Imagine seeing a specific molecular target in a live animal, following a drug’s distribution in the same animal and quantitating the drug’s direct effect on the target, all in a matter of minutes. Although this prospect might have seemed utopian a few years ago, enabling technologies, such as novel imaging modalities and molecular probes, are being developed at a rapid pace and should allow these questions to be addressed routinely in the not-too-distant future. The widespread availability of mouse imaging systems is not as far off as many researchers might think. These systems (TABLE 1) are generally cheaper than their clinical counterparts and can be housed in basic science laboratories (see REF. 1 for a review). Given the increasingly common use of mouse models of disease to validate potential drug targets, to assess therapeutic efficacy and to identify and validate biomarkers of drug efficacy and/or safety, the ability to image mouse models non-invasively would have far-reaching applications in drug discovery and development.

**MAGNETIC RESONANCE IMAGING** (MRI). A powerful diagnostic imaging method that uses radio waves in the presence of a magnetic field to extract information from certain atomic nuclei (most commonly hydrogen). It is primarily used for producing anatomical images, but also gives information on the physico-chemical state of tissues, flow, diffusion, motion and, more recently, molecular targets.

**MOLECULAR IMAGING IN DRUG DISCOVERY AND DEVELOPMENT**

Markus Rudin* and Ralph Weissleder‡

Imaging sciences have grown exponentially during the past three decades, and many techniques, such as magnetic resonance imaging, nuclear tomographic imaging and X-ray computed tomography, have become indispensable in clinical use. Advances in imaging technologies and imaging probes for humans and for small animals are now extending the applications of imaging further into drug discovery and development, and have the potential to considerably accelerate the process. This review summarizes some of the recent developments in conventional and molecular imaging, and highlights their impact on drug discovery.

It has become possible to image specific molecules and targets, a field that is often referred to as molecular imaging. Indeed, molecular imaging can be used to either image the administered drug directly (for example, following its distribution and target binding) or the target itself (for example, receptor expression and modulation of downstream targets). For the newer molecular imaging tools to be useful, two prerequisites must become available: first, they must have the high sensitivity that is required to monitor interactions at a molecular level and also have sufficiently high spatial resolution to image mouse models of human disease; and second, more target-specific molecular probes must become available. The goal of this paper is to briefly review the available imaging modalities, highlight some uses of anatomical and functional imaging and then focus on exciting advances in molecular imaging (FIG. 1) and how they will affect drug discovery and development. For more in-depth reading on specific imaging modalities and diseases, the reader is referred to several recent review articles1–7.

**Imaging modalities**

Imaging technologies exploit the interaction of various forms of energy with tissues to non-invasively visualize the body. Some technologies, such as magnetic resonance imaging (MRI) and X-ray computed tomography (CT), rely solely on energy–tissue interactions, whereas others, such as positron emission tomography (PET), require the
administration of imaging agents or reporter probes (which can be targeted to specific cells or receptors; see below).

The choice of imaging modality in drug development depends primarily on the specific question to be addressed and different imaging techniques are, in general, complementary rather than competitive. The versatility of MRI has made it a widely used tool in pharmaceutical research. Owing to its excellent soft-tissue contrast properties, MRI allows for the sensitive detection of soft-tissue pathologies and, in addition, yields valuable physiological information. Today, MRI has evolved to be the imaging modality of choice for studies of the central nervous system, such as stroke, neurodegenerative disorders and multiple sclerosis, and provides qualitative diagnostic and quantitative morphometric information, as well as functional/physiological readouts (FIG. 2). The technique is also widely used to diagnose and stage visceral pathologies (for example, neoplastic structures and cardiovascular diseases) or musculoskeletal diseases (for example, rheumatoid arthritis). CT is the classical anatomical imaging modality and is particularly suited for the study of skeletal structures and of the lung. Nuclear imaging techniques such as PET offer the sensitivity required to study small animals, such as mice, other rodents and primates and can ultimately be used in clinical trials. From the perspective of drug development, such tools will be highly valuable. In the following sections, we consider the integration of non-invasive imaging methods into modern drug discovery and development (FIG. 1).

**Structural and functional imaging**

**Characterizing disease models and evaluating efficacy.** High-resolution structural and functional imaging has become increasingly important in drug development, both experimentally and clinically. Its main advantages over other biomarkers (for example, tissue sampling, excision and fluid analysis) are the direct visualization

### Table 1 | Overview of high-resolution, small-animal imaging systems

<table>
<thead>
<tr>
<th>Technique</th>
<th>Resolution</th>
<th>Depth</th>
<th>Time</th>
<th>Imaging agents</th>
<th>Target*</th>
<th>Cost†</th>
<th>Primary small-animal use</th>
<th>Clinical use</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR</td>
<td>10–100 µm</td>
<td>No limit</td>
<td>Minutes–hours</td>
<td>Gadolinium, dysprosium, iron oxide particles</td>
<td>A, P, M</td>
<td>$$$</td>
<td>Versatile imaging modality with high soft-tissue contrast</td>
<td>Yes</td>
</tr>
<tr>
<td>CT</td>
<td>50 µm</td>
<td>No limit</td>
<td>Minutes</td>
<td>Iodine</td>
<td>A, P</td>
<td>$$</td>
<td>Lung imaging</td>
<td>Yes</td>
</tr>
<tr>
<td>Ultrasound</td>
<td>50 µm</td>
<td>Millimetres</td>
<td>Minutes</td>
<td>Microbubbles</td>
<td>A, P</td>
<td>$$</td>
<td>Vascular and interventional imaging</td>
<td>Yes</td>
</tr>
<tr>
<td>PET</td>
<td>1–2 mm</td>
<td>No limit</td>
<td>Minutes</td>
<td>$^{18}F, ^{11}C, ^{15}O</td>
<td>P, M</td>
<td>$$$</td>
<td>Versatile imaging modality with many different tracers</td>
<td>Yes</td>
</tr>
<tr>
<td>SPECT</td>
<td>1–2 mm</td>
<td>No limit</td>
<td>Minutes</td>
<td>$^{99m}Tc, ^{11}In chelates</td>
<td>P, M</td>
<td>$$</td>
<td>Commonly used to image labelled antibodies, peptides and so on</td>
<td>Yes</td>
</tr>
<tr>
<td>FRI</td>
<td>2–3 mm</td>
<td>&lt;1 cm</td>
<td>Seconds–minutes</td>
<td>Photoproteins (GFP), NIR fluorochromes</td>
<td>P, M</td>
<td>$</td>
<td>Rapid screening of molecular events in surface-based tumours</td>
<td>Development</td>
</tr>
<tr>
<td>FMT</td>
<td>1 mm</td>
<td>&lt;10 cm</td>
<td>Seconds–minutes</td>
<td>NIR fluorochromes</td>
<td>P, M</td>
<td>$$</td>
<td>Quantitative imaging of targeted or ‘smart’ fluorochrome reporters in deep tumours</td>
<td>Development</td>
</tr>
<tr>
<td>BLI</td>
<td>Several millimetres</td>
<td>Centimetres</td>
<td>Minutes</td>
<td>Luciferins</td>
<td>M</td>
<td>$$</td>
<td>Gene expression, cell and bacterial tracking</td>
<td>No</td>
</tr>
<tr>
<td>Intravital microscopy (confocal, multiphoton)</td>
<td>1 µm</td>
<td>&lt;400 µm</td>
<td>Seconds–minutes</td>
<td>Photoprobes (GFP), Fluorochromes</td>
<td>P, M</td>
<td>$$$</td>
<td>All of the above at higher resolutions but at limited depths and coverage</td>
<td>Limited development (skin)</td>
</tr>
</tbody>
</table>

*Primary area that a given imaging modality interrogates: A, anatomical; M, molecular; P, physiological. †Cost of system: $<100,000; $$100–300,000; $$$: >300,000.

BLI, bioluminescence imaging; CT, X-ray computed tomography; FMT, fluorescence-mediated molecular tomography; FRI, fluorescence reflectance imaging; GFP, green fluorescent protein; NIR, near-infrared; MR, magnetic resonance; PET, positron emission tomography; SPECT, single-photon emission computed tomography.
of disease processes, the ability to quantify changes over time and the non-invasive nature of the tests. As an example, FIG. 2 shows how MR can be used to image the development of pathology in a rodent model of human embolic stroke. MR techniques allow the precise localization of the site of vascular occlusion, the quantification of the ensuing perfusion and the oxygenation deficits, leading to energy failure, membrane breakdown and cytotoxic edema. Later steps in the pathophysiological cascade include the breakdown of the blood-brain barrier, the formation of vasogenic edema and the infiltration of inflammatory cells, all of which are detectable by MRI. The efficacy of cytoprotective therapy has been commonly assessed using structural readouts, that is, using estimates of infarct volumes coupled with the assumption that structural damage is a surrogate for clinical outcome. More recent data, however, indicate that structural integrity (that is, normal appearance in anatomical images) is a necessary but not sufficient criterion for functional integrity as revealed by functional MR imaging (fMRI) studies of brain function. Both anatomical and functional read-outs have therefore become established in research to determine the efficacy of newer thrombolytic and cytoprotective therapies. For clinical applications, however, some of these surrogate markers of drug development have not yet been accepted by the US FDA. Similar structural and functional imaging approaches have been used to determine the efficacy of anti-angiogenic therapies, anti-inflammatory treatments, apoptosis-inducing agents and many other areas of drug action.

Labelling the drug

**Imaging biodistribution and pharmacokinetics.** Although the biodistribution and pharmacokinetics of new agents in rodents are still commonly measured by blood and tissue sampling or autoradiography, nuclear imaging techniques have gained in importance. Nuclear techniques — in particular, quantitative PET imaging — can now be carried out in small rodents and are routinely used in canine and primate models, as well as being used clinically. A particularly exciting aspect of PET is the fact that many drugs can be labelled with 11C or with 18F (REFS 7,29–34), which means that labelling only minimally affects, if at all, the chemical/physicochemical properties of the compound, allowing the monitoring of the drug biodistribution. As an example, a study showing the distribution of fluconazole, a fluorene-containing anti-fungal agent, is shown in FIG. 3 (REF. 35). PET imaging was used to obtain detailed quantitative information on fluconazole kinetics and dynamics in various tissues, including the human brain. Another approach frequently used in PET imaging is to analyse the inhibition of specific binding of a well-characterized PET radioligand by an unlabelled drug.

**Labelling the target**

**Imaging target distribution and function.** Advances in genomic, proteomic and chemical sciences have accelerated the development of ever-more-precise therapeutics aimed at specific molecular targets associated with disease. Examples of such therapies include inhibitors of specific kinases (for example, Glivec/Gleevec, which targets the BCR-ABL receptor tyrosine kinase), receptors or proteinases. Ideally, we would like to monitor these directed therapies by visualizing the intended drug target and then image the functional consequences of drug-target interactions in live animals and, ultimately, in patients. Specifically, we would like to know whether a putative drug reaches the target, whether it affects target expression and/or function (up- or down-regulation, activation or inactivation) and, ultimately, whether the drug has a disease-modifying effect. Such information can potentially be provided by molecular imaging techniques. The central challenge for molecular imaging is to develop specific reporter probes and amplification strategies to differentiate target information from non-specific background noise so as to be able to cope with low (sub-nanomolar) target concentrations.

The design of molecular reporter probes is variable, but typically involves either ‘targeted agents’ or ‘activatable agents’. Targeted agents are essentially small molecules, peptides, metabolites, aptamers, antibodies or other molecules labelled with a reporter moiety that can be detected by a given imaging modality (for example, 11C- and 18F-labelled PET ligands, 111In- or 99mTc-labelled ligands, fluorochrome-labelled ligands and magnetic ligands). In vivo
visualization of a target requires specific enrichment of the reporter probe at the target site, that is, we have to wait until the non-bound fraction of the reporter probe is eliminated to minimize the background signal. Many types of targeted imaging agent have been developed for different imaging techniques (BOX 1).

During the past several years, there has been an increasing interest in PET imaging as a tool in central nervous system drug discovery and development\textsuperscript{30,45–53}. This has been primarily due to a growing list of neuroreceptor-specific PET tracers, improvements in PET camera resolution, the availability of small-animal PET cameras\textsuperscript{25} and improved communication between academia and drug companies. A significant number of small-molecule receptor ligands have been labelled with \(^{11}\text{C}\) and \(^{18}\text{F}\), and some of these ligands readily cross the blood–brain barrier and bind to their intended targets. In particular, the dopamine and serotonin (5-HT) receptor systems have been investigated\textsuperscript{32,50,54}. Using such specific ligands, PET studies have provided information on the visualization of a target requires specific enrichment of the reporter probe at the target site, that is, we have to wait until the non-bound fraction of the reporter probe is eliminated to minimize the background signal. Many types of targeted imaging agent have been developed for different imaging techniques (BOX 1).

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amount of a therapeutic drug that gets into the brain, the minimum effective dose, the duration of action or the binding-site occupancy required to elicit a particular therapeutic or behavioural effect. Fig. 3 shows one example of how imaging of the 5-HT₂ receptors in the human brain can be used to evaluate the effect of an oral dose of the antipsychotic agent ziprasidone within 4 h of administration.21,32

Activatable, or smart, probes are fundamentally different from targeted probes in that they undergo chemical or physicochemical changes on target interaction and, as such, have a built-in amplification strategy. Examples of activatable imaging agents include caged near-infrared fluorochromes (NIRF)41, paramagnetic agents that change spin-lattice relaxivity on activation or superparamagnetic sensors.42 One example of a pro tease activatable agent is shown in Fig. 4, in which matrix metalloproteinase (MMP) expression in tumours is visualized using an activatable NIRF agent.43 Importantly, the efficacy of the model MMP2 inhibitor AG3340 (prinomastat) could be imaged directly after the initiation of drug therapy, and the dose could be tailored accordingly.44 Activatable NIRF agents have now been developed for several proteases (Box 1), and the number of available imaging agents is continuously growing. A recently established NIH database — the Molecular Imaging (MOLI) database (see online link) — links the rapidly growing number of imaging agents to the respective targets.

Targeted and activatable imaging probes are key enablers for visualizing drug–target interactions. From a drug developer’s point of view, this is certainly a highly attractive feature; however, additional downstream read-outs of drug efficacy are equally important. These surrogate markers might include the activation of individual signalling pathways or markers of metabolic or physiological processes. In an ideal scenario, we would like to have both a direct target-specific read-out and a downstream effector read-out, a result that could be achieved with multichannel imaging.45 Although the recently reported armamentarium of newer imaging probes is welcome, there remain two methodological hurdles: improving intracellular delivery and developing better amplification methods. Many of the potential molecular imaging targets are located intracellularly. In general, MRI and optical imaging use large reporter moieties, and although such probes can easily target endovascular receptors, interstitial targets or the lysosomal compartment, cytoplasmic or other intracellular locations are more difficult to access. More recently, signalling peptides have been used for intracellular targeting and directing imaging agents.46,47 An alternative solution is to use radionuclide-labelled small molecules with improved cellular permeation. Another area of potential improvement concerns the development of more efficient biocompatible amplification strategies (both chemical and biological). These include, for example, multivalency to improve affinity,48 cellular internalization and trapping of imaging ligands,49,50,51 MR imaging agents with higher relaxivity and lower detection thresholds, chemical-shift reagents52,53 or fluorescent lifetime agents to reduce background noise54.

**Optical technologies for molecular imaging**

Continued advances in fluorescent probe design, photo-proteins and detection systems are facilitating the application of novel imaging technologies in drug discovery. The adaptation of these tools to the imaging of deep tissues in live animals is now changing the way we visualize molecular processes in vivo and, ultimately, in the clinic. The primary enablers have been progress in mathematically modelling photon propagation in tissue, expanding biologically compatible near-infrared (NIR) probes and the introduction of highly sensitive photon-detection technologies. Fluorescence- and bioluminescence-imaging techniques are of particular interest to the drug discovery and development process because of their low cost,
Box 1 | Some examples of existing imaging targets/probes used for in vivo imaging

- Proteases: cathepsin B, cathepsin D, cathepsin K, matrix metalloproteinase (MMP1, MMP2, MMP7), cytoomegalovirus protease, human immunodeficiency virus protease, herpes simplex virus protease, hepatitis C virus protease, caspase-1, caspase-3 and thrombin.
- Receptors: somatostatin, bombesin, dopamine D1, D2, serotonin, benzodiazepine, opioid, acetylcholine, adrenoceptor, oestrogen, cholecystokinin, epidermal growth factor receptor, vascular endothelial growth factor receptor (VEGFR), glycoprotein Ib/IIa, folate, insulin, neurokinin, transforming growth factor, asialoglycoprotein and adenosine 2.
- Enzymes: herpes simplex virus thymidine kinase, farnesyl transferase, topoisomerase, cytochrome p450, hexokinase, 3-hydroxyacyl-coenzymeA dehydrogenase (HAD), choline metabolism, citrate metabolism, protein synthesis (amino acids), Akt kinase, β-galactosidase and glutamate carboxypeptidase.
- Angiogenesis: E-selectin, α/β, VEGFR, human vascular cell adhesion molecule 1, endoglin (CD105), thrombin and endostatin.
- Cellular tracking: CD8, CD4, CD34, neural progenitor cells, stem cells, macrophages, dendritic cells and tumour cells.

versatility and high-throughput capability. There are several other optical techniques being developed, such as NIR spectroscopy, in vivo Raman spectroscopy and multiphoton imaging.

In fluorescence imaging, the energy from an external source of light is absorbed and almost immediately re-emitted at a longer wavelength of lower energy. Fluorescence imaging can be carried out at different resolutions and depth penetrations ranging from micrometres (intravitral microscopy) to centimetres (fluorescence-mediated molecular tomography; FMT). One of the key strategies for imaging deeper tissues (that is, more than a few millimetres) has been to use NIR light combined with NIR fluorochromes. Imaging in the NIR region has the advantage of minimizing tissue autofluorescence, which will improve target/background ratios. Fluorescence reflectance imaging (FRI) can be a useful technique when probing superficial structures (<5 mm deep), for example in small animals, during endoscopy, dermatological imaging, intravascular catheter-based imaging or intraoperative imaging. FMT, the newest optical imaging technology, has recently been shown to three-dimensionally localize and quantify fluorescent probes in deep tissues at high sensitivity. Indeed, it has become possible to image and, importantly, quantitate fluorochrome concentrations at femtomolar levels and at a sub-millimetre spatial resolution of point sources in small animals. In the near future, FMT techniques are expected to markedly improve in spatial resolution by using higher-density detector systems and advanced photon technologies, such as modulated-intensity light or very-short photon pulses. Clinical FMT imaging applications will ultimately require highly efficient photon-collection systems, but penetration depths of up to 10 cm are theoretically achievable depending on tissue type.

Bioluminescence imaging (BLI) detects luminescence generated by a biochemical reaction during which a photon is released. Firefly luciferin (a benzothiazole) and photinus luciferase are the most commonly used substrate–enzyme pairs, although several other luciferase–luciferin combinations can be used for image generation. Unlike fluorescence techniques, there is no inherent background signal, which means that BLI is highly sensitive. In contrast to radionuclide imaging techniques, the interpretation of bioluminescence images can be more challenging because of the frequent positional uncertainty of the light-emitting cells. Hence, the primary applications of BLI have so far been either qualitative (“Is luciferase expressed or not?”) or as a semiquantitative imaging tool to follow the same animal under identical conditions. BLI has been used to monitor the efficacy of antibiotic or chemotherapeutic agents, to identify transgenic mice that use luciferase as a reporter gene and to visualize the activation of specific pathways and cellular processes. BLI is less likely to be used in human patients, owing to limitations in light penetration and the fact that stable expression of luciferase (or an analogous system) is required to generate a signal.

Impact of imaging on drug discovery

The above examples illustrate the potential of established and emerging imaging technologies in drug discovery and development. When applied properly, imaging methods offer several advantages over other current practices. The use of imaging end points instead of time-consuming dissection and histology can significantly decrease the workload involved in tissue analysis and thereby speed up the evaluation of drug candidates. Imaging might provide biomarkers of a disease process and therefore help to define stratified study groups. As imaging methods are non-invasive, they allow for longitudinal studies in a single animal. This increases the statistical relevance of a study, allows for more clinically relevant study designs and decreases the number of animals required. Imaging will also provide important information on the optimal timing and dosing of drugs. Finally, emerging molecular-imaging tools can provide much earlier surrogate markers of therapy success than is at present possible.
In view of these arguments, it is reasonable to assume that imaging might reduce the development time of new drugs and provide tools for faster proof-of-concept testing in clinical studies. The latter is of key interest to the pharmaceutical industry, so why are molecular-imaging biomarkers not already being widely used as end points in clinical trials? First, regulatory agencies have historically relied on end points that require lengthy trials (for example, survival) rather than embracing new read-outs (for example, imaging vascular endothelial growth factor (VEGF)-receptor expression or a downstream target for a VEGF-receptor tyrosine kinase inhibitor). This is in part due to the fact that many of the newer imaging tools have not yet been sufficiently validated, at least for regulatory purposes. Second, molecular-imaging biomarkers, at present, exist only for a few targets and/or pathways and substantial development is still required. Third, molecular-imaging agents have to undergo lengthy approval processes (often longer than for a therapeutic agent) before their clinical use. Fourth, despite the efforts of various organizations in Europe and the United States (for example, the Society for Noninvasive Imaging in Drug Discovery (SNIDD); see online links), the interactions

![Figure 4](image-url)  
**Figure 4 | Fluorescence molecular imaging.** a | Visualization of matrix metalloproteinase (MMP)2 inhibition in a mouse tumour model using an MMP2-activatable, near-infrared (NIR) imaging probe and fluorescence reflecting imaging (FRI). Colour-coded in tumours maps of MMP2 activity are shown merged onto white-light images. b | Before treatment, in tumours MMP2 activity is high and decreases markedly within 48 h of an intravenous dose (150 mg kg⁻¹) of the MMP inhibitor prinomastat. Reprinted with permission from REF. 57 © (2001) Macmillan Magazines Ltd. c | Quantitative fluorescence-mediated tomography (FMT) imaging in a mouse model of lung tumour. The animal had been treated with cisplatin and treatment efficacy is shown as binding of NIR fluorochrome-annexin V to apoptotic tumour cells. The bottom row shows reconstructions of the tumour in the three orthogonal planes. Images courtesy of V. Ntziachristos and R. Schulz. d | Accuracy of reconstructed fluorochrome concentrations from within tissue phantom.
between the larger imaging and drug development communities have been limited. This is partly due to intellectual property issues but also because of National Institutes of Health priorities in funding disease detection and characterization rather than the development of biomarkers.

It is clear that closer and more widespread interactions between these communities would be of mutual interest. Finally, imaging competes with alternative technologies that can provide similar decision-making information; genomic and proteomic sciences detect altered expression levels associated with disease and/or therapy response, and metabolomics potentially yields biomarkers for drug efficacy or for potential safety issues.

Given the broad possibilities, it seems obvious that the pharmaceutical industry must invest in conventional and novel imaging technologies — indeed, we believe it should drive specific developments for its unique needs. This is particularly true for molecular-imaging applications, for which imaging and therapeutic targets are often the same. A highly specific therapy depends crucially on surveillance strategies (diagnostic kits), which allow for patient selection and close monitoring of the therapy response. Obtaining objective readouts for a patient might also help in tailoring the dosing regimen. The prerequisites for new imaging agents and approaches are clear: the techniques have to be quantitative, reproducible, specific, sensitive, applicable to clinical practice and safe. Developing novel imaging techniques and agents as part of the drug discovery process therefore seems a logical choice. Not only will this speed up drug discovery, but it will also ultimately reduce costs and result in better medicines.