomatic or even as a follow-up tool to evaluate disease progression. Many biomarkers such as those used in nuclear applications have the advantage of not only providing structural information but also quantitative data concerning organ and/or tumor metabolism and function. Moreover, this kind of biomarker provides unique selectivity and sensitivity to measure drug interactions with specific pathways. As depicted in Fig. 3, 2-deoxy-2-[18F]fluoro-D-glucose (18F-FDG), an analog of the glucose molecule, is a widely used PET imaging biomarker for assessing tumor metabolism. In the same panel, a SPECT tracer (99mTc-exendin-4) was used to determine the efficacy of streptozotocin in insulinoma treatment. Imaging is also useful in areas such as inflammation detection (Fig. 3), in which the efficacy of an anti-inflammatory drug can be evaluated by bioluminescence. The main advantage of molecular imaging biomarkers is the possibility to measure activity at the site of action (e.g. receptor occupancy) in a noninvasive way, allowing longitudinal studies to be performed in a very convenient, practical and cost-effective way.
Figura 3. Examples of some molecular imaging modalities in the characterization of tumor biology (a) and in the evaluation of drug effects over the target (b,c). (a) Tumor heterogeneity depicted by 2-deoxy-2-[18F]fluoro-d-glucose (18F-FDG), a biomarker for clona selection, tumor stem cell and biology-modifier drug studies. Images were acquired in a positron emission tomography/molecular resonance imaging (PET/MRI) scan (NanoScan, Mediso, Hungary), 50 min after the intravenous injection of 18F-FDG (3.5 MBq) in a systemic mouse leukemia model. MRI acquisition was T2-weighted. Both imaging modalities show the presence of a highly complementary pattern of heterogeneous tumor tissue (white arrows). (b,i,ii) Whole body single-photon emission computed tomography and computed (SPECT/CT) images (NanoScan SPECT/CT, Mediso, Hungary) of two male C57Bl6 mice were acquired, after the intravenous injection of 24 MBq of 99mTc-exendin-4, glucagonlike peptide-1 (GLP-1) receptors ligand. On the left, maximum intensity projection is shown and, on the right, a sagittal slice. (bi) Healthy mouse.
Molecular imaging has been applied to drug development aiming to improve disease characterization and early evaluation of drug efficacy and safety, drug pharmacokinetic profile characterization, image drug distribution in the target and assessment of target function. Many compounds are eventually selected for their excellent target binding and pharmacodynamic effects, the possibility to influence selected signal transduction pathways and other biological control processes. However, there are specific traits that allow the prediction of a potential failure in the development process that can be assessed using modern and advanced qualitative and/or quantitative imaging methodologies. Possible examples could be the in vivo imaging of transport over barriers in an organism-wide context and/or tracking known traits of toxicities such as cardio, liver or kidney toxicities.

PET and SPECT

The underlying principle of PET and SPECT modalities resides in the use of tracer amounts of radioactive compounds to produce biodistribution images of the drug candidate and/or the resulting effect on organ or body function. This requires two distinct approaches: in the first case, the drug candidate must be labeled with a suitable radionuclide that does not significantly change the structure of the original molecule; whereas in the second case, the drug candidate is administered and its effect on organ function or tumor size and metabolism can be assessed using molecular imaging biomarkers that accumulate in the organs under study (target organs). PET and SPECT radionuclides have distinct physical properties, mainly related to the type of emission and energy: the first group emits positrons that, after annihilation by electrons, generate two high energy photons (511 keV); the latter group emits one photon per decay, usually with much lower energy (25–250 keV). In both cases, the emitted photons will be detected by scintillation crystals, producing light that will be used to obtain electronic signals. Images are then formed, representing the biodistribution of the radiotracers. In the case of positron emitters, because two high energy photons are originated, PET imaging requires the placement of detectors on opposite sides of the emitting source to detect the coincident photons simultaneously. Currently, PET scanners are composed of detector blocks, assembled in a full ring configuration gantry. The scintillation material of PET detectors is coupled to a photomultiplier tube (or avalanche photodiode) to produce electrical pulses, the amplitude of which is proportional to the energy of the incident photon. The signal is then amplified and analyzed to check whether they fall inside the predefined energy window centered at 511 keV. Moreover, a parallel signal path is propagated from the amplifier to a timing single-channel analyzer on the opposite detector to determine if the detected photons originated from the same annihilation event. If the pulses are detected in
the predefined temporal window (8–12 ns in most cases) and the energy criteria are met, then the event is considered coincident and recorded to form the final image.

Single or multiple images (including whole body) can be acquired without increasing the amount of radiotracer given to the subject or the radiation exposure. Recently, more intercompatible albeit less stable silicon-based sensors have been paving the way for breakthrough future combinations of methodologies. In the field of neuroscience, for instance, measurements of receptor occupancy with radiotracers usually involve the acquisition of a ‘baseline’ [i.e. scanning with an adequate radiotracer, for instance D2 receptor occupancy imaged with $^{11}$C-raclopride or brain metabolism depicted with $^{18}$F-FDG – this scan takes place before treatment with the candidate drug to characterize the basal state and is then repeated during or after one (or several) session(s) of drug administration] PET or SPECT scan and a MRI scan providing anatomic references. Then, the subject is treated with the drug candidate (single- or multi-dose regimen), followed by the acquisition of another PET or SPECT scan, often paralleled by blood sampling to determine plasma pharmacokinetics of the drug, allowing measurement of the displacement of tracer by the drug candidate. The chance to perform these studies in species ranging from mice to non-human primates, prior to the first human administration, makes it possible not only to establish prioritization of which candidate drug will go forward to the clinical studies but also to confirm the dose range and regimen, or even to discontinue a research program if insufficient concentration of the drug accumulates in the desired target 35 and 38, or even if maximal receptor occupancy has been reached but no efficacy is observed. At this stage the kinetics of receptor occupancy by the candidate drug should be fully understood (including maximal occupancy) and compared with drug efficacy observed in preclinical models to establish targets for clinical studies. A common example that is usually given is the study of neurokinin 1 (NK1) receptor occupancy by PET, which provided an understanding about the relationship between the blood levels of aprepitant (an antagonist of the NK1 receptor) and the receptor occupancy. This drug was thought to be effective as an antidepressant and only at Phase III trials was PET introduced to image normal subjects and patients under treatment. Imaging revealed that the aprepitant dosing regimens provided continuously high levels of NK1 receptor blockade but no efficacy.

Another approach that has been explored to a lesser degree so far is to label the drug candidate directly with a suitable radionuclide, collecting information about the pharmacokinetic profile of the drug candidate such as the percentage of the injected dose that accumulates in the organs, its regional distribution and kinetics as well as the relationship with plasma levels. Even though this might be a valuable approach, it could see some challenges related to the feasibility, complexity and optimization of the labeling procedure. Moreover, although the uptake of the drug by the target organ can be imaged and quantified, it might not tell enough about a drug's action on the target site. For example, the antipsychotic drug clozapine was labeled with carbon-11 and its regional brain distribution was imaged by PET. Although a high level of striatal uptake was observed, it was not clear whether the drug bound to serotonin (5-HT2), dopamine (D2) or cholinergic receptors. Later on, through the use of target-specific radioligands, it was possible to study the ability of clozapine to compete with the receptors and, only then, to characterize its pharmacologic profile. In fact,
PET and SPECT have been extensively used to study antipsychotic drugs in the preclinical and clinical context, not only due to the availability of suitable dopamine D2 receptors but also to the high sensitivity of these imaging modalities 44 and 45. It is known that a certain level of occupancy is required to achieve efficacy and that levels that are too high will induce extra-pyramidal side effects. Imaging has played a relevant part in providing data to establish the dose required to maximize efficacy while minimizing side effects. Of paramount importance is the fact that the amount of administered PET or SPECT ligands is far from the pharmacologic doses (high specific activity), allowing safe image acquisition without radiotracer interference on the biological processes under study (because tracer doses are in the range of nano- or micro-grams, presenting similar pharmacokinetic characteristics but in the range of no therapeutic effects, whereas pharmacological doses are in the range of milligrams or grams and associated with pharmacodynamic effects). Additionally, a good molecular imaging ligand must have other properties such as: high affinity for the receptor of interest; ability to reach the equilibrium within the time-frame for the measurement, in order to determine the binding potential; low nonspecific binding, allowing background noise to be kept as low as possible; high selectivity for the target; and a ‘simple-as-possible’ radiochemistry, which allows its use in a multicentric study at reasonable cost. Although not having in vivo microscopic spatial resolution, nuclear imaging has a high sensitivity, detecting less than 10^{-1} to 10^{-12} M of tracer molecules and 10^{-15} M target protein concentrations per mg of imaged tissue (for PET) in vivo. Preclinical SPECT has submillimeter (0.3–0.5 mm) resolution, and preclinical PET has now also entered the submillimeter range (unpublished). The clinical counterparts have about 10–15 mm and 4–5 mm, respectively. Moreover, an each-day wider range of radioactive probes is available for nuclear imaging, allowing quantitative data to be obtained, without depth penetration limitations. Owing to its higher sensitivity, PET imaging might be more accurate for quantification purposes. By contrast, a great advantage of PET is that the most common and relevant biological elements, such as carbon, oxygen, nitrogen and fluorine, have positron emitter isotopes, implicating a much better access to labeled biologically active molecules, because they are easily attached via covalent bonds to an huge number of biochemical and drug structures, leading to a greater versatility in the development of PET biomarkers compared with the application of ^{99m}Tc (attached via bulky chelating linker groups) or ^{123}I (which forms a biologically unstable bond with carbon). In fact, if the radioisotope can directly substitute the stable isotope of a certain molecule, the molecular structure and consequently the chemical properties will be almost identical.

The major advantages of SPECT are the possibility to use and detect a variety of radioactive agents based on radioisotopes with different energies (making it possible to image simultaneously two or more molecular pathways), a relatively simple and stable chemistry allowing the synthesis of ligands on site and their relatively long physical half-lives (ranging from hours to days), making them easily accessible to many research groups and suitable to be used in such investigational contexts where the need for longer periods for data acquisition is verified; for instance, slower kinetic processes of antibody and fragment accumulation 47 and 50.
As mentioned, PET radionuclides are usually isotopes of carbon, fluorine, oxygen and nitrogen occurring abundantly in tissues, but they have shorter physical half-lives (ranging from minutes to hours), ideally requiring an ‘on-site’ solution for radiotracer production. In the case of the most widely available fluorinated compounds (fluorine-18) they are easier to transport as a result of the physical half-life of $^{18}$F (110 min). Although the use of carbon-11 compounds might be compatible with local solutions, it is most frequently limited to laboratories with an on-site cyclotron ($^{11}$C half-life: 20 min) with even harder conditionings applying to nitrogen-13 (half-life: 10 min) and oxygen-15 (half-life: 2 min). Ideally, the physical half-life of the radionuclide should be comparable with the biological half-life of the kinetic process to be quantitated and visualized, meaning that the use of controlled delivery formulations, liposomes, particulate drugs and antibody-based solutions will require longer half-life nuclides for radiolabeling in PET. These cyclotron products could be zirconium-89 and iodine-124 (and to a lesser extent technetium-94 m) where one of the main advantages of PET concerning biogenic isotopes is lost but better quantitation and kinetic modeling characteristics are maintained. Table 1 summarizes the main radionuclides used in PET and SPECT imaging. Specific activity is a very important parameter, corresponding to the proportion of radioactive (probe) atoms to the whole amount of the atoms of the element applied for radiolabeling the test compound or biomarker molecule. Therefore, the specific activity of the radiolabeled molecule (final compound) will be determined not only by the specific activity of the element but also by the labeling efficiency of the molecule, which is indeed a key issue for accurate measurements. As shown in Table 1, SPECT radionuclides have greater specific activity compared with PET tracers. Higher specific activity probes are essential to obtain as low as possible injected test compound masses, to ensure pharmacodynamic inertness of the imaging process.
Other imaging modalities

Other imaging modalities can complement data from nuclear techniques. Morphologic techniques such as CT and MRI have been particularly useful owing to the lack of anatomic detail of nuclear modalities (Fig. 2). Computed tomography is a morphological imaging technique that measures differences in tissue density, being particularly useful for the study of bone structures and lung imaging. A typical CT system consists in an X-ray tube and an X-ray detector assembled in opposite positions in a rotating gantry. X-rays traversing the subject deposit energy in an inverse proportion to the electron density of the body tissue; the remaining energy is detected by the detector. The main advantage of CT is the high spatial resolution (30–100 μm for in vivo preclinical devices and 400–600 μm in high performance clinical scanners). Besides having low sensitivity, the main drawback of CT is related to radiation burden for the subjects involved in the procedures, even more crucial when longitudinal studies will be conducted. CT has an established role in the assessment of tumor response to cytotoxic therapies. The size of tumors is measured using either the sum of the perpendicular diameters or the sum of the largest diameters [response evaluation criteria in solid tumors (RECIST)], providing a morphologic evaluation of tumor growth and response to therapy. Additionally, using contrast agents, it might be possible to obtain some extra information related to tumor perfusion.

MRI relies on magnetic properties of tissues and their interactions with strong external magnetic fields. Hydrogen nuclei (1H) from water are the most used in MRI, owing to their paramagnetic properties and abundance in the body. Briefly, the underlying principle is that, when a sample within a magnetic field is
subjected to a radio-frequency pulse, protons absorb energy and generate a detectable signal during the relaxation phase which can be digitally encoded through magnetic field gradients to generate digital images. MRI is a very versatile imaging modality, providing morphological images with excellent contrast and spatial resolution (<50 μm for preclinical devices and 300 μm in ultra-high field experimental clinical devices), as well as information regarding tissue composition, perfusion, oxygenation, tissue elasticity, metabolism and detection of molecular probes within a single acquisition session without radiation exposure. One of these MRI modalities is designated as functional MRI (fMRI) or blood oxygen level dependent (BOLD) imaging, based on the distinct magnetic properties of oxyhemoglobin and deoxyhemoglobin, which might be considered particularly useful for brain activation studies. Additional parameters such as brain or tumor perfusion and vascular permeability can be assessed using dynamic contrast enhanced imaging (DCE-MRI), where an intravenous bolus injection of a contrast agent is detected during its first passage through the organs or the arterial spin labeling (ASL) in which the arterial blood water magnetization itself functions as an endogenous contrast agent. Nowadays, DCE-MRI is the most commonly used modality for the preclinical and early clinical evaluation of antiangiogenic agents. Some examples are Avastin® (bevacizumab, Roche), Nexavar® (sorafetinib, Pfizer), Sutent® (sunitib, Pfizer).

Magnetic resonance spectroscopy (MRS) provides information about the concentration of certain chemicals in the organs and so far has been useful in brain studies for the quantification of N-acetyl aspartate, choline, creatine, myo-inositol, lactate, lipids, glutamine, glutamate and amino acids. However, compared with PET, MRI has a lower sensitivity (three to six orders of magnitude), requiring an amount of $10^{-3}$ to $10^{-5}$ M of imaging probe to be detected within a single voxel. The higher sensitivity of nuclear techniques makes them a unique approach when low density binding sites are the drug targets.

OI includes a variety of techniques based on the use of a set of light sources and very sensitive detecting devices that capture the photons transmitted through tissues. To improve image contrast, targeted fluorescent or activatable probes were developed, making it possible to measure the activity of the chosen molecular targets. Imaging of such probes involves excitation at a certain wavelength and the detection of the specific signal emission at a significantly different wavelength. The most relevant OI techniques are bioluminescence, fluorescence and near infrared fluorescence imaging. In the context of fluorescence imaging, quantum dots (i.e. inorganic fluorescent semiconductor nanoparticles) are becoming increasingly popular. Overall, the most important advantages of OI are the high sensitivity, low cost, relatively high throughput and short acquisition time (typically 10–60 s), as well as the visualization of physiological and pathophysiological processes at the cellular and molecular level in vivo with high specificity. An additional advantage of OI is the fact that several probes with different spectral characteristics can be used for multichannel imaging. The main drawbacks of OI include: the limited transmission of light from tissues, which limits spatial resolution and the depth of imaging, resulting in a very limited signal quantification. Moreover, so far, OI has limited clinical application, but in the preclinical arena OI has been very useful in the detection and functional characterization of tumors through the application of fluorescence enzyme-mediated probes that bind to proteases that are expressed by tumors (cathepsin, matrix metalloproteinases 2 and 7 and caspase 1), as well as in the detection of infection and inflammation ( Fig. 3).
The intrinsic limitations of each imaging modality (Table 2) have led to the idea of combining two or more modalities to image the same biological process in the same animal. Multimodality imaging provides complementary information upon the subject and/or pathophysiological process being studied. The most common are the combinations between high sensitivity and functional modalities, such as PET and SPECT, with high spatial resolution and morphological techniques, such as CT and MRI. The rationale underlying the preference for multimodal imaging using one or several types of devices (either single or dual-modality: PET/CT, SPECT/CT and PET/MRI the most used by order of relevance, or tri-modality: PET/SPECT/CT the most popular configuration, or even four-modality devices that are under study by some manufacturers) is multifactorial, and should consider the relevant issues for each specific case. In fact, there are pros and cons for each option and, if no single imaging modality can fulfill the research needs of drug development industry entirely, the access to multimodality might be obtained directly by hybrid imaging—when the same device uses distinct imaging modalities—and/or co-registration with other imaging modalities. Compact benchtop systems might be considered better, essentially because of their practical physical aspects (i.e. can be placed almost anywhere in a research laboratory) but also because of their increased easiness of use (normally presenting lower complexity that might allow newcomers to the field of imaging to be quickly autonomous in their operation) and higher cost:efficiency ratios (owing to the integration process); nevertheless, the final throughput might not be the most favorable (and this might be a very relevant issue when the number of subjects to be evaluated is considerable). In fact, multimodality imaging has been gaining notable popularity in the preclinical and in the clinical context. An increasing number of authors defend that, in the (near) future, multimodality imaging will be the prevailing concept of in vivo imaging 13, 63 and 64.
<table>
<thead>
<tr>
<th>Imaging modality</th>
<th>Sensitivity</th>
<th>Relative cost</th>
<th>Type of probe</th>
<th>Major advantages</th>
<th>Major limitations</th>
<th>Major applications in drug development</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPECT</td>
<td>pM</td>
<td>0.4–0.6</td>
<td>Molecules labeled with low energy gamma emitters</td>
<td>- Longer physical half-lives</td>
<td>- Low sensitivity than PET</td>
<td>- Pharmacokinetics and pharmacodynamics</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Multiple radionuclides can be detected simultaneously</td>
<td>- Relatively low spatial resolution of clinical devices</td>
<td>- Receptor-occupancy studies</td>
</tr>
<tr>
<td>PET</td>
<td>pM</td>
<td>0.8–1.0</td>
<td>Molecules labeled with positron emitters</td>
<td>- High sensitivity</td>
<td>- Short-lived radionuclides</td>
<td>- Pharmacokinetics and pharmacodynamics</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Accurate quantification; diversity of biological probes available</td>
<td>- Probe availability</td>
<td>- Receptor-occupancy studies</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Relatively expensive equipment and overall procedure</td>
<td>- Cellular metabolism</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Genes expression</td>
</tr>
<tr>
<td>CT</td>
<td>nm</td>
<td>0.2–0.4</td>
<td>Radiopaque contrast agents</td>
<td>- High spatial resolution, particularly for lung and bone imaging</td>
<td>- Poor soft tissue contrast</td>
<td>- Phenotyping</td>
</tr>
</tbody>
</table>
The decision for an imaging modality should be done taking into consideration firstly the question to be addressed and secondly the material conditions available, as well as possible logistic limitations relevant for the experimental procedures. Although the challenge of data translation from animals to humans is indeed a huge one, it can be more easily done if the chosen modalities (and respective imaging probes) for small animal imaging have clinical homology, thus reducing the gap between preclinical and clinical studies. Nuclear modalities (PET or SPECT), through the use of the radiolabeled form of the drug candidate at an early stage, provide valuable information about drug pharmacokinetics in a small group of rodents. Usually, the same studies are then held in a small group of humans and, if results between animal and human studies are similar, then the drug candidate is tested in larger populations of animals and finally in more humans. This demonstrates how imaging techniques and particularly nuclear modalities fill the gap between preclinical and clinical research. The requisites for imaging methods in translational medicine are well defined: the techniques must be quantitative, reproducible, specific, sensitive, applicable to clinical practice and safe. PET is the imaging modality that best meets all these requirements. Although SPECT has lower sensitivity and is based on less physiological probes, it might as well represent an interesting possibility, namely when the physiological process under study requires the use of radionuclides with longer half-lives.

Examples of applications

As mentioned in the previous sections, molecular imaging can play a part in multiple steps of drug development (i.e. disease characterization to drug target validation as well as the characterization of drug pharmacokinetic and pharmacodynamic profiles). The first key step in the drug development process is target selection; the role of molecular imaging here is to provide information about the presence of specific proteins or biomarkers associated with the disease.
targets, their spatial and temporal distribution, as well as the effect resulting from the interaction between the drug and the target. One of the main challenges at this stage is to develop specific probes and amplification strategies to distinguish target signal from non-target (background) signal, which might be tricky for low target (subnanomolar) concentrations. There are some examples available in the literature describing the successful use of imaging in drug development, particularly in the fields of neurology and oncology. Nuclear techniques have long been used for the study of receptor expression and occupancy in central nervous system drug development 7 and 10. A significant number of receptor ligands have been labeled with $^{11}$C, $^{18}$F and $^{123}$I for the study of several receptor systems, especially dopaminergic and serotonergic ones. Other target-specific probes have also been developed such as the near infrared fluorescent dye Cy5.5 and magnetic contrast agents such as iron oxide. Microbubbles that bind to vascular endothelial growth factor receptor 2 (VEGFR2) and αβ3 integrin have been used in US imaging for tumor angiogenesis evaluation.

Paclitaxel (a chemotherapeutic agent that inhibits mitosis) was labeled with $^{18}$F to characterize its pharmacokinetics and, because it is a substrate for the P-glycoprotein (Pgp) efflux pump, the dynamics of the pump and the consequences of multidrug resistance were also accessed. Moreover, through the use of imaging it was possible to understand that there are significant differences between brain and plasma kinetics. Gefvert et al. used $^{11}$C-raclopride and $^{11}$C-N-methyl-sipiperone as ligands to show that the antipsychotic drug, quetiapine, despite its short half-life in plasma (2.5–5.0 h), has much a longer half-life in terms of receptor occupancy (10 h), which allowed changing the treatment regimen based on two-times a day dosing instead of three times (thought to be optimal based on plasma level data).

The action of vortioxetine was assessed using $^{11}$C-N,N-dimethyl-2-(2-amino-4-methylphenylthio)benzylamine ($^{11}$C-MADAM) and $^{11}$C-WAY100635 for the quantification of 5-HTT and 5-HT$_{1A}$ occupancy, respectively. Vortioxetine is a recently approved (September 2013) antidepressant for the treatment of major depressive disorders, acting by a combination of two mechanisms: receptor activity modulations and reuptake inhibition. Table 3 shows some examples of recently approved drugs where imaging was used during development phases.
In the field of oncology, owing to its superior sensitivity, 18F-FDG has been the classical agent for glycolytic metabolism evaluation, because tumor uptake reflects the level of glucose transporter 1 (GLUT1) expression and the enzymatic activity of hexokinase. During the approval process, all patients with gastrointestinal stromal tumors (GIST) treated with imatinib (Gleevec®) who responded to anticancer therapy exhibited metabolic changes that preceded, by weeks or months, a significant decrease in tumor size seen on CT [71].

Concluding remarks

Over the past decade, molecular imaging has gained increasing acceptance by the academic and industry communities regarding drug discovery and development. It has been useful in the acquisition of a deeper knowledge of biology regarding a variety of diseases, allowing the design of better candidate drugs in the identification and validation of new therapeutic targets, in the selection of candidate drugs that must move forward to the clinic or those where research should be stopped and in the establishment of dose range and regimens. Imaging has brought modern drug development to a superior level, not only by providing a deeper understanding of normal physiology, molecular mechanisms of disease and drug effects but also by allowing significant savings (in terms of time and resources). Molecular imaging in particular has driven not only the development of safer and innovative drugs but also strengthens the concept of personalized medicine.
References

1. K.I. Kaitin, J.A. DiMasi
2. J.A. DiMasi, H.G. Grabowski
The cost of biopharmaceutical R&D: is biotech different?
3. P. Preziosi
Science, pharmacoeconomics and ethics in drug R&D: a sustainable future scenario?
In vivo mouse imaging and spectroscopy in drug discovery
5. X. Qiu, S.S. Abdel-Meguid
Protein crystallography in structure-based drug design
A. Makriyannis, D. Biegel (Eds.), Drug Discovery Strategies and Methods, Marcel Dekker (2004), pp. 1–22
Integrated drug product development—from lead candidate selection to life-cycle management
PET and drug research and development
8. M. Rudin, R. Weissleder
Molecular imaging in drug discovery and development
Molecular-genetic imaging based on reporter gene expression
10. Y. Waerzeggers, et al.
Multimodality imaging: overview of imaging techniques (CT, MRI, PET, optical imaging) and impact of multimodality imaging on drug development
Drug discovery in the next millennium
Molecular imaging in drug development
Small-animal SPECT and SPECT/CT: important tools for preclinical investigation
14. J.A. DiMasi
The value of improving the productivity of the drug development process: faster times and better decisions
Pharmacoconomics, 20 (Suppl. 3) (2002), pp. 1–10
15. R.S. Uppoor, et al.
The use of imaging in the early development of neuropharmacological drugs: a survey of approved NDAs
Positron emission tomography molecular imaging for drug development
17. E. Ashton
Can medical image analysis change the economics of drug development?
Pharm. Discov. (2005) Available at:
http://www.spectroscopyonline.com/spectroscopy/article/articleDetail.jsp?id=180274&sk=&date=&pageID=3
18. FDA
The Center for Drug Evaluation and Research Handbook

19. X. Zhang, et al.
Application of microPET imaging approaches in the study of pediatric anesthetic-induced neuronal toxicity
Compressing drug development timelines in oncology using phase ‘0’ trials
21. S. Marchetti, J.H. Schellens
The impact of FDA and EMEA guidelines on drug development in relation to Phase 0 trials

22. N. Rick
Clinical trials
Drugs: From Discovery to Approval Wiley–Blackwell (2009), pp. 176–207

23. L.M. McShane, et al.
Effective incorporation of biomarkers into Phase II trials

The contemporary drug development process: advances and challenges in preclinical and clinical development

25. D.C. Anderson, K. Kodukula
Biomarkers in pharmacology and drug discovery

26. W.S. Richter
Imaging biomarkers as surrogate endpoints for drug development

27. J. Woodcock, R. Woosley
The FDA critical path initiative and its influence on new drug development
Annu. Rev. Med., 59 (2008), pp. 1–12

Use of biomarkers and surrogate endpoints in drug development and regulatory decision making: criteria, validation, strategies

29. L.W. Goldman
Principles of CT: radiation dose and image quality

30. O.S. Tehrani, A.F. Shields
PET imaging of proliferation with pyrimidines

18FFLT: an imaging biomarker of tumour proliferation for assessment of tumour response to treatment
32. J.R. Allport, R. Weissleder
In vivo imaging of gene and cell therapies

33. M.S. Berridge, et al.
Imaging studies of biodistribution and kinetics in drug development

34. G.C. Kagadis, et al.
In vivo small animal imaging: current status and future prospects

35. R.P. Maguire
Molecular imaging biomarkers as a tool in development of novel medicines

36. R.D. Hichwa, J.R. Halama
Principles of PET and PET/CT imaging

37. A. Rahmim, H. Zaidi
PET versus SPECT: strengths, limitations and challenges

38. B. Andree, et al.
Positron emission tomographic analysis of dose-dependent NAD-299 binding to 5-hydroxytryptamine-1A receptors in the human brain
Psychopharmacology (Berl.), 167 (2003), pp. 37–45

Lack of efficacy of the substance p (neurokinin1 receptor) antagonist aprepitant in the treatment of major depressive disorder

40. P. Herrero, et al.
Assessment of myocardial blood flow using 15 O-water and 1-11 C-acetate in rats with small-animal PET

41. S. Kapur
Neuroimaging and drug development: an algorithm for decision making

42. T. Lundberg, et al.
Specific binding of 11C-clozapine to frontal cortex and striatum in drug-free schizophrenics and healthy controls studied by positron emission tomography
D1, D2, and 5-HT2 receptor occupancy in relation to clozapine serum concentration: a PET study of schizophrenic patients

44. P.D. Acton, et al.
Occupancy of dopamine D2 receptors in the mouse brain measured using ultra-high-resolution single-photon emission tomography and 123 IBF

PET tracers for imaging of the dopaminergic system

46. L. Farde, et al.
Positron emission tomographic analysis of central D1 and D2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine. Relation to extrapyramidal side effects

47. S.R. Meikle, et al.
Small animal SPECT and its place in the matrix of molecular imaging technologies

48. D.L. Bailey, K.P. Willowson
An evidence-based review of quantitative SPECT imaging and potential clinical applications

49. S.M. Moerlein
Radiopharmaceuticals for positron emission tomography
R.J. Kowalsky, S.W. Falen (Eds.), Radiopharmaceuticals in Nuclear Pharmacy and Nuclear Medicine, American Pharmacists Association (2004), pp. 337–339

50. L. Fass
Imaging and cancer: a review
Mol. Oncol., 2 (2008), pp. 115–152

X-ray and X-ray CT

52. P. Therasse, et al.
New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada
Advances in multimodal neuroimaging: hybrid MR-PET and MR-PET-EEG at 3T and 9.4T
Imaging devices for use in small animals
55. A. Leroy-Willig, G. Geldwerth-Feniger
Nuclear magnetic resonance imaging and spectroscopy
56. J.P. O’Connor, et al.
DCE-MRI biomarkers in the clinical evaluation of antiangiogenic and vascular disrupting agents
57. W.C. Eckelman
The use of positron emission tomography in drug discovery and development
58. C. Bremer, et al.
Optical-based molecular imaging: contrast agents and potential medical applications
59. E.R. McVeigh
Emerging imaging techniques
60. T.F. Massoud, S.S. Gambhir
Molecular imaging in living subjects: seeing fundamental biological processes in a new light
Genes Dev., 17 (2003), pp. 545–580
61. V. Ntziachristos
Tumor imaging
62. J. Comley
In vivo preclinical imaging: an essential tool in translational research
63. E.D. Agdeppa, M.E. Spilker
A review of imaging agent development
AAPS J., 11 (2009), pp. 286–299

64. AIPES
State-of-the-art Nuclear Medicine: SPECT and SPECT/CT imaging—an introduction to mainstay diagnostic testing in non-invasive medicine

65. A. Kristian, O. Alstrup
Small animal imaging for accelerating drug development, and comparison to other techniques

Molecular imaging in drug discovery and development: potential and limitations of nonnuclear methods

Dual-targeted contrast agent for US assessment of tumor angiogenesis in vivo
Radiology, 248 (2008), pp. 936–944

68. K.A. Kurdziel, et al.
Biodistribution, radiation dose estimates, and in vivo Pgp modulation studies of 18 F-paclitaxel in nonhuman primates

69. O. Gefvert, et al.
Time course of central nervous dopamine-D2 and 5-HT2 receptor blockade and plasma drug concentrations after discontinuation of quetiapine (Seroquel) in patients with schizophrenia
Psychopharmacology (Berl.), 135 (1998), pp. 119–126

70. P. Stenkrona, et al.
5-HTT and 5-HT1 A receptor occupancy of the novel substance vortioxetine (Lu AA21004). A PET study in control subjects

71. A.D. Van den Abbeele
The lessons of GIST—PET and PET/CT: a new paradigm for imaging
Oncologist, 13 (Suppl. 2) (2008), pp. 8–13

72. B. Tavitian, et al.
In vivo radiotracer imaging

73. J.P. Holland, et al.
Standardized methods for the production of high specific-activity zirconium-89
Technetium-94 m-teboroxime: synthesis, dosimetry and initial PET imaging studies

75. E.J. Knust, et al.
Preparation of 124I solutions after thermodistillation of irradiated 124 TeO2 targets

76. C.L. Yasuda, et al.
The effect of topiramate on cognitive fMRI

77. C.F. Muccio, et al.
Heat-stroke in an epileptic patient treated by topiramate: follow-up by magnetic resonance imaging including diffusion-weighted imaging with apparent diffusion coefficient measure

78. J.P. Szafarski, J.B. Allendorfer
Topiramate and its effect on fMRI of language in patients with right or left temporal lobe epilepsy
Epilepsy Behav., 24 (2012), pp. 74–80

79. K.K. Roggin, M.C. Posner
Modern treatment of gastric gastrointestinal stromal tumors
World J. Gastroenterol., 18 (2012), pp. 6720–6728

FDG PET and FDG PET/CT in patients with gastrointestinal stromal tumours

81. D. Scheller, et al.
Rotigotine treatment partially protects from MFTP toxicity in a progressive macaque model of Parkinson's disease

82. J.L. Glade Bender, et al.
Phase I pharmacokinetic and pharmacodynamic study of pazopanib in children with soft tissue sarcoma and other refractory solid tumors: a children's oncology group Phase I consortium report

83. F.G. Blankenberg, et al.
Noninvasive assessment of tumor VEGF receptors in response to treatment with pazopanib: a molecular imaging study
Transl. Oncol., 3 (2010), pp. 56–64

84. E.A. Punnoose, et al.
Evaluation of circulating tumor cells and circulating tumor DNA in non-small cell lung cancer: association with clinical endpoints in a Phase II clinical trial of pertuzumab and erlotinib


85. I. Vaneycken, et al.

Preclinical screening of anti-HER2 nanobodies for molecular imaging of breast cancer


86. L.F.M. Van Zupten

Introduction