Review Article

Theme: Imaging in Drug Development and Therapeutics
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A Review of Imaging Agent Development

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Abstract. This educational review highlights the processes, opportunities, and challenges encountered in the discovery and development of imaging agents, mainly positron emission tomography and single-photon emission computed tomography tracers. While the development of imaging agents parallels the drug development process, unique criteria are needed to identify opportunities for new agents. Imaging agent development has the flexibility to pursue functional or nonfunctional targets as long as they play a role in the specific disease or mechanism of interest and meet imageability requirements. However, their innovation is tempered by relatively small markets for diagnostic imaging agents, intellectual property challenges, radiolabeling constraints, and adequate target concentrations for imaging. At the same time, preclinical imaging is becoming a key translational tool for proof of mechanism and concept studies. Pharmaceutical and imaging industries face a common bottleneck in the form of the limited number of trials one company can possibly perform. However, microdosing and theranostics are evidence that partnerships between pharmaceutical and imaging companies can accelerate clinical translation of tracers and therapeutic interventions. This manuscript will comment on these aspects to provide an educational review of the discovery and development processes for imaging agents.

KEY WORDS: drug development; imaging agent development; PET; SPECT.

INTRODUCTION

Imaging biomarkers hold much promise for diagnosing disease, monitoring disease progression, tracking therapeutic response, and enhancing our knowledge of physiology and pathophysiology. With the continued momentum towards specialized therapies and personalized medicine, there will be an increasing need to monitor the physical state of an individual in a noninvasive manner with increased specificity. Medical imaging has been put forth as one of the major players in such assessments, with an emphasis on molecular and functional imaging in addition to anatomical imaging.

Molecular imaging is also expected to play an increasingly important role in drug discovery and development

(1–5). A seminal review by Frank and Hargreaves describes the type 0 biomarkers along the continuum of the natural history of disease, type I biomarkers for detecting a therapeutic drug's mechanism of action, and type II biomarkers that are equivalent to surrogate end points (6). Molecular imaging probes have been designed to target all three types of biomarkers. To better understand their utility, imaging probes also can be grouped according to the markers they are interrogating: target, mechanism, efficacy, and surrogate markers. Probes can interact with target biomarkers to temporally determine the presence, quantitative level of expression, and spatial localization of specific targets for a therapeutic drug. A mechanism biomarker may be interrogated by specific molecular imaging probes to assess the therapeutic's modulation of the drug target. Visualization of efficacy markers involves the use of molecular imaging to monitor drug action. Surrogate markers can be imaging biomarkers if the related probe concentration predicts the effect of a therapeutic drug in lieu of a clinical end point for regulatory decisions. It can be appreciated from these groupings that imaging biomarkers can provide valuable information in preclinical and clinical stages of drug development.

While the outlook for medical and molecular imaging is quite promising, the commercial development of imaging agents can be as challenging as the development of therapeutics. In fact, current imaging agent development shares much in common with standard drug discovery and development practices (Fig. 1) (7). This is especially true for molecular imaging agents that bind to a specific target *in*

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ABBREVIATIONS: CMS, Centers for Medicare and Medicaid Services; CT, computed tomography; IP, intellectual property; MRI, magnetic resonance imaging; NOPR, National Oncologic PET Registry; PD, pharmacodynamics; PET, positron emission tomography; PK, pharmacokinetics; PPP, public-private partnership; RDRC, Radioactive Drug Research Committee; SNM, Society of Nuclear Medicine; SPECT, single-photon emission computed tomography.

vivo. For example, target validation, identification of suitable candidate compounds with high affinity and uptake at the target site, adequate clearance, and low potential toxicity are key considerations for therapeutic and imaging compounds. In addition, there are similar stages such as hit identification and lead generation as well as multiple phases of clinical trials before approval. Despite these similarities, there are differences in imaging agent discovery and development processes that can be critical to the ultimate success of an imaging agent, some of which will be discussed in this review.

The role of academia and industry in the development of therapeutics and diagnostics is one aspect that can differ. The main players in the development of diagnostic imaging agents include an active and far-reaching community of academic investigators as well as a handful of companies involved in their commercialization. The academic community is invaluable for exploring new high-risk areas and initially showing the feasibility of new imaging approaches. For example, early feasibility studies around targeted magnetic resonance imaging (MRI) (8,9) are opening the door for MRI to be used as a molecular imaging modality. Additionally, the academic community directly develops new agents and is credited with the majority of positron emission tomography (PET) and single-photon emission computed tomography (SPECT) tracers discovered to date. These efforts provide significant licensing opportunities for diagnostic companies, which in turn provide a route of commercialization for the tracers discovered in academia, although academics also can drive the development process from basic research to limited clinical studies without industry sponsorship.

Beyond the academic community, there are companies which focus solely on the commercial development of diagnostic imaging agents, while, in other cases, these efforts are part of larger therapeutic or technology companies. In general, the size of these companies/divisions is on par with smaller biotechnology companies. This is likely a reflection of the smaller market potentials and profits from diagnostic imaging agents compared to those associated with pharmaceuticals. Based on 2004 numbers, it was estimated that the total imaging agent market was only 1% of the total therapeutic market (10). This means that the majority of imaging agents would likely fall into the small/specialty market size. While reliable estimates of time and costs for the development of diagnostic agents are not often reported, Nunn calculated that a diagnostic imaging agent takes approximately 10 years to develop at a cost of \$150 million; yet generates only \$200–\$400 million in sales annually, even for a highly used diagnostic agent, such as Omnipaque™ (iohexol, *N,N'*-bis(2,3-dihydroxypropyl)-5-[*N*-(2,3-dihydroxypropyl)-acetamido]-2,4,6-triiodoisophthalamide, GE Healthcare) or Cardiolite™ ([Tc-99m]*N*-(2-methoxy-2-methyl-propyl)methanimine, Lantheus). As a comparison, he reported that a therapeutic costs approximately \$850 million to develop over 14 years but could attain as much as \$3.4 billion in sales for a blockbuster drug (Fig. 1) (10). It is evident from these numbers that the commercialization of diagnostic imaging agents will not generate the same return on investment achieved in the pharmaceutical industry. Therefore, diagnostic companies are exploring partnerships with academia and Pharma to improve the viability of agent development in light of the increasingly personalized treatment of patients.

Fig. 1. Discovery and development of therapeutics and diagnostics. **a** Therapeutic process modified from Fig. 4 of the FDA's Critical Path Initiative; \$3.4 billion annual revenue is representative of a typical top ten selling drug. **b** The best selling diagnostic imaging agent has an annual revenue of \$400 million. Cost and timeline numbers taken from Nunn (10)

These partnerships will likely continue to be important in the creation of new imaging agents. Yet, the development of imaging agents has unique aspects that are critical to its success that may not be well known outside of the imaging community. Therefore, this review outlines the processes, opportunities, and challenges encountered in the discovery and development of imaging agents.

DISCOVERY

Identification of Diagnostic Opportunities

Early in the commercial development of an imaging agent, several factors must be considered beyond the scientific challenges. One of the first considerations is to identify how the agent will be used. This will then define performance metrics required for success. For agents used to diagnose disease, their performance criteria include sensitivity and specificity requirements. For example, Cardiolite™ ([Tc-99m]-sestamibi), which is used to detect coronary artery disease (11), has similar sensitivity and specificity compared to Thallium-201, the gold standard at the time of its approval (12). Yet the advantageous properties of the Tc-99m label improved image quality and allowed for the use of cardiac gating, which resulted in significant improvements in specificity for female patients (13,14).

Agents also can be used as efficacy markers to monitor therapy, as exemplified by the use of 2-[F-18]fluoro-2-deoxy-D-glucose ([F-18]FDG) PET to monitor the reduction of tumor metabolism resulting from the cytostatic drug imatinib mesylate (Gleevec, Novartis) (15). When considering probe development for therapeutic monitoring, the noise level of the modality as well as the expected target modulation due to therapy and disease progression should be considered in early feasibility assessments.

Preclinical molecular imaging of targets and mechanisms is increasingly used in drug discovery and development. Imaging probes (e.g., [Tc-99m]scVEGF/Tc) for vascular endothelial growth factor receptor have been used for determining target expression in tumor-bearing mice (16). In addition, bioluminescence of live mice was recently used to monitor the inhibition of lymphocyte proliferation by rapamycin, specifically the interaction between rapamycin-binding domain and cytosolic protein FK506-binding protein 12 (17). Development of such imaging agents needs to provide convincing evidence that they are specifically marking the targets and mechanisms they are designed to measure.

In addition to defining the use of the imaging agent, other factors to assess include the market size, optimal modality, intellectual property (IP), competing diagnostics, and the existence of disease-modifying therapies. With relatively small revenues for even large market segments, market size is an important factor when prioritizing projects. This may lead to technically feasible projects being deprioritized due to considerations of return on investment (10). Market size also may be a factor in the selection of the imaging modality, which can be driven by the current/future install bases as well as factors associated with clinical usage (e.g., repeated dosing) and modality constraints (resolution and sensitivity). For example, SPECT and SPECT/computed tomography (SPECT/CT) equipment has a larger installed

base (~12,530 stationary multihead SPECT scanners in the US in 2008) compared with PET and PET/CT (~1,000 stationary PET and PET/CT in 2008) in the US (18,19). Therefore, if the performance of SPECT imaging is adequate, then the development of a SPECT tracer may be preferred over a PET tracer. This is especially true as advancements in SPECT technology are likely to shorten the current resolution and sensitivity gaps between the two modalities (20,21). To reach users of both modalities, there are examples of PET and SPECT tracers under development for the same biological targets, such as amyloid deposits (22–24) or $\alpha\beta3$ expression (25,26).

As with therapeutics, knowing the IP landscape before project inception helps to identify opportunities and competition. For diagnostic companies, competition can come from technology advancements, nonimaging biomarkers, and other applicable imaging agents, regardless of modality. Given the long development times for new imaging agents and the continuous technology improvements in the various imaging modalities, it is important to consider the emerging technologies that could displace the agent's market potential. For example, MRI researchers are ever-expanding opportunities to leverage endogenous signals to gain information without a contrast agent (27–29). Blood biomarkers and nonimaging diagnostics are another form of competition that could limit the use of an imaging agent. These diagnostics are typically cheaper than imaging tests but lack the spatial information that can be gained from imaging.

Finally, imaging companies are beginning to carry out health economic analyses at the front end of development for molecular imaging agents and technology. Health economics is the science of value and, when applied to imaging agents, determines if there is additional benefit from a new agent worth the cost to the health care system. Health economics and outcomes-based research are expected to assist in the identification and commercialization of new medical technologies in light of the reimbursement issues.

Once these concerns have been addressed, the project is ready to begin the discovery and development process.

Target Identification/Validation

Standard drug discovery often begins with target identification and validation by providing evidence that the drug target plays an important mechanistic role in the disease process such that inhibiting the target may modify the disease (30). Since the drug will have a functional impact, a validated target then serves as the basis for early-stage screening of potential drug compounds (31). A key difference between screening for drugs and imaging agents is that imaging agents can be aimed at targets that play critical roles in disease but do not need to be functional targets that modify the disease. There are several instances where nonfunctional targets are markers of the disease process, such as extracellular matrix proteins, membrane lipids, structural proteins, or extracellular deposited peptides (32–35). Target validation for imaging agents also requires determining the amount of target accessible for high signal-to-noise images. Therefore, the absolute amount of accessible target *in vivo* is important to imaging agent performance.

While PET displacement studies have helped receptor binding determinations of drugs *in vivo*, there is growing evidence that modeling and simulation of molecular imaging help characterize nonreceptor nonenzymatic ligand binding. For example, saturation binding of a highly specific imaging agent can be used to calculate the receptor density ($B_{\max}$) empirically and has been particularly useful for well-understood receptor–ligand interactions, which pharmaceutical companies have leveraged in receptor occupancy studies (36,37). However, interest in complex nonreceptor imaging targets related to disease processes is growing. For example, imaging beta-amyloid plaques requires ligand binding to a complex heterogeneous target with multiple independent binding sites (33,38,39). The complexity of some imaging targets, such as beta-amyloid, also arises from a target's trafficking pattern *in vivo* (40,41). In such cases, computational and systems biology may provide useful guidance. This approach was recently applied to understand the relationship between the heterogeneous microenvironment of plaque and imaging kinetics (42,43). Further, recent data indicates that combining models of pharmacokinetics with physiological models of beta-amyloid production and clearance has value in understanding the relationship between target concentrations, affinity, and image quality (44). The use of mathematical models and quantitative assessments early in a project's development can play an important role in assessing the feasibility and risk of the imaging approach (45,46). As with the pharmaceutical industry, the use of *in silico* models and computation will be important tools to help diagnostic companies become more efficient in bringing new imaging agents to market.

Iterative Discovery Processes

The discovery process for traditional small-molecule drugs has typically been represented as a fairly linear process, progressing along the major milestones outlined in Fig. 1. This is not to say that therapeutic discovery is not iterative, as certainly there are times when iterations are necessary to achieve the required *in vivo* therapeutic performance. In major pharmaceutical companies focused on small-molecule drugs, a large number of chemical compounds can be generated and then screened for performance against specific assays to arrive at a reasonable number of compounds to take forward into *in vivo* testing. Diagnostic companies often do not have a large library of chemical compounds to select from. Furthermore, *in vitro* results do not always translate well to *in vivo* performance (47–49). For example, in the search for a SPECT agent equivalent to [F-18]FDG, an iodinated mannose analog was investigated. While the stability of the tracer looked good *in vitro*, *in vivo* performance suggested that the label was rapidly deiodinated (47) and therefore would not satisfy imaging requirements. Since the pharmacokinetic behavior and signal generation are major contributors to performance, chemical synthesis, and screening, preclinical studies and lead selection can be quite iterative for diagnostics (Fig. 2). This is especially true for novel imaging approaches that involve complex mechanisms and targets assessed by nontraditional (nonsmall molecule) compounds. In these cases, the chemical and *in vitro* screening assays may not fully capture *in vivo* performance. Therefore,

Fig. 2. The iterative discovery process allows for the early *in vivo* assessment of novel imaging compounds and targeting mechanisms

early studies in simple animal models may be used to understand and optimize the performance of the new agents. Information learned through these studies is then fed back to the chemists for modifications to improve performance before taking them into more rigorous preclinical studies. Once a lead compound has been identified, the compound will then be optimized and formulated for use in humans.

Chemical Synthesis

The historical success of neurological tracers for nuclear medicine was governed in part by the same principles followed in therapeutic drug development. Parameters for optimization included affinity for the target, selectivity, metabolism, lipophilicity, and molecular weight/size (50–54). Equally important is the selection and incorporation of the signaling moiety into the chemical construct, which can significantly alter the delivery, retention, binding, and clearance of an imaging agent. Nuclear tracers can be grouped into three broad classes according to how the radiolabel is integrated into the imaging agent. The first class of radiolabeled tracer is the radionuclide itself (Fig. 3). For instance, sodium [Tc-99m]pertechnetate is simply the oxidized gamma-emitting technetium metal used for SPECT blood pool imaging. Likewise, sodium [F-18]fluoride is a bone-seeking PET agent used in imaging osteosarcoma. The second class involves a pendant radiolabel extending from the molecular scaffold that imparts targeting of molecular markers. This second grouping is traditionally applied to radiometals and their chelators for SPECT imaging (e.g., [Tc-99m]TRODAT-1; Fig. 3) (55); however, the pendant can be applied to PET agents, where the pendant label is a radiometal (e.g., Ga-68 DOTA in [Ga-68]DOTATOC, Fig. 3) or an [F-18]alkyl fluoride, such as in 3-*N*-(2-[F-18]fluoroethyl)piperone (Fig. 3). The third class of tracers incorporates the radiolabel within the structure of the targeting molecule. For example, experimental estradiol derivatives for SPECT imaging integrate the receptor binding sites of the molecule on the exterior of the technetium radiometal (Fig. 3). For PET imaging, positron-emitting radionuclides C-11, N-13, and O-15, which are isotopes to endogenous elements like C, N, and O, have been incorporated into molecules without changing chemical properties. For example, a comparison between L-[C-11]DOPA and L-6-fluoro[F-18]DOPA clearly shows that the *in vivo* decarboxylation rate differs (56). While fluorine is not as ubiquitous in endogenous biomolecules, F-19 is a common element in many drug pharmacophores. Therefore, substitution of F-19 with F-18 may be ideal for the labeling of some small-molecule drugs. However, Pharma scientists should note that F-

Fig. 3. Three classes of tracers (modified from Dilworth and Parrott (55)). **a** Class 1 tracers are those where the radionuclide is the imaging agent itself. Class 2 tracers have pendent radiolabels. Class 3 incorporates the radionuclide within the targeting molecule. **b** (1) [Tc-99m]TRODAT-1, (2) Experimental [Tc-99m]estradiol. **c** (3) [Ga-68]DOTATOC, (4) [C-11]DOPA, (5) 3-N-(2[F-18]Fluoroethyl)piperone

18-labeled drugs are analogs with different pharmacokinetic and pharmacodynamic properties *in vivo* unless there is F-19 in the drug structure that is amenable to F-18 substitution.

Early probe development in academia has utilized C-11 chemistry due in part to the synthetic opportunities using a radioisotope of carbon relative to the other positron-emitting radionuclides. However, fluorine-18 and other relatively longer-lived radioisotopes have been used clinically and commercially. The 110-min half-life of F-18 (compared with

the 20-min half-life of C-11) allows for longer radiosynthesis of tracers and imparts greater potential for regional distribution of tracers to clinics without cyclotrons. It is estimated that 90% of all PET scanners do not have facilities that can produce C-11 tracers (24), thereby being dependent on local distribution of tracers with longer half-lives.

There also can be advantages to using SPECT radio-labels (e.g., Tc-99m, I-123) for biomolecules and drug labeling and there is a long history of various published techniques for

radioiodine labeling of proteins, nucleic acids, and small molecules (57). The relatively facile labeling with radioiodine, its longer half-life (13.2 h for I-123), and gamma emissions ideal for sodium-iodide-based SPECT detectors are all reasons why I-123-labeled SPECT tracers are still being introduced to the clinic (e.g., I-123 MIBG). However, the workhorse radioisotope for nuclear medicine continues to be technetium-99m (Tc-99m) due to its convenient production, half-life of 6 h, and 140- and 142-keV gamma emissions which are ideal for NaI detectors. In addition, Tc-99m is produced by a molybdenum-99:technetium-99m generator which can be conveniently located in small hot laboratories in nuclear medicine clinics.

Radiolabeled molecules for PET and SPECT imaging are produced and administered in very low concentrations, which allow for detection of ligand–target interactions without prompting a pharmacological effect. The high sensitivity of scanners for the radioactive emissions from the tracers enables detection of picomolar concentrations of C-11-labeled PET tracers, for instance (58,59). Therefore, the molecular and biochemical system can be interrogated using PET imaging without perturbing the system. This so-called tracer method is made possible with highly specific radioactivity (radioactivity per mass of radioactive and nonradioactive molecules) of the cyclotron- or generator-produced radionuclide. The challenge of achieving and maintaining high specific activity should not be underestimated because of the effects of isotopic dilution (particularly for C-11) and the promise of microdosing (discussed below) that is incumbent on the high specific activity of a radiotracer. As new imaging biomarkers are identified, new radiotracers need to be developed with high specific activity in order to target the typically low-concentration markers of early disease. This also is a consideration in preclinical studies, where the mass of injected tracer can lead to much greater receptor occupancy levels than anticipated (60).

Preclinical Testing

Preclinical studies are undertaken to assess the pharmacokinetic behavior of the tracer, as well as provide evidence for proof of mechanism (POM) and/or proof of concept (POC), assess potential toxicity risks, and address translation to humans. As with pharmaceuticals, most imaging agents available today are small-molecule compounds that rapidly distribute and clear from the body. However, this is changing as labeling of antibodies and antibody fragments is being explored as well as macromolecules and nanoparticles for various applications (61–65).

Imaging agents are typically intravenously administered, which simplifies their pharmacokinetic characterization. As stated earlier, since the pharmacokinetics of an imaging agent is particularly important to its performance, the chemical compound may go through modifications based on preclinical imaging results to improve its pharmacokinetic behavior. In addition, prior to human use, the complete pharmacokinetics of the tracer is typically evaluated in preclinical studies. Using biodistribution or dynamic imaging studies, the time-dependent concentration of an agent in the major body tissues and excrement is recorded. This information is then used to assess dosimetry, identify the dose-limiting organ for toxicity and radiation exposure, and can be used in the translation to humans (66–68). Similar studies in humans are performed in

the first phase of the clinical trials to determine definitive human pharmacokinetics since results in preclinical species do not always translate well to humans. While nuclear tracers usually have limited toxicity issues due to the low mass dose administered, MRI and CT contrast agents are dosed at much greater amounts and therefore carry a greater risk of toxicity. Thus, safety assessment is a critical factor in the development of contrast agents for these modalities.

Since the signal of a nuclear tracer is due to the radiolabel, a thorough understanding of the parent/intact agent along with any metabolites, biotransformations, and free label is important to understand and quantify if possible. For new tracers originating in academia, mathematical models are often reported to describe the distribution of the tracer in select tissues based in preclinical or clinical studies (69–74). The mathematical model can then be used to quantify specific parameters of interest, such as a binding potential or distribution volume for neurotracers (75,76).

POM studies should demonstrate the mechanism by which the agent is acting, while POC studies should have relevance to the clinical disease of interest and may also be used to show performance against gold standards or competitor agents. POM does not necessarily require *in vivo* studies, as long as the mechanism can be convincingly demonstrated (77–79). In cases where *in vivo* studies are used, the expression of the target may be amplified and modulated to provide evidence that the imaging agent is hitting its target. Since PET and SPECT imaging agents do not elicit a pharmacologic effect, a pharmacodynamic response is not measurable. The burden of proof therefore lies in the ability to correlate uptake of the imaging agent with the modulated target density. Modulation of a target can be accomplished through the use of therapeutics or genetically modified animals. In addition, competition and inhibition studies are important to show that the observed uptake is due to specific binding to a target and not an alternative mechanism.

A key step in this process is validation of target modulation. The validation of imaging biomarkers is traditionally dependent on histologic assays despite the wave of recent genomic and proteomic discoveries in medicine. Medical and molecular imaging is still very much focused on the precise localization of an imaging marker in the context of anatomy. Hence, autoradiography and radiologic–pathologic correlates are gold standards for validation of *in vivo* images (24,80–83). Expression profiling of RNA and DNA, while valuable for the development of *in vitro* diagnostics and molecular therapies, is not as useful in providing a measure of an imaging probe's likelihood of success.

The discovery process for molecular imaging probes is exemplified by the lack of success with Congo red and Thioflavine T derivatives and the eventual recognition of benzothiazole analogs as *in vivo* imaging agents for the visualization and detection of beta-amyloid plaques. As previously mentioned, beta-amyloid aggregates form plaques in the Alzheimer's disease brain and contain multiple independent binding sites for small-molecule probes. Congo red and Thioflavine T are gold standard bright-field and fluorescent dyes, respectively, used for *in vitro* histological staining of beta-amyloid aggregates in *ex vivo* brain specimens. Early attempts to translate these charged *in vitro* dyes into *in vivo* probes for the beta-amyloid aggregates in plaques were not entirely successful or optimal for eventual clinical

molecular imaging. Congo red is a charged molecule that lacks sufficient hydrophobicity for diffusion through the blood–brain barrier (84,85). A lipophilic analog of Congo red was synthesized and demonstrated improved blood–brain barrier permeation of the probe (86). However, the *in vivo* two-photon confocal microscopy showed unfavorable pharmacokinetics for optimal signal to background necessary for routine PET imaging in mammals. Similarly, a lipophilic radioiodinated analog of Thioflavine T also was synthesized and demonstrated *ex vivo* to enter the brain of normal mice (87). But the analog reached maximal uptake at 30 min in the mouse brain which suggests less than optimal uptake in the brain for *in vivo* imaging in humans.

The iterative improvement of Thioflavine T derivatives resulted from the recognition of molecular requirements for the binding of this family of compounds to beta-amyloid plaques and eventually led to successful *in vivo* PET imaging of plaques in Alzheimer's disease patients using a C-11-labeled derivative called 6-OH-BTA-1 (88–91). The development of an F-18 analog of the C-11 derivative continues the iterative improvements with the goal being a PET probe with the longer 110-min radioactive half-life for F-18 rather than the 20-min half-life of C-11 (92). This will result in less radioactive decay of the probe when it is commercially distributed from regional PET tracer distribution sites to imaging clinics without onsite cyclotrons for F-18 radioisotope production. In general, the longer half-life of F-18 over C-11 also allows for the imaging of relatively longer biological

processes or probes with slow distribution and clearance of free probe. Thus, it is evident that the typical iterations in the imaging agent development process are similar to feedback loops in drug development except for the radiolabeling of potential probes which includes additional recursive steps in order to optimize the *in vivo* image.

DEVELOPMENT

Once an imaging agent has passed through preclinical testing and shows an efficacious response, it now enters the development phases and proceeds through the regulatory process. Currently, the US Food and Drug Administration (FDA) evaluates imaging agents in the same manner as therapeutics. Thus, the current regulatory requirements are quite similar (Table I). Several recent reviews detailing the regulation of diagnostic imaging agents in the US and Europe are available (93–96). A notable opinion is that the role of the European Medicines Agency in regulating radiopharmaceuticals cannot be compared to that of the FDA because European directives are implemented by the individual Member States that may introduce changes and timelines varying from other Member States (93). Therefore, the following section will provide a brief historical description of the basic process and current challenges in the US.

Since radiotracers are administered at tracer doses and not expected to elicit a pharmacologic response, developers initially worked with the Radioactive Drug Research Com-

Table I. Clinical Trial Phases for Therapeutics and Diagnostics

Phase	Therapeutic	Diagnostic
RDRC approval	n/a	Limited first-in-human studies Gain pharmacokinetic, metabolism, dosimetry data
Exploratory	5–30 subjects Nontherapeutic doses PK, metabolism, Reduced preclinical toxicity data requirements	5–30 subjects Nondiagnostic doses PK, metabolism, dosimetry Reduced preclinical toxicity data requirements
1	Healthy volunteers (except oncology) Evaluate safety Dose selection General PK/PD Evidence of efficacy, if possible	Healthy volunteers Evaluate safety Dose selection General PK Record tissue distribution (whole-body studies) for dosimetry and protocol design Studies with excess unlabeled tracer—"worst case scenario"
2	Several hundred patients Show efficacy in target population Evaluate safety and side effects Controlled studies, closely monitored	Refine dose, imaging protocol, and analysis criteria to support phase III Gain additional PK if necessary Gain additional efficacy and safety data Define population and clinical setting for phase III Use formulation that will be marketed; if not, may need bridging study
3	Several hundred patients Controlled and uncontrolled trials Show benefit–risk ratio based on efficacy and safety in large population	Demonstrate efficacy and safety Efficacy by confirming principal hypothesis Recommends use of different investigators, centers, readers for blinded reads to improve generalization
4	Postmarketing studies Monitor safety data	Postmarketing studies Monitor safety data

Modified from Hoffman *et al.* (96)

mittee (RDRC) to begin first-in-human studies. This was one mechanism through which academic institutions could evaluate a tracer in humans without having to formally file an Investigative New Drug (IND) application with the FDA. These studies provided a basic understanding about the metabolism, distribution, and dosimetry of a compound. Unfortunately, the RDRC was limited in scope and proposals have been put forth to extend its functions and control over the regulatory process for diagnostic agents (97,98).

In 2006, the FDA established the exploratory IND for therapeutics and diagnostics. This IND allows for initial studies in a limited number of human subjects with no therapeutic or diagnostic intent. The exploratory IND provides an early look at the potential success of a new tracer by monitoring its distribution and metabolism through screening or microdosing experiments. This phase does not require as much animal toxicity and safety data as the traditional IND and, therefore, can be performed with fewer financial and time losses if the compound fails.

If a diagnostic agent shows potential in these limited human studies, a formal IND can be filed and the compound must still proceed through the traditional clinical trial phases. As with therapeutics, the Phase I study involves a small number of healthy volunteers with the primary objectives of establishing safety, defining the human pharmacokinetics and establishing an initial dose range. During this phase, agent distribution in multiple tissues is captured by imaging at multiple times post-administration of the compound to evaluate the radiation dosimetry of the tracer and also to help optimize the imaging protocol for subsequent phases. Phase II studies are performed in the target population to get an estimate of the diagnostic accuracy of the agent and include a few hundred patients. These studies also involve protocol refinement and standardization of data interpretation by blinded readers for the phase III study. They should also be designed to provide information about the appropriate patient population for the phase III trials. Phase III should provide evidence of efficacy in a large clinical trial that involves hundreds to thousands of patients and is performed at multiple sites. The study should continue to show safety data and validate the usage and appropriate patient population for final approval of the diagnostic. In general, the phase III trials attempt to generalize the method and show the risk–benefit ratio by providing metrics of efficacy and safety in comparison to existing gold standards. Phase IV trials are typically postmarketing studies that continue safety surveillance for the diagnostic as well as trials to show efficacy for different indications to expand the approved usage of the diagnostic agent (95,96,99,100).

Given that only two new PET/SPECT tracers have been approved by the FDA in the last 10 years, many are commenting on the inefficiencies of this regulatory approach for diagnostic imaging agents, in particular radiotracers (97,98). Radiotracers are given at doses that do not elicit a pharmacologic event (orders of magnitude below therapeutic doses), are infrequently administered, and are designed to measure molecular processes, not modify the disease (97,98). These factors reduce the safety risks associated with radiotracers compared to therapeutics, yet they are regulated as though they carry the same risks. The reduced risks and different usage of imaging tracers support development of alternatives to the current regulatory process. The Society of Nuclear Medicine (SNM), through a workshop with academic, industry, and government participants, has put forth the elements of an approval process specifically for

diagnostic imaging agents (98). This initiative outlines a two-step process, whereby first the agent is shown to be safe and efficacious in measuring a molecular process. This would entail validation against a reference or gold standard. The second step would be to show clinical utility and efficacy. This step would involve phases II and III clinical trials. The exploratory IND processes may cover some of the early studies but fall short in providing approval for the larger clinical trials that would be part of the second step in the regulatory process.

Existing FDA guidance provides four broad indications for imaging agents: (1) structural delineation, (2) disease or pathology detection or assessment, (3) functional, physiological, or biochemical assessment, and (4) diagnostic or patient management (101). In general, the imaging industry prefers the third indication as the label for a new tracer of a molecular process; however, the FDA guidance requires new “functional” tracers to demonstrate beneficial outcomes (fourth indication above). It then becomes necessary to show the patient benefit of a new tracer because usefulness of functional measurements is unknown. Unlike SNM’s proposed two-phase approach, the FDA guidance makes it difficult to link patient outcome to imaging information alone, especially considering that patient management includes imaging and nonimaging patient data. Alternatively, another path forward for new tracers could be to expand on the exploratory IND and parallel the orphaned drug paradigm.

While [F-18]FDG is a well-known and widely used radiotracer today, it by no means had a smooth transition from a research tool to clinical use and highlights the difficulties in the clinical adoption of a radiotracer. With the availability of cyclotrons and streamlined methods for [F-18]FDG cGMP (pharmaceutical grade) production, the compound seemed poised for adoption into clinical diagnostics. However, since the compound had been within the public domain, no company was willing to support a clinical trial or take the compound to the FDA for approval (102). Therefore, a long FDA-imposed moratorium kept it within the domain of research until clinical safety regulations could be provided. In addition, another problem became apparent: because [F-18]FDG never went through the rigors of commercial product development, its imaging techniques were never standardized. Consequently, there was confusion in making accurate measurements and interpreting imaging results. For example, there were difficulties in extrapolating imaging metrics for clinical evaluation of anticancer agents. Another consequence from lack of standards was that insurers were reluctant to reimburse for [F-18]FDG PET services (103).

While it can be difficult to execute the large multicenter trials with standardized procedures, in the US, there are ongoing efforts to streamline tracer development and the use of imaging biomarkers in drug development. In 2008, the SNM created the Molecular Imaging Clinical Trials Network with centralized INDs for nonproprietary radiolabeled tracers as its centerpiece. Imaging and pharmaceutical industries can cross-reference the centralized IND for multicenter trials (104). 3'-Deoxy-3'-[F-18]fluorothymidine is the first SNM-sponsored IND, which should help pharmaceutical companies measure the effectiveness of antiproliferative drugs. The participating imaging sites' adherence to standardized methods was another key element of the SNM network in order to maintain imaging quality, standardization, and consistency. Such guidelines will need to be established with other tracers as well.

The American College of Radiology Imaging Network also has been created to help address this issue (105).

For an imaging agent to be used clinically, the imaging test must be reimbursed by the Center for Medicare and Medicaid Services (CMS) and private insurance companies. This can be especially challenging in a time when imaging has been cited as a factor in the increased health care costs (96). Diagnostic developers must work with these agencies to provide the evidence-based data they require to assess the technology and show the value of the imaging tests so that it is economically feasible to continue developing diagnostic imaging agents. In the area of oncology, if imaging agents can be shown to serve as biomarkers, they will certainly become cost-effective in directing treatments, especially when those treatments are much more expensive than the imaging tests (105). The National Oncologic PET Registry (NOPR) opened in 2006 as an example of clinical data collection to assess the effect of [F-18]FDG PET on patient management for cancer diagnosis, staging, restaging, recurrence, and monitoring treatment (106). In October 2007, the CMS stated that the NOPR and similar registries are good models for [F-18]FDG PET reimbursement of expanded coverage for cancer indications as well as others.

DISCUSSION

The previous sections have highlighted the processes involved in the development of imaging agents. It is evident that similarities exist with the development of therapeutics, but there are also aspects that are unique for imaging agents. The remainder of this review will discuss additional challenges and opportunities that are present in the use and development of imaging agents.

Challenges in the Use of Molecular Imaging Agents in Drug Development

Response evaluation of a therapeutic intervention for cancer exemplifies the challenge of validating molecular imaging biomarkers to existing surrogate markers. In oncology, the quantitative assessment of the overall cancer burden can help characterize the treatment effect as being a complete response, partial response, stable disease, or progression of disease (107). While the Response Evaluation Criteria in Solid Tumors guidelines for the evaluation of anticancer agents were adequate when anatomical imaging dominated the assessment of oncology, the rise of personalized medicine and the development of new molecular imaging probes require new ways to assess response from a targeted therapeutic (108). It has been argued that 90% of anticancer drugs in development have failed in the clinic because new cytostatic therapies are not cytotoxic with no change in tumor volume being observed (109). PET/CT and other hybrid imaging modalities, which allow for the overlaying of consecutively acquired molecular and anatomical images, may enable imaging of the molecular response to cytostatic therapies with PET at the same time as measuring tumor size using CT (110). New clinical trial end points based on molecular markers of anticancer activity, including molecular imaging biomarkers, may readily show the modification due to therapy while reducing severe toxicities.

Another challenge is that the integration of PET and SPECT into the clinical research workflow requires an understanding of the drug's mechanism of action. Von Hoff

refers to the preclinical-clinical uncoupling where clinical trial end points of complete tumor shrinkage or partial response neglect assessments of the mechanism modulating the therapeutic target (109). [F-18]FDG PET may help identify earlier the downstream effects of cancer drugs before tumor shrinkage occurs, yet routine clinical use of [F-18]FDG PET outside of clinical trials involves static imaging 2 h post-FDG administration. If localization of tumors and metastases is all that is needed, then static imaging may be useful. However, dynamic imaging, which acquires PET data during the PET scan starting at the initial injection of [F-18]FDG, could provide more information about the pharmacokinetics of the tracer and in turn the pharmacodynamics of the anticancer drug for the individual at that point in time (111).

Intellectual Property

The therapeutic drug and imaging agent industries rely on exclusivity of IP to gain an advantage over the competition; however, the lower return on investment for imaging agents causes the imaging industry to be very selective of in-licensed IP. The Pharma industry, imaging industry, and academic institutions have developed a more sophisticated knowledge of the current imaging agent IP landscape and are more vigilant in protecting the IP of their agents. Some academic centers are becoming so protective of their technology that the institutions adopt the same IP protection as for-profit companies (112). However, the markets for new targeted tracers appear to be for niche indications, making it difficult for imaging companies to justify the costs associated with in-licensing and developing new tracers, especially those that are not far enough in the development process. Possible

Fig. 4. Stages in preclinical and clinical therapeutic development paired with measurable end points using molecular imaging (modified from Workman *et al.* (113))

solutions to the challenges with tracer IP include public-private partnerships (PPP) to secure tracer IP ownership of potentially small-market indications (112). A recent SNM workshop also discussed working with the Biomarkers Consortium which is developing a document on the issues of biomarker IP (98). The SNM workshop also recommended separate IP criteria for the research use of tracers and for their use in diagnosis and treatment based on the reasoning that research applications do not require the same level of protection as clinical applications. Partnering between academics, industry, and societies like SNM could lead to patent pooling of agents with small indications.

Partnering Between Academia, Pharma, and Imaging Companies

In addition to addressing IP issues, PPPs allow for the accelerated utilization of imaging agents as theranostics in drug development. A theranostic is an imaging agent that not only has diagnostic uses but also has the ability to guide therapeutic intervention. Theranostics aid in the selection of the appropriate drug for an individual patient, at the right

dose and the right time. The personalized administration of a drug can rely on the tracer and what is measurable with molecular imaging (Fig. 4) (113).

One commonly known theranostic approach is found in radioimmunotherapy. Ibritumomab tiuxetan (Zevalin, Cell Therapeutics) is an anti-CD20+ antibody that carries either the gamma-emitting indium-111 for SPECT imaging or the beta-emitting yttrium-90 radioisotope used for treating non-Hodgkin's lymphoma. The indium-111-labeled antibody is used to confirm the organ-specific accumulation of the drug and determine dosimetry before treatment with the [Y-90] ibritumomab tiuxetan (114).

An alternative theranostic paradigm is the use a molecular imaging probe to select potential responders to a therapeutic. An example of such a theranostic would be the hypoxia tracer, [F-18]fluoromisonidazole ([F-18]FMISO), used with tirapazamine to demonstrate drug targeting hypoxic tumor cells (115). Figure 5a shows a baseline [F-18]FDG PET of a patient with a primary squamous cell carcinoma and a nodal mass on the left side of the patient. Hypoxia imaging with [F-18]FMISO shows that the primary tumor at baseline is not hypoxic while the left node is hypoxic (Fig. 5b). After

Fig. 5. **a** Baseline [F-18]FDG PET images of a patient with squamous cell carcinoma of the pyriform fossa with left nodal mass. **b** [F-18]FMISO PET at baseline with nonhypoxic primary tumor and hypoxic node. **c** [F-18]FDG PET 12 weeks after chemotherapy, complete response in nonhypoxic primary tumor and poor response in hypoxic node. Hypoxic node identified for tirapazamine treatment (images from Rischin *et al.* (115))

chemotherapy, the primary tumor gave complete response whereas the hypoxic node had poor response (Fig. 5c). The [F-18]FMISO imaging helped guide the patient to tirapazamine treatment, which is cytotoxic in hypoxic conditions.

PPPs combine expertise and resources from academia and industry which otherwise would be difficult to marshal separately. A PPP for imaging in drug development could leverage the academic network within a PPP to carry out efficient, standardized clinical trials (e.g., the SNM Molecular Imaging Trials Network). Imaging companies could provide the platform technologies for standard GMP synthesis, imaging, image analysis, and data handling, while Pharma's therapeutic drugs and GCP clinical trials provide the therapeutic intervention. Collectively, all three parties bring their own experiences and resources into dealing with the FDA and other health authorities.

Partnering Between Pharma and Imaging Companies

The failure rate of new drug candidates and increased development costs are well known but could be better addressed with molecular imaging. Three out of four new chemical entities under development as drugs fail in clinical trials; development costs are increasing and there are increasing demands for reducing preclinical animal experiments. These factors are promoting concepts to improve early screening procedures in humans. The imaging companies' development of semi-automated rapid synthesis of PET tracers allows a potentially large number of new drug candidates to be used as probes in drug trials using PET studies. Additionally, PET microdosing could be a means for imaging experts and Pharma to work together to select or reject labeled drugs based on *in vivo* performance in man. Because only very low amounts of drugs are used in PET microdosing studies, a complete preclinical package with limited toxicity assessment has been proposed for the regulatory framework of PET microdosing (111,116). PET microdosing of nonhuman primates and human subjects along with tracer kinetic modeling of labeled drugs can accelerate pharmacokinetics/pharmacodynamics studies with an emphasis on understanding drug mechanisms of action. Collaborations between Pharma and imaging companies involving PET microdosing will hopefully drive the development process towards an increased learning mode thereby improving cost-effectiveness.

Another compelling opportunity for imaging and Pharma companies to cooperatively enhance probe development is through the conversion of failed therapeutic drugs into molecular imaging probes. Abandoned drug candidates and families of compounds, which failed due to toxicity or short biological half-life, can be considered as potential PET or SPECT probes. The sensitivity of PET imaging, for instance, allows for the safe use of very low concentrations (picomolar) of compounds that are toxic at higher concentrations. Rapid clearance of a compound also is ideal for a PET tracer that should have rapid uptake and clearance by target organs in order to quickly remove background signal in PET images. Considering the large chemical libraries underlying a therapeutic drug candidate, molecular imaging probe development could be accelerated if failed drugs and related drug libraries can be mined for potential imaging probes (5).

CONCLUSION

The parallel paths for the development of therapeutic drugs and diagnostic agents raise exciting opportunities for technology convergence, where applications from both domains converge (e.g., theranostics) and technologies from one domain can accelerate processes in the other (e.g., PET microdosing). The iterative discovery process for PET and SPECT tracers allows for optimization of new probes via preclinical studies using the same tracers that are translatable to human studies. Similarly, therapeutic drug development can raise the value of imaging by incorporating tracers and imaging biomarkers into pharmaceutical drug trials. The unbundling of the drug and diagnostic agent value chains allows for possible innovations through cooperation between Pharma and the imaging industry.

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