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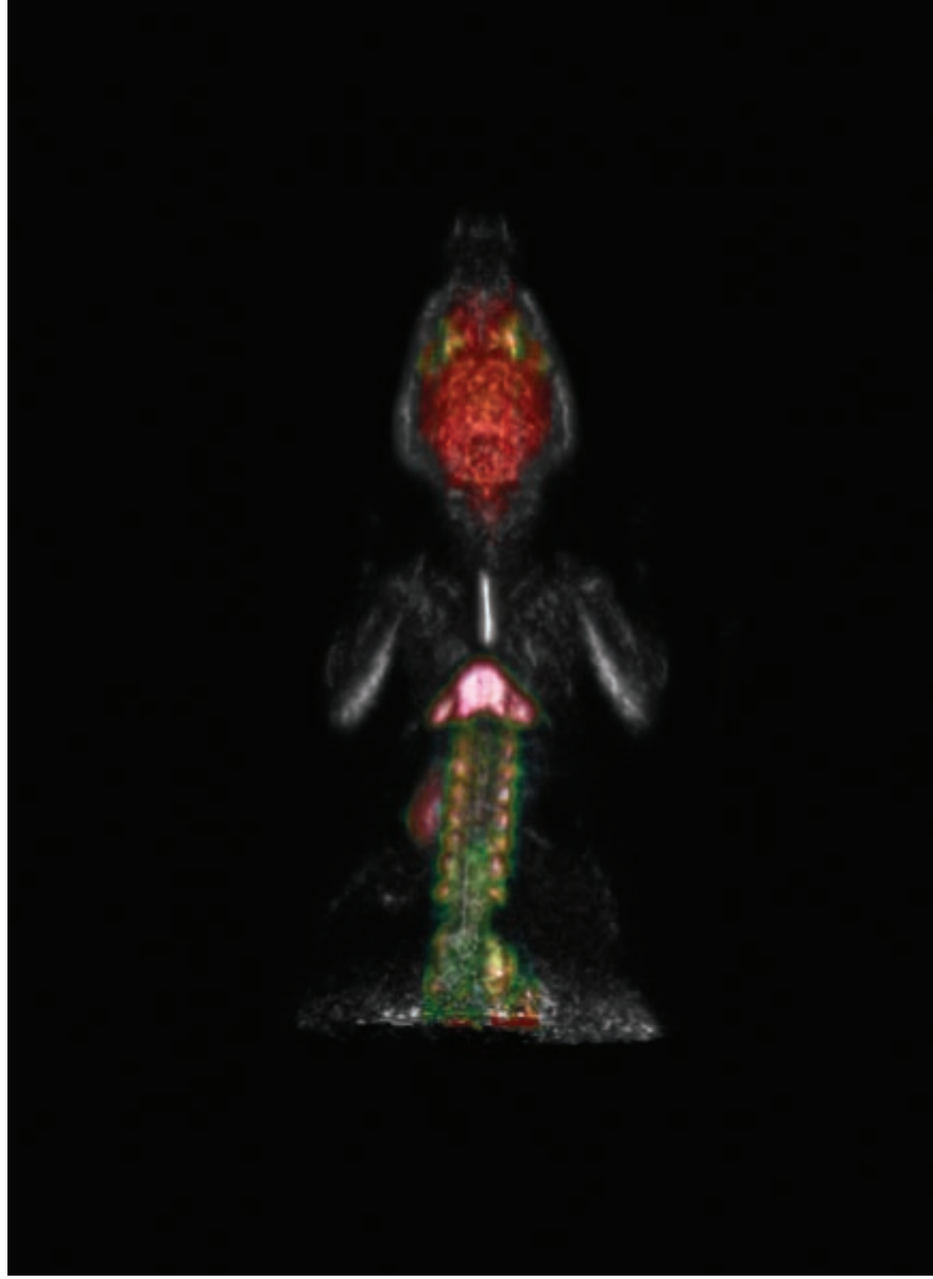

Applications of preclinical imaging and biomarkers in drug discovery and development

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Editorial Summary

Santosh Arcot, Ph.D

Marketing Manager
Molecular Imaging, Siemens Healthcare

Siemens is proud to sponsor the following four mini-review articles that will educate the reader on the emerging role of molecular imaging in the drug development process. Currently, the cost of bringing a single drug to market from conception to regulatory approval can run upwards of $1B, and the costs continue to rise! This cost is ultimately passed to the consumer, and contributes to the worldwide trend of rising costs in healthcare (healthcare costs in 2020 are projected to be 30% of GDP in the U.S. alone!) Molecular imaging is starting to move from the early adopter stage to a proven, cost-effective way that can drive the costs of drug development down, with no compromise to the safety and efficacy of the process.

The first review article by Dr. J. Paul Shea provides an excellent overview of the fundamental questions associated with using molecular imaging in the drug development process, both during the preclinical (small animal) and clinical phases. Dr. Ward Digby provides a general overview of biomarker development, describes the important role it plays in drug development, explains the current state-of-the-art molecular imaging techniques and gives us a glimpse of what the future may bring in the second article. In the third article, Dr. Paul Acton explains the benefits of using molecular imaging from a large pharmaceutical perspective, while Olivier Duchamp gives us the CRO perspective in the fourth article. The last two articles also address the challenges, needs and future directions that still need to be overcome before molecular imaging is a fully proven technology and adopted fully by this market segment. We are indebted to the authors for sharing their unique perspectives and ideas on this topic.

Siemens will continue its excellence in innovation by offering the most comprehensive product portfolio in the molecular imaging field. From cyclotrons, radioisotope distribution and biomarkers, to small animal and clinical imaging scanners, we provide all the essential tools for applications of imaging technology in drug discovery and development.

We hope you will find these articles both educational and beneficial.

Non-invasive Imaging in Drug Discovery and Development — Where to Begin?

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Introduction

There has been much publicity and discussion concerning the role of non-invasive imaging techniques in drug discovery and development. I, like many, believe these are process transforming tools that have the potential to fundamentally alter our approach to the challenges we face as drug developers. As I travel and discuss these technologies with my colleagues in therapeutics discovery and development, a common set of questions emerges.

- **What will be the long term utility of these technologies in therapeutics development?**
- **In which technology do I make my initial investment?**
- **Where in the discovery — development timeline do these technologies belong?**
- **What are the current uses of these technologies in the field?**

What will be the long term utility of these technologies in therapeutics development?

Over the past few decades, multiple new technologies have been introduced into the field of drug discovery and development. These have included computer aided drug design, combinatorial chemistry, high throughput screening, high sensitivity analytical tools and a whole host of -omics. In each case, their introductions began a sequence of euphoria, predictions of revolutionary impact, dismay and doubt and, finally, a proper role in the process. It is a logical progression, given that these tools originated in academic settings, where initial objectives were more pure than applied science. Accuracy, reproducibility, ruggedness and applicability to the drug development process were initially unanswered questions. Is non-invasive imaging a different story?

Consider that anatomical and functional imaging technologies have seen clinical use for decades. Indeed, anatomical technologies such as X-ray, CT (computed tomography), ultrasound and MRI (magnetic resonance imaging) are diagnostic staples of current clinical practice. Likewise, functional imaging modalities have found broad clinical use in cardiac function testing and in oncology disease diagnosis and staging. These decades of experience have progressed an understanding of the utility of these methodologies to clinical assessment, disease progression monitoring and treatment response. Advances in clinical understanding have been accompanied by technological advances in instrument performance (speed, sensitivity, resolution and image reconstruction software — resulting in improved image quality, visibility and interpretation. Non-invasive imaging is a mature technology with proven clinical applicability. What is new is the ability to extend this technology to in vivo specimens as small as a mouse, with species scaled sensitivity and resolution comparable to the clinical setting.

The obvious opportunity, then, is the direct translational applicability of these technologies. With the existing clinical infrastructure, it can be a straightforward process to introduce imaging endpoints early in a clinical development plan. From this translational perspective, one can envision the preclinical setting as ideal for establishing the relationship between disease, treatment and imaging endpoint. What technology/biomarker to scan with, what parameters to measure, the optimal scanning protocol, etc. can all be addressed in a well designed preclinical imaging plan. Proper late stage use of imaging endpoints in the preclinical environment not only provides critical clinical planning data, but also firmly establishes a link between the preclinical safety and efficacy database and parallel early clinical data.

The less obvious role, then, becomes molecular imaging's involvement in the discovery process. While perhaps less obvious, it is also potentially the point of greatest impact on any drug discovery program — i.e. establishing the validity and relevance of novel biological targets. As discussed below, throughput issues prevent imaging from contributing to large scale compound screening, but do not hinder its ability to probe early biological data. Drug discovery programs, particularly those aimed at unique, novel targets, proceed with a hypothesis of the relationship between target, target modulation and impact on disease. Since functional imaging presents the opportunity to measure physiological processes in intact, living animal, it can clearly play a role in demonstrating target validity and cross species comparability. Molecular imaging endpoints focused on measuring target modulation effects, i.e. drugability, can contribute greatly to ensuring proper investments in unproven targets.

So, while non-invasive imaging has a clear role in supporting translational research efforts, it also represents a uniquely powerful method of establishing the validity of novel therapeutic intervention. As ongoing clinical research further establishes molecular imaging's role in translational medicine, it will take more time, effort and, most importantly, data to establish molecular imaging's final utility in the overall process.

In which technology do I make my initial investment?

The commitment to creating an in-house molecular imaging center is a large one, particularly if it is to be a multi-modality facility. The power of multimodality imaging makes it difficult to envision a single technology laboratory. In addition to dedicated laboratory space, there are significant costs associated with instrumentation and hiring of skilled personnel. If functional imaging is an objective, there will be significant investments in radiochemistry facilities, trained radiochemists and isotope generation. The starting point must be potential applications in your organization's areas of interest, as early proof of value will support future growth and investment.

Choices for anatomical imaging modalities include CT, MRI and ultrasound. While ultrasound is the low cost entry point, its applicability is limited by depth of penetration, image quality and no opportunity for image fusion with functional imaging platforms. CT represents the intermediate cost option and can visualize bone and, with contrast agent, soft tissue. CT is also the most common anatomical reference data fused with functional data from PET (positron emission tomography) or SPECT (single photon emission computed tomography) instrumentation. Indeed, multiple manufacturers are now offering a single platform capable of acquiring hybrid PET/CT or SPECT/CT images. MRI represents the high cost option, offering the highest special resolution and best soft tissue imaging. There is currently no available instrumentation capable of single-pass MRI/PET or MRI/SPECT images, although image fusion software is available to create these images.

Functional imaging modalities include optical (bioluminescence and fluorescence), PET

and SPECT. Optical imaging represents the low cost option and is capable of efficient, high throughput functional assays in rodents. Optical technology is an excellent introduction into the conceptual arena of molecular imaging, but is limited by depth of penetration, 2-D images and a lack of applicability in translational studies. The nuclear imaging methodologies PET and SPECT represent high resolution, high sensitivity functional methodologies well suited for translational applications from mouse to man. PET and SPECT each possess relative strengths and weaknesses. PET isotope and tracer availability is more limited than in SPECT, but PET is essential for imaging small molecule drugs, due to its available isotopes. SPECT, on the other hand offers easier access to longer lived isotopes which are well suited for labeling large biomolecules (peptides and antibodies).

For an introduction into the power of functional imaging, optical imaging would be hard to argue against. For the most general range of applications, CT combined with PET or SPECT would be the most appropriate, with PET offering small molecule studies and SPECT more applicable in a large biomolecule oriented laboratory. For the uncertain, the anxious or the under funded, outsourcing opportunities in commercial and university entities are available that would allow the incorporation of imaging endpoints into any preclinical development program.

Where in the discovery — development timeline do these technologies belong?

The discovery – development timeline has very different requirements as the compound selection process progresses. In early lead discovery, the number of compounds screened can be on the order of 10^5 or 10^6. While high throughput screening is impedance-matched to these numbers, molecular imaging technology is not. Animal handling, image collection times and image processing all contribute to the limited throughput of imaging technology. Imaging is not an appropriate general screening tool. It does, however, represent an ideal platform for early systems biology measurements. A drug discovery program is a triad of therapeutic, disease and target. Novel targets resulting from genomic or proteomic studies often have an uncertain relationship to a specific disease. Additionally, a perfectly acceptable target from a biological perspective is of little value if it is not drugable, i.e. if its action cannot be modulated by an external therapeutic. Imaging is an ideal platform to assess these target biology questions. By focusing functional imaging endpoints on presumed target effects, the validity of the target can be probed in normal and diseased models, across species and, potentially, in early clinical trials. The longitudinal, whole system datasets generated by non-invasive imaging provides a platform for investigating the time course relationship between target, disease state and therapeutic. Such data can be extremely valuable in interpreting early clinical results.

On the other end of the timeline, the translational potential of molecular imaging technologies is evident. As development programs mature, key questions of pharmacodynamics, efficacy and toxicity often emerge. These key questions and the data generated to answer them often determine the success or failure of the program.

- Is the dose-response curve in man similar to that in the preclinical model species?
- What is the dose relationship between preclinical models and clinical disease state?
- What stage of disease, if any, is optimal for therapeutic intervention?
- Is a side effect as likely to be seen in man as in the toxicology species?
- What are my clinical patient selection criteria? How uniform is my disease population?

A well designed preclinical imaging program has the potential to establish quantifiable endpoints upon which to answer these types of translational questions. Early or late in the process, non-invasive imaging has the potential to contribute uniquely and concretely to the therapeutics discovery process.

What are the current uses of these technologies in the therapeutics discovery field?

Non-invasive imaging techniques are seeing increasing utilization in the therapeutics discovery field. Interest in the technology has been spurred by the FDA Critical Path Initiative and Exploratory IND guidelines. Large pharmaceutical companies are making investments in preclinical and clinical imaging centers to facilitate the inclusion of imaging endpoints throughout the development process.

Perhaps the most versatile platform for probing biology with imaging is that of gene expression monitoring. The unique flexibility of this platform is the result of linking molecular biology with a quantifiable visualization method. Utilized with functional imaging technologies (optical and nuclear), the expression of a particular enzyme is directly monitored. The expression can be linked to a specific promoter or to a sequence of interest. Since the visualization method is used repeatedly (i.e. luciferase or thymidine kinase expression), the use of a single tracer can probe multiple processes quite efficiently. Due to the fact that flexibility and targeting is the result of molecular biology and not chemistry, new inquiries can be generated rather quickly, utilizing a thorough understanding of the underlying biology and not tied to issues of chemistry (i.e. finding a ligand/substrate with appropriate biopharmaceutical profile and imaging signal).

In oncology research, preclinical imaging is being used both for early efficacy verification and for later stage clinical planning. Using primarily functional imaging (optical, PET), researchers are establishing non-invasive imaging endpoints as measures of efficacy in primary tumor treatment and metastatic disease. In our laboratory, we have used PET imaging in mice to assist clinicians in determining the optimal radiotracer and optimal imaging protocol for use in early clinical efficacy trials. Anatomical methodologies (CT, MRI) are being used to assess efficacy in bone diseases and other anatomically defined diseases. CT, in particular, has been used to assess compound efficacy in osteoarthritis, osteoporosis, and bone healing. Laboratories are investigating the use of CT scanning as a tool for fetal skeletal analysis.

Compound distribution and pharmacokinetics are areas of concentration for nuclear methodologies. These methods provide unique, continuous distribution datasets that can be extended into the clinic. Using appropriately labeled compounds, targeted antibody therapies can be rapidly assessed in preclinical and clinical settings. Labeled small molecules can provide early clinical distribution data to support compound selection, i.e. Phase 0 studies in man. Labeled small molecules are also seeing wide application in CNS (central nervous system) research, where brain receptor occupancy studies are being used to establish clinical dosing regimens. In our laboratory, we have used PET distribution studies to make a rapid assessment of compound behavior in the rat. These data provide a time and cost-effective means to establish key time points and organs for more traditional and/or detailed distribution studies.

Summary

Non-invasive imaging technologies are seeing increasing utilization in the drug discovery and development process. Across therapeutic areas, across program timelines, imaging endpoints are showing promise as quantifiable measures of compound efficacy and disease response to treatment. Access to these technologies is increasing, through the establishment of in-house imaging resources and CRO's (contract research organizations). Choose a challenge, select a technology and start collecting data.

Imaging Biomarkers in Drug Discovery

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Introduction

Imaging biomarkers play a central role in the application of preclinical imaging to drug discovery. The biochemical characteristics of the imaging biomarker determine the nature of the molecular process being assessed. Molecular imaging can play a role in several different aspects of drug development, with each aspect potentially requiring different characteristic for the tracer. There are a variety of different molecules, radioisotope labels, and techniques to fit a wide range of applications.

Interconnected landscape

Molecular imaging is a powerful yet complex technique requiring a number of different aspects to come together in a coordinated fashion. The landscape of entities developing and using molecular imaging is also complex (Figure 1). The application of molecular imaging in drug discovery in particular requires close coordination with radioisotope production and radiopharmaceutical synthesis, to enable characterization of the desired molecular process, and preclinical imaging systems, for acquiring data on the animal models of disease. Advanced domain knowledge in one area of molecular imaging can help drive innovation in the other related aspects.

Nuclear medicine / PET (positron emission tomography) techniques in drug development benefit from translation to clinical studies, so the ability to go easily from animal studies to humans is also very important. This synergy, with linked development all under one roof, helps move new technology ideas from the small animal world to the full size imaging systems. It is important to be in a leadership position in each of these areas in order to be able to drive progress for the field as a whole. In particular, the new technology developed in preclinical imaging systems can be adapted for use in the human systems, and research tracers may in some cases evolve to become clinically used. The fundamental understanding in how these parts interconnect also leads to better development of a complete solution.

Roles for molecular imaging in drug discovery

There are several aspects to molecular imaging in drug development (see Table 1). Molecular imaging has played an important role in understanding many disease processes from early discoveries on cardiac metabolism (1) to recent findings on addiction and the brain (2).

Lead optimization involves new candidate drugs. One of the areas where molecular imaging can add to the understanding is by directly labeling the drug (generally with ^{11}C) and following the time course of where the drug goes in the body. If the radioisotope can directly substitute for the stable isotope in the molecular structure then the chemical properties will be virtually identical. One of the key advantages of preclinical imaging in biodistribution studies is that individual animals can be used to track how the distribution evolves over time. Traditional methods involve many animals, each sacrificed at a specific time point, to gain the knowledge on the kinetics.

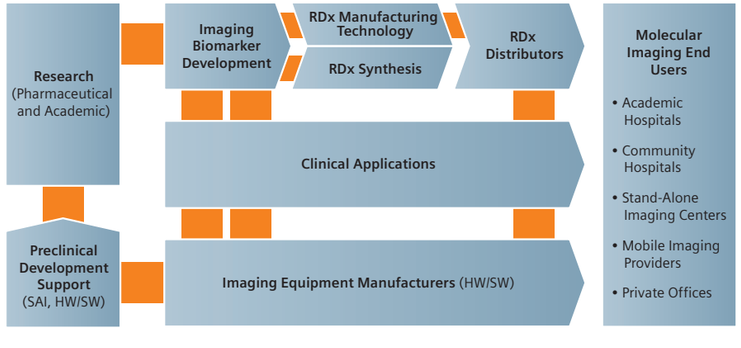

Figure 1. Molecular imaging landscape

Another aspect of early drug development that can be enhanced with preclinical imaging is determining the route and rate of clearance of the drug candidate. Since the images will show the accumulation in the bladder (for renal clearance) this can be quantified by acquiring a series of images over time and analyzing the activity levels. Binding can also be determined for drugs, both by measuring the rate the imaging biomarker attaches to the target of interest and characterizing the rate of decoupling (if any). This can be further enhanced through the use of competitive binding, where other established ligands are used with the same receptor system – the relative rate of "blocking" of the receptor can determine the binding affinity for the new drug. One of the most common applications of this method is in neuroreceptor binding where a PET imaging agent is used to compete with the drug, which allows the relationship between blood concentration of the drug and receptor occupancy to be established. Once relatively high occupancy is achieved if there is little or no efficacy, then the candidate can be rejected for this application.

In the clinical development world, there is a great deal of interest in surrogate endpoints which are "expected to predict clinical benefit (or harm, or lack of benefit) based on epidemiologic, therapeutic, pathophysiologic or other scientific evidence" (4). Short of a validated surrogate endpoint, a biomarker can provide early insight into what is going on with a drug. These include the use of FLT (^{18}F fluorothymidine) to characterize the transport of nucleosides as a measure of cellular proliferation (6). A number of pharmaceutical companies are using FLT during clinical research to gain an early perspective on the effectiveness of new drugs in development.

Basic science	Understanding biology, target identification
Lead optimization of new drug candidates	Biodistribution, clearance, and binding in preclinical studies
Clinical development	Biomarkers and eventually surrogate end points to simplify and accelerate clinical trials
Commercialization	Identification of the appropriate patient population
Clinical use	Monitoring therapeutic response

Table 1. Various steps in the drug development process and corresponding molecular imaging applications in each step of the process.

A now "classic" example of the use of an imaging biomarker in drug development is Merck's study of NK1 receptor occupancy for a drug that was hoped to be effective in depression — even at high receptor occupancy levels it did not show efficacy (7). Without the PET imaging it would not have been possible to directly link the blood levels of the drug with receptor occupancy. The drug was effective, however, to control nausea caused by chemotherapy and the PET data is mentioned in the package insert (8).

For commercialization of a new drug, selecting the optimum patient population to maximize treatment effectiveness is a worthy objective. While this is becoming more common using tissue-based testing (HER2 characterization before Herceptin treatment, for example) it is still an emerging area for molecular imaging. It may be possible at some point to characterize the estrogen receptor status of a primary breast cancer and various distributed metastases (9) or to determine if a patient is appropriate for anti-VEGF therapy based on the rate of angiogenesis (10).

Therapy monitoring is becoming more extensively used clinically with the widespread availability of FDG (fluoro deoxy glucose), a glucose analog used in PET. This imaging biomarker has been shown to provide valuable feedback in a number of cancers, although consensus on protocol specifics and formal validation is still needed (11). Some of the new tracers in development are also hoped to provide valuable information during therapy such as the hypoxia or angiogenesis status, cellular proliferation, and apoptosis.

Imaging biomarkers

Most of the common PET radioisotopes are produced in low energy cyclotrons, either on site or at a nearby commercial supplier (Table 2).

The most commonly used radioisotope is ^{18}F and the nearly two hour half life allows for distribution within and around most metro areas in the U.S. Internationally, there are a number of smaller suppliers so commercial access is still frequently possible. The shorter half-life cyclotron products need to be produced close to the imaging system, and the chemistry needed to attach the isotope to the molecule of interest needs to be rapid and efficient. Most of these products, with the notable exception of [^{13}N]NH_3, are used only at research centers.

Several PET isotopes are available from a generator, so the parent compound is supplied on a regular basis and the generator daughter product can be obtained whenever needed – this is the model for ^{82}Rb used for myocardial perfusion studies. The other generator products are not yet FDA-approved and commercially available.

Most single photon nuclear medicine isotopes tend to have longer half lives and are ordered from commercial suppliers (Table 3).

The most common single photon isotope is ^{99}mTc, although for preclinical work the longer half lives of ^{123}I and ^{111}In can be valuable. The radioisotope is sometimes used by itself, as is the case for the ^{82}Rb and ^{18}F negative ions in PET and ^{201}Tl in SPECT, but generally they are incorporated into biologically relevant molecules. This is done either in an automated radiochemistry synthesis system (PET) or in a kit (single photon) based on a freeze-dried precursors. A large number of interesting molecules can be made, to characterize just about any biological system. The National Institutes of Health maintains a database with characteristics of many of these imaging agents (3).

Current imaging biomarker availability

PETNET Solutions, a Siemens company, offers FDG at all 50 cyclotron-equipped radiopharmacies worldwide. FDG is a powerful imaging agent with broad applications in drug development (5). PETNET also offers other imaging biomarkers that may be useful for drug discovery efforts at selected locations (Table 4).

Challenges

Utilizing PET as a molecular imaging technique in drug development has several key advantages:

- availability of positron-emitting isotopes of C, N, O, and F* allows the preparation of labeled drugs with chemical composition close to or identical to the actual drug substance or a key substrate
- high sensitivity means low amount of tracer needed for PET and allows the translation of promising animal techniques into human studies
- new generation of preclinical imaging systems deliver performance equivalent in scale to clinical systems in humans*

* F is often substituted for a H atom in the original molecule, which can change the chemical properties.

Isotope	Half-life	Production
^{124}I	4.1 days	Cyclotron
^{64}Cu	12.8 hr	Cyclotron
^{18}F	110 min	Cyclotron
^{11}C	20.4 min	Local cyclotron
^{13}N	9.96 min	Local cyclotron
^{15}O	120 sec	Local cyclotron
^{68}Ga (^{68}Ge)	68 min(278 days)	Generator
^{82}Rb (^{82}Sr)	75 sec (25 days)	Generator
^{62}Cu (^{62}Zn)	9.7 min (9.3 days)	Generator

Table 2. Common PET isotopes

Isotope	Half-life	Energy
^{131}I	8.1 days	364 keV
^{67}Ga	3.3 days	93, 185, 300 keV
^{201}Tl	3.0 days	60-80 keV x-rays
^{111}In	2.8 days	172, 247 keV
^{123}I	13 hr	159 keV
^{99}mTc	6.0 hr	140 keV

Table 3. Common single photon radioisotopes

There are, however, certain challenges associated with PET in drug discovery-

- isotope production, radiochemistry, and in vivo imaging each require complex equipment and specialized skills
- all these disciplines must work together in a highly efficient manner due to the short half lives of PET isotopes
- full capability remains restricted to leading academic institutions and large pharma – except for FDG the ability to obtain other tracers commercially is currently limited.

Future solutions

Siemens is committed to providing solutions that address these challenges to enable further growth in the use of molecular imaging biomarkers in preclinical imaging for drug discovery. We are expanding the number of sites in the network, both in the U.S. and internationally, and increasing availability of interesting new imaging biomarkers through PETNET Solutions and through local synthesis solutions.

Siemens also recently announced that the National Cancer Institute has filed a cross-reference letter to a Type II Drug Master File that Siemens submitted to the U.S. Food and Drug Administration for its investigational 3'Deoxy-3'[^{18}F] Fluorothymidine (^{18}F-FLT) imaging biomarker. The cross reference allows ^{18}F-FLT to be produced by Siemens / PETNET and used by any principle investigator in an NCI multi-center clinical trial. In addition, the imaging biomarker will be available to independent investigators if they receive NCI approval. FLT is produced by Siemens Molecular Imaging Biomarker Research (MIBR) and PETNET Solutions — subsidiaries of Siemens Medical Solutions USA, Inc. The imaging biomarker has been used in studies monitoring the proliferative activity of cancer cells and it is currently being used in Phase I clinical trials at several research sites.

For preclinical imaging, there are a number of additional research compounds available from the Siemens MIBR group at several sites. We also offer fully automated radiochemistry synthesis systems for users that wish to produce compounds of interest locally using delivered ^{18}F. We see the future of preclinical imaging for drug discovery to be very promising, and will continue to develop solutions that enable our pharma and academic partners.

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Biomarker	Applications
^{18}F-NaF	Higher sensitivity than planar MDP bone scan and better image quality
^{18}F-F-DOPA*	Parkinson's disease, neuroendocrine tumors, brain tumor imaging
^{18}F -FLT	Investigational new drug for measuring tumor proliferation, treatment monitoring
^{82}Rb	Myocardial perfusion imaging

Table 4. Other PET imaging biomarkers from PETNET, in addition to FDG, depending on location and their applications

* Not recognized by FDA as being safe and effective for diagnosis of Parkinson's disease.

Preclinical Imaging and Increasing Efficiency in Drug Development

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Introduction

The classical role for imaging was as a diagnostic tool in the clinic, particularly for diseases such as cancer where it could be used to detect and stage tumors, and measure response to therapy. More recently, advances in molecular imaging technology, and the development of specialized small animal imaging equipment, has led to a major shift in the role of imaging. In addition to being used for clinical diagnosis, molecular imaging now has a major role to play preclinically in the study of animal models of disease. The rapid advances in transgenic models of human disease have been the driving force behind the development of preclinical imaging equipment which complements that available in the clinic.

One of the biggest sectors to take up preclinical imaging is the pharmaceutical industry. The power of non-invasive, in vivo imaging for the development of novel drugs was recognized very early by pharmaceutical industry leaders. The driving force behind much of the development of imaging in drug discovery has been the need to reduce the costs and improve the success rate of the pharmaceutical industry. With the costs of developing a new drug reaching $2 billion (1), and the chances of success for a new drug submitted for regulatory approval dropping to 1 in 12, many pharmaceutical companies have turned to imaging to improve the success rate, while lowering costs. This has required a paradigm shift in philosophy on the part of many therapeutic area teams, requiring imaging to be applied early enough in the development pipeline for it to make an impact, and for the results that are generated to be accepted and acted upon. However, care must be taken in choosing suitable targets and applications in which imaging can make a contribution, keeping in mind that it may not be applicable everywhere.

Imaging in drug development can be applied to measure drug target expression and activity, using a suitable radiolabeled ligand. Alternatively, using a "label the drug" approach, the biodistribution and pharmacokinetics (PK) of the drug can be measured directly. The interaction of the drug with the target can be monitored, and the dose-response relationship derived, which will guide dose optimization in future clinical trials. From a pharmacodynamic (PD) perspective, imaging can be used to measure early response to a novel therapeutic, provide a biomarker of efficacy, and monitor disease progression or response to treatment over time. The key to molecular imaging having an impact on the drug development process is the ability to translate these results directly to the clinic, with little or no modification. This translation of molecular imaging from preclinical to clinical is virtually unique, enabling the chasm between animal and human studies to be bridged.

Drug discovery and development is an inherently inefficient process, requiring hundreds of targets to be tested and validated, and sometimes millions of compounds to be screened for activity at that target (2). Of those compounds screened, a few show some activity at the target, while fewer still will demonstrate efficacy in an animal model of the disease. Drug safety and tolerability whittles this number down to a handful, with perhaps one or two making the leap to human trials. Clinical trials are the most expensive aspect of drug development, and can take many years and hundreds of millions of dollars before the candidate is ready to be submitted for regulatory approval. Most will fail this final hurdle.

Now imagine if an imaging program was established to measure the behavior of the drug at the target in parallel with the development of the drug itself. It is important to realize that imaging is not a "high throughput" technique – it will never replace screening of compounds in traditional HTS (high throughput screening) methods. However, the selection and validation of the target can be enhanced by adding an imaging component to the process. For relatively little cost, a molecular imaging probe can be developed for the target, which will be used in subsequent in vivo studies to test the interaction of the drug candidates at the target, and may provide a novel biomarker for later clinical trials. Once the high throughput methods have whittled the candidates down to a few potential molecules, an additional imaging program could be established to label the drugs themselves to measure biodistribution. This can have a tremendous impact on the likelihood of the drug moving forward, both from a PK and a safety perspective. The advantage of initiating the imaging process at such an early stage in drug development is that as soon as the candidates move into in vivo animal models, imaging is right there to provide vital information on PK, PD, target interaction, mechanism of action, safety, and dose-response. Some of the development required for imaging must be done at risk, but the costs are fairly minimal. The danger of leaving this development until much later is that the imaging program is always playing "catch up" to the pharmacology program, and is less likely to make a significant impact.

Ultimately, preclinical imaging should provide sufficient information on a candidate drug that the investigators have confidence in moving forward to clinical trials, or can make the decision to kill the drug before the expense of a clinical trial is incurred. In addition, once the step has been made into human subjects, imaging follows the drug into the clinic, and provides vital information at every phase of clinical trials. Now the aim is to add to the safety and efficacy package, or to give a rapid readout that the drug is not working as expected. Like preclinical imaging, clinical imaging studies can enhance the likelihood of success, or prevent unnecessary, and expensive, trials from moving forward.

Examples of imaging in drug development

Biodistribution and PK

The standard method for using molecular imaging to monitor the biodistribution and PK of a drug is to label the drug, either with radioactive isotopic substitution, or possibly using a fluorescent tag. In this way, imaging with PET (positron emission tomography) or optical techniques can be used to measure the distribution throughout the body, or in a specific tissue or organ of interest. The data are collected over time in an anesthetized animal, usually accompanied by blood sampling and metabolite analysis.

An interesting and slightly unconventional application of this technique was to study the distribution in the lungs of aerosol-delivered drugs, such as steroids for asthma (3, 4). The traditional methods of measuring in vivo PK and PD often yield inconclusive results when applied to aerosol delivery, due to the difficulty relating local delivery to systemic plasma exposure. Using imaging, a direct, three-dimensional, quantitative measure of aerosol delivery can be obtained in vivo. These studies developed a radiolabeled version of triamcinolone acetonide (TAA), an inhaled steroid used for topical treatment of allergic rhinitis and asthma, using ^{11}C (5). The PET images were combined with anatomical CT (computed tomography) data to provide an accurate map of the distribution of ^{11}C-TAA in the airway tree (6). The most significant finding of this study was that the inhalation spacer built into the inhaler had a dramatic impact on the delivery of drug to the lungs, increasing deposition of ^{11}C-TAA by a factor of 2 – 5 times (Figure 1). Consequently, the manufacturer was able to demonstrate a clear benefit of the spacer, which has now become the standard of care. It is worthwhile emphasizing that the data required to completely changing the way aerosol delivery was performed for this product came from as few as five PET scans. Although these studies were done in human subjects, if similar studies had been performed much earlier in the development cycle using preclinical imaging, it could have given a significant improvement in the way this product was used by patients.

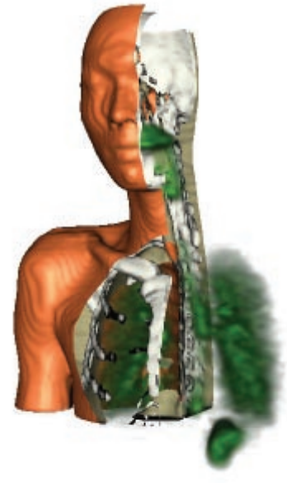

Figure 1. Three-dimensional visualization of aerosol deposition of radiolabeled TAA. The green area indicates the aerosol distribution after inhalation as seen by PET, overlaid on a surface rendering of the upper torso derived from CT data. Image courtesy of Dr. Marc Berridge, 3D Imaging, Little Rock, AR, and Dr. Zhenghong Lee, Case Western Reserve University, Cleveland, OH.

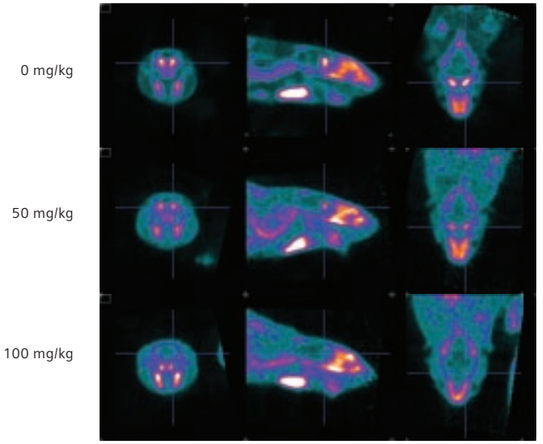

Figure 2. Occupancy of dopamine D_2 receptors by the atypical antipsychotic clozapine in rat striatum, measured using ^{18}F-fallypride and PET (the cross hairs are centered on the striatum). At increasing doses of drug, more receptors are occupied, leaving fewer receptors available for binding by the PET tracer, until at the highest dose the PET signal in the striatum is almost completely eradicated. The fitted ED50 (dose required to occupy 50% of striatal receptors) was approximately 50 mg/kg.

Receptor occupancy

Measurement of the occupancy of the target binding site or receptor at different doses of drug is one of the most important applications of molecular imaging, enabling the efficacious dose to be established with a high degree of accuracy. This has the potential to optimize the dose for efficacy rather than optimizing for tolerability, which should require lower doses and reduced exposure. If this type of study can be performed preclinically, the dose range for effect can be set long before the drug is used in human subjects, and the same dose-occupancy imaging study can be performed in a clinical trial, using the results of the animal studies as a guide.

Antipsychotic medications have been the most widely studied drugs using PET and SPECT (single photon emission computed tomography) imaging, both preclinical (7, 8) and clinical (9-17), mainly due to the widespread availability of suitable dopamine D_2 receptor (D_2R) radioligands (18, 19). With the higher affinity D_2R tracers, such as ^{18}F-fallypride (20), occupancy of both striatal and extra-striatal receptors can be visualized. A typical example of the measurement of preclinical receptor occupancy is shown in Figure 2, for the classic atypical antipsychotic clozapine. It is well known that a certain occupancy is required in the striatum to achieve efficacy, while too high an occupancy leads to extra-pyramidal side effects (9). Imaging provides the key piece of data which enables these drugs to be dosed to maximize efficacy, rather than dosed for acceptable tolerability.

Mechanism of action and PD

In some cases drugs may exhibit efficacy in an animal model of a particular disease, but there is no known or obvious target with which the drug is interacting. Indeed, the drug may exhibit low affinity for multiple targets, but no clear binding to any specific site. However, when taken as a whole, the small effects on a range of targets could combine to give a large efficacious response. Further, due to this complex interaction with multiple binding sites, the drug may exhibit efficacy for a number of different diseases over a range of doses. Consequently, an imaging biomarker of mechanism of action and PD response would add considerable value to the program, and potentially accelerate the development cycle.

The conventional method for establishing brain penetration of a drug to the target site would be to label the drug and measure the biodistribution. However, this does not necessarily indicate target engagement, nor does it demonstrate any functional change in state of the brain in response to the therapeutic. Consequently, an imaging technique that can provide information on brain penetration and functional response would add significant value to the program, and provide clues to the mechanism of action.

Figure 3 shows an example of this technique, using PET and ^{18}F-FDG (fluoro-deoxy glucose) to measure changes in cerebral glucose metabolism in a rat in response to a painful stimulus. These images show increased metabolic activity in the thalamus, a brain region known to be involved in pain

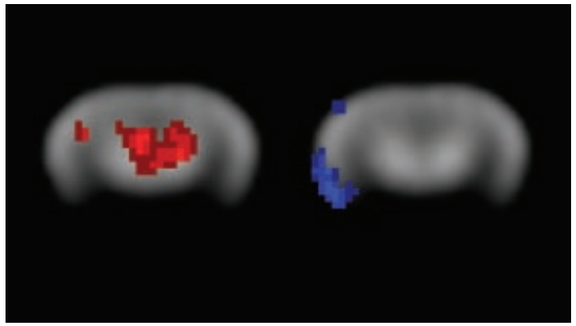

Figure 3. Statistical parametric map (SPM) of the uptake of ^{18}F-FDG in the rat brain in response to a painful stimulus. The image represents a single coronal slice through the brain (shown in gray-scale), overlaid in color with those brain regions that exhibit statistically significant changes in glucose metabolism in response to the pain (red increased metabolism, blue decreased).

processing, and decreased metabolism in the somato-sensory cortex on the side contralateral to the stimulus. This could be used as a potential imaging biomarker of response to pain, which may be attenuated by novel analgesic drugs. While this technique is non-specific, and does not tell us precisely which targets are being engaged, it does provide valuable information on the excitatory and inhibitory mechanisms of action of the painful stimulus, and those brain regions which need to be targeted by analgesic drugs. Without this information it would be difficult to justify moving an analgesic pain program forward due to the absence of a known target or mechanism.

Anesthesia is known to have a significant impact on the uptake and retention of PET tracers (21-26), and on the underlying physiology of the animal. One additional benefit of using ^{18}F-FDG and PET is that the injection of tracer can be done while the animal is still awake, and the pattern of activity in the brain locks-in over a period of several minutes. Once the distribution is fixed, the animal can be anesthetized and scanned, with minimal impact on ^{18}F-FDG distribution. This effectively eliminates the confounding effects of anesthesia on the results, which helps bridge the gap between preclinical and clinical imaging studies.

Drug-drug interactions and drug safety

One of the most important applications for molecular imaging is in the evaluation of drug safety, and potential drug-drug interactions. During the drug development process, the interaction with various liver enzymes (cytochrome P450) is tested to demonstrate the likelihood of a drug interfering with the metabolism of other drugs. Similarly, if a drug is a substrate for certain transporters, such as P-glycoprotein (P-gp), there is the strong possibility that it may change the uptake and retention of other P-gp substrates (27). While reaction with any of these targets is likely to lead to drug-drug interactions, in vivo imaging can be applied to measure either the direct binding to the target, or the effects of that interaction in a preclinical animal model.

PET imaging of P-gp interaction has been performed using radiolabeled substrates of P-gp, such as ^{11}C-verapamil (28-31). Since verapamil is a known P-gp substrate, the interaction with another drug at the transporter will lead to increased uptake of the radiolabeled tracer into the brain. Consequently, higher brain uptake of ^{11}C-verapamil can be interpreted and quantified as binding of the drug to P-gp. The advantage of this technique, rather than an in vitro test of P-gp activity, is the measurement is performed in a living animal under real-world conditions, which will mimic those in future clinical trials. If P-gp interaction is observed in an in vivo imaging study, it is highly likely this will be an issue for potential drug-drug interaction in the future.

Future needs for streamlining and standardizing imaging

While imaging is an extremely powerful technique for studying the in vivo behavior of drugs and their targets, as mentioned previously it is not a high-throughput process. Further, the start-up costs for an imaging facility are high, particularly if radiochemistry costs are included. Consequently, there is a strong desire to improve the efficiency of imaging, by increasing the numbers of animals studied, and maximizing the return on the investment.

Increasing throughput for small animal imaging can be achieved by scanning multiple animals simultaneously – many larger-bore PET scanners can accommodate 4 rats at a time (Figure 4). This has the additional advantage of injecting multiple animals with a single dose of tracer, which is particularly important considering the short half-life tracers labeled with ^{11}C. Care must be taken with the radioactive dose in the field of view of the scanner, and with corrections applied to the data to account for photon attenuation and scatter. The rewards can be quite significant, reducing typical imaging studies from several weeks to just a few days, and reducing the costs of PET tracers by a factor of four.

This method for increasing the numbers of animals scanned now places the burden of throughput to the animal handling rather than the time taken to perform the scans. However, even minor changes in animal handling can have a profound impact on the uptake and retention of the PET tracer (25). Standardization of the entire imaging process, from the way animals are acclimated, handled, anesthetized, injected, and maintained in the scanner is absolutely vital (24, 26, 32, 33). In fact, one of the biggest hurdles to the acceptance of preclinical imaging is the lack of standard and uniform methods for performing imaging studies. Further standardization of the analysis of preclinical imaging data is required, using population-derived samples (34), template- or atlas-based methods (35-37), or automated techniques, such as SPM (statistical parametric mapping) (37-39). Until these studies are performed and analyzed using accepted standards, it will be impossible to compare results across sites or between different centers, leading to needless duplication of studies and inefficiencies.

Conclusion

Imaging has tremendous potential to impact the drug development process, although much of that has yet to be fully realized. Information on target engagement, safety, efficacy, PK, PD, and potential drug-drug interactions can be derived from imaging studies. Given careful selection of imaging targets and probes, the results generated by preclinical imaging studies can influence decisions on whether to move forward with a promising candidate, or to kill a potential drug early in the pipeline. If a drug moves into clinical development, imaging has an almost unique opportunity to follow it into humans, using the same techniques developed during the preclinical phase. However, the lack of standardization across the imaging industry, particularly in preclinical studies, could hinder the more general application of imaging to drug development.

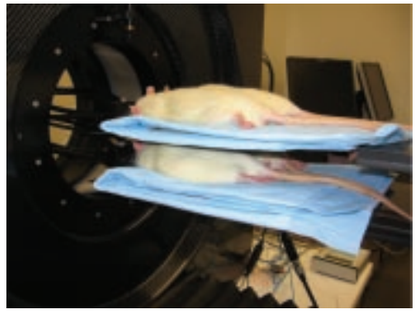

Figure 4. Multiple animal imaging in a large-bore small animal PET scanner, where 4 rats are scanned simultaneously in a 2×2 arrangement

Acknowledgements

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Integrating Pharmacology and Imaging in Preclinical Oncology Drug Development

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Introduction

The last ten years have seen major discoveries in cancer research particularly in the field of investigation techniques. The identification of original targets on which a large number of compounds are being tested in vitro leads to the emergence of new active drugs. The drug selection process is partly performed using animal models that are as close as possible to the targeted malignancy. In this context, imaging techniques using small-animal dedicated imaging devices play an essential role.

Imaging techniques designed for in vivo use include: X-ray computerized tomography (CT), ultrasound (US), magnetic resonance imaging (MRI) and spectroscopy (MRS), positron emission tomography (PET), single photon emission computed tomography (SPECT), and new optical technologies such as near-infrared fluorescence imaging (NIRF). These non-invasive modalities are increasingly used in preclinical studies using animal models to assess drug distribution or biomarker levels for tumor staging or treatment follow-up. All of these imaging modalities can accelerate the preclinical development of new drugs and some are also directly transferable from the animal model to the clinic. Among these, MRI / MRS and PET are complementary technologies allowing quick and repeated access to morphological and functional information in vivo. The selection of the imaging modality varies with the question to be answered and the performance of the imaging device (sensitivity, spatial and temporal resolutions). The main objective is to deliver new active drugs to the clinicians earlier and with more accuracy.

Clearly, there is a need to produce new drugs with novel mechanisms of action. Today, it takes approximately 12 years and $1-2 billions to bring a drug from laboratory to FDA approved product. The drug development process needs to move more efficiently and quickly while minimizing costs, to rapidly identify the most promising candidates and to identify and cease those projects that are failing before too much money has been invested. In the development of new targeted therapies, a number of key issues need to be addressed:

- **Does the drug reach active concentrations in blood and tumor to induce the intended biological effect?**
- **Does the drug hit the selected molecular target? (1, 2, 3) (Figure 1).**

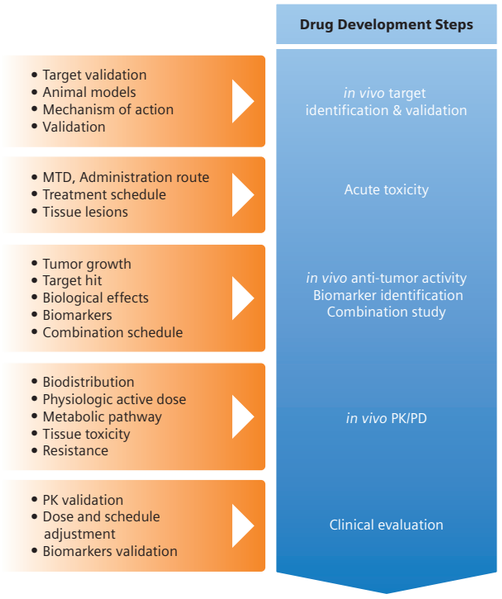


Figure 1. Drug development process. MTD is the maximum tolerated dose.

The use of imaging biomarkers in cancer drug development is rapidly being adopted by pharmaceutical and biotech companies to obtain improved pharmacological endpoints. It is especially important to establish a well-defined relationship between pharmacokinetic (PK) and pharmacodynamic (PD) properties to select the best drug candidate for clinical development. Many pharmacological endpoints in clinical routine are invasive, requiring repetitive sampling. To reduce this invasiveness and to choose the best timing for sampling, we argue the importance and potential value of functional and molecular non-invasive imaging techniques. The purpose of this review is to discuss, on the basis of examples focusing on MRI and PET, the ability of functional imaging to meet researchers' requirements and to evaluate all possibilities offered by translational research to validate and transfer these techniques from the preclinical field to the clinic.

Non-invasive imaging technologies to support the drug development process

For decades, anatomical imaging with CT or MRI has facilitated drug development in oncology by providing quantifiable and objective evidence of response to therapy. In recent years, metabolic imaging with [^{18}F] fluorodeoxyglucose–PET (FDG-PET) became an important tool for oncologists to detect treatment response earlier. MRI can assess tumor size and structure and provide functional information such as tumor perfusion and permeability of the microcirculation. Dynamic Contrast-Enhanced MRI (DCE-MRI) is based on the temporal and spatial changes in signal intensity following the rapid injection of low molecular weight Gadolinium chelates to provide information on tumor perfusion, vessel density and permeability, and blood volume. Larger molecular weight Gd-based contrast agents or iron oxide nanoparticles may also be used to evaluate blood volume, vessel size and permeability, but are not yet available for clinical trials of anticancer drugs. DCE-MRI is now systematically used for biomarker identification of the efficacy of anti-angiogenic and anti-vascular compounds (12). Diffusion-weighted MRI (DW-MRI) measures changes in the diffusion properties of water molecules in living tissue and could be used to study tumor microenvironment at a physiological level. It has been used as an early indicator of response to classical cytotoxic, chemo- or radio-therapies (4, 5, 6). At cellular and molecular levels, the current clinical imaging techniques are MRS and PET. Both techniques can be used to directly monitor drugs pharmacokinetics and biodistribution when containing appropriate nuclei with magnetic properties (MRS) e.g. 5-FU detected by ^{19}F-MRS (7) or a radionuclide (PET) e.g. ^{11}C-temozolomide (8). Endogenous metabolites measured by ^{1}H-MRS (N-acetylaspartate, citrate, choline, lactate) or to a lesser extent by ^{31}P-MRS (adenosine triphosphate, inorganic phosphate) have been used particularly in brain and prostate malignancies to quantify tumor metabolism and bioenergetic status changes during treatment (9). FDG-PET, reflecting tumor glucose metabolism, or with ^{18}F-fluorothymidine (FLT), reflecting DNA synthesis, provides relevant information regarding treatment response. Changes in tumor PET tracers uptake may precede changes in tumor size. Both FDG and FLT-PET enable early prediction of success in the treatment course and enable the determination of the viability of residual masses (10). PET can also be used to measure specific biological endpoints that are directly relevant to a particular target, for example using ^{124}I or ^{64}Cu-labeled anti-erb b2 antibody to select patients for therapy with Herceptin in the treatment of breast cancer (11). To further illustrate the role of imaging technologies in drug development, examples of our own and collaborative works will be described in more detail.

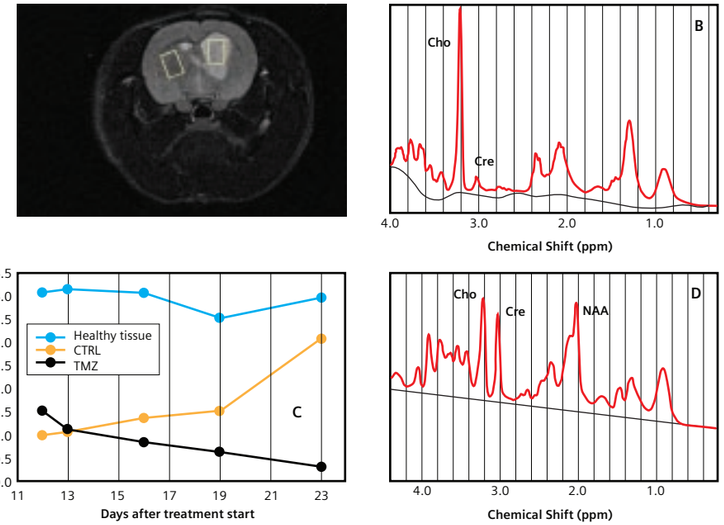


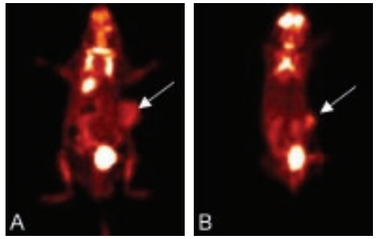
Figure 2. T2-weighted anatomical images showing localization of U-87 MG orthopically xenografted glioma in nude rats along with tumor and contralateral voxels for spectroscopic data acquisition (A). Evolution over time of ^{1}H metabolites, NAA over choline ratios (C). The NAA/choline ratio increased in the temozolomide-treated rats, while it decreased in the non-treated rats. ^{1}H MR spectrum from glioma without treatment (B) and after treatment with temozolomide (D).

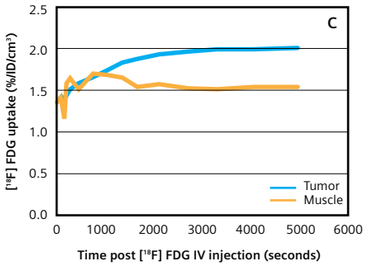
Tumor metabolism and cellular proliferation inhibition

Many anticancer treatments affect cell cycle and cellular metabolism. The most appropriate techniques to evaluate these biologic processes are proton MRS (^{1}H-MRS) and the FDG-PET for tumor metabolism and FLT-PET for tumor cellular proliferation. ^{1}H-MRS measurements of decreases in the levels of choline-containing compound following treatments have been shown to be predictive of response in brain, breast and prostate cancers (18,19). As an example, single voxel ^{1}H-MRS was used successfully to evaluate the anti-tumor activity of Temozolomide (TMZ) and radiotherapy (RT) in human orthotopic glioblastoma models in nude rats (Figure 2). A strong inhibition of tumor growth and prolonged survival were observed by TMZ treatment in both models while RT treatment had no or moderate effect on survival. The N-acetylaspartate to choline peak ratios increased significantly in TMZ treated rats, whereas it decreased in control and RT-treated rats. Monitoring tumor metabolism using ^{1}H-MRS was well suited to follow the growth of glioma and quantify the anti-tumor effect of TMZ with choline being the most pertinent biomarker (20).

FDG tumor uptake is correlated with the level of glucose transporter GLUT1 expression to take up into the tumor cells where FDG is phosphorylated by hexokinase. Glycolysis could be evaluated by FDG-PET reflecting the effect of drugs on cell metabolism. All have in mind the FDG-PET images of the first patients treated by Gleevec® where FDG uptake was significantly decreased as early as 24 hrs after the first dose, whereas tumor size reduction appeared several weeks later (21). From this day, many drugs have been evaluated by PET-FDG though this technique has some limitations. For clinical application, high uptake of FDG is measured in some normal tissues, i.e. the brain, and accumulation in inflammatory zones could influence the evaluation of tumor response to treatment. The main limitations are probably for preclinical applications where the fasting period for approximately 6-12 hours before FDG injection in addition to anesthesia maintenance between FDG injection and image acquisition are very stringent conditions that could definitely modify the tolerance of small animals to the tested drug.

Some investigations have reported significant differences in ^{18}F-FDG and ^{18}F-FLT uptakes in various subcutaneous tumor xenografts. In tumors where radiotracer uptake is low, it may not be possible to assess the anti-tumor efficacy of a drug, as radiotracer uptake variations may be hardly detectable (22). Tumor cell proliferation and response to treatment have been assessed by PET using FLT trapped in the cells after phosphorylation by thymidine kinase 1, which is up-regulated during the S phase of the cell cycle (23). The potential advantage of FLT over FDG could be the possible increased sensitivity to cytostatic properties of targeted therapies, which often block cell division with a low influence on glucose metabolism (24), but this hypothesis needs additional supporting data.





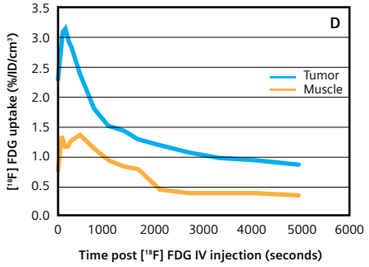


Figure 3. [18F]FDG uptake in human CWR-22 prostate tumors subcutaneously xenografted in Nude mice. Static images were recorded before (A) and eight weeks after surgical castration (B). [18F]FDG uptake period was 1 hour. The tumor is indicated by a white arrow. [18F] FDG dynamic scan recorded before (C) and eight weeks after castration (D). For both scans, mice received a single IV injection of 200 µCi [18F]FDG after a 6 hour fasting period.

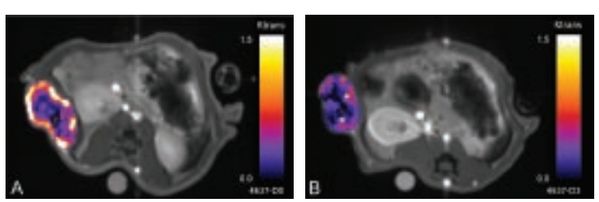
As FDG-PET has lower sensitivity for slow growing and metabolically less active tumors like hormone-dependent prostate tumors, new PET tracers are needed. One research program of the Laboratory for Preclinical Imaging and Imaging Technology of the Werner Siemens-Foundation (Tuebingen, Germany) is the selection of novel PET tracers for prostate cancers. They demonstrated that human hormone-independent tumor xenograft models, also compared to clinical findings in humans, showed very different pharmacokinetics and uptake characteristics for [18F] FLT, [18F]FDG, [11C]Choline and [18F]FECh. Subsequently, they investigated PET tracers uptakes in xenografted hormone-dependent human prostate tumor models. In baseline studies, they found faint uptake in tumors imaged with [18F]FECh, no tumor tracer uptake with [11C]choline and moderate [18F]FLT and [18F]FDG uptakes. Surgical castration induced a decrease of [18F]FDG tumor-to-muscle ratios (Figure 3) and variable [18F]FLT tumor-to-muscle ratios depending on the tumor model (25).

Angiogenesis and vascular function inhibition

Angiogenesis, the process whereby new blood and lymphatic vessels are formed from pre-existing vasculature, plays a pivotal role in tumor development and metastasis. Inhibiting angiogenesis represents the first strategy for development of anticancer targeted therapies (12). As mentioned in previous section, DCE-MRI allows for the quantification of pharmacodynamic effects of anti-angiogenic agents and their relationship to the administered dose (Figure 4). In DCE-MRI studies, images are acquired rapidly to dynamically follow the extravasation of an injected contrast agent into the tumor tissue. It is now the most widely used technique in the preclinical and early clinical evaluation of anti-angiogenic and anti-vascular agents (12), with 75 anti-angiogenic agents in clinical trials at present (13). Avastin® (Bevacizumab, Roche, Switzerland), Nexavar® (Sorafenib, Bayer, Germany), and Sutent® (Sunitinib, Pfizer, USA) are the first three FDA-approved compounds where DCE-MRI was documented in both preclinical and early clinical phases.

Vascular endothelial growth factor (VEGF) plays a key role in tumor angiogenesis by stimulating the proangiogenic signaling of endothelial cells via activation of VEGF receptor (VEGFR) tyrosine kinases, making VEGF and VEGFRs attractive therapeutic targets. KRN951, a novel multiple tyrosine kinase inhibitor (Kirin Pharma, Japan and Aveo Pharmaceuticals, USA), showed a significant anti-tumor activity against a wide variety of human tumor xenografts (14). DCE-MRI revealed a correlation between Ktrans reduction, reflecting a modification of tumor perfusion/vascular permeability, and the anti-tumor activity of KRN951. Furthermore, in a dose-escalation phase I clinical trial, KRN951was active against renal, colon and lung cancers. DCE-MRI also indicated a decrease in tumor perfusion in selected patients (15). These studies suggest that DCE-MRI is useful in detecting early responses to KRN951 in a clinical setting.

In collaboration with the Grenoble Institute for Neurosciences (France), we have recently investigated the use of multiple MRI biomarkers to explore the vascular changes associated with the anti-tumor activity of Carmustine and Sorafenib in a human orthotopic glioblastoma model in nude rats. Blood volume (BV), vessel size index (VSI), apparent diffusion coefficient (ADC) and blood brain barrier permeability to a contrast agent (BBB perm.) were mapped in the whole tumor, at different time-points after treatment onset. VSI/BV and BBB perm. parameters were computed from T2, T2* and T1-weighted images using an intravascular contrast agent (Feruloxtran-10, Sinerem®) and P846 (Gd-based contrast agent, Guerbet/AMAG Pharmaceuticals). Despite poor effects of Sorafenib and Carmustine treatments on survival, MRI demonstrated a tumor growth inhibition induced by these drugs. ADC is affected by both treatments while VSI and BV were sensitive to the effect of Sorafenib only. Histological data confirmed the mean vessel density was highly decreased by Sorafenib treatment. Together, these results indicate that VSI, BV and ADC parameters would be of value to combine anti-angiogenic with cytotoxic therapies in glioblastomas (16, 17).



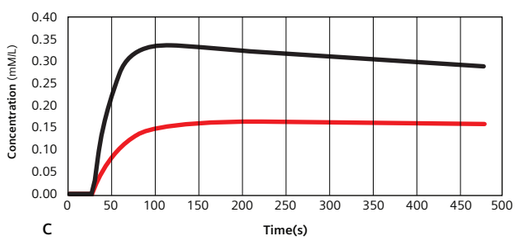


Figure 4. Results from a DCE-MRI experiment performed on nude rats bearing MDA-MB-231 human breast tumor xenografts and treated with Sorafenib. Image acquisition was performed just before the first treatment and 3 days after treatment onset. K^{trans} parameter maps superimposed on morphological images before (A) and after (B) treatment. Mean Gd-DTPA uptake curves in tumor rim with fitted PK model (solid line) before (black) and after (red) treatment (C). K^{trans} values are 1.23 s^{-1} before treatment and 0.32 s^{-1} after treatment.

Perspectives of functional and molecular imaging for personalized medicine

Translational research aims at moving basic discoveries from preclinical research into clinical evaluation to better select the right drug for the right person and to help the clinician to rapidly adapt therapeutic strategy to tumor response. The two most famous examples of targeted cancer drugs, Gleevec® and Herceptin®, highlight the necessity of imaging biomarkers and surrogate pharmacological endpoints adapted to the mechanism of action of each drug. Even as pharmaco-imaging is now becoming an important tool in drug development, we believe that some major advances need to occur in order to evolve from a research endeavor to a high-throughput production system. This requires the integration of multiple imaging modalities (26), with huge volumes of data and the standardization of protocols through the construction of dedicated international consortia.

There are many possibilities to combine complementary data from multiple imaging modalities. Combining functional MRI and spectroscopy with PET paves the way for a new perspective in molecular imaging with great potential for clinical applications (27). Combined or hybrid technologies, such as PET/CT and SPECT/CT, incorporate both imaging modalities into one machine but conduct the two scans sequentially. The lack of uniformly structured data affects drug discovery and individualized medicine, all of which rely heavily on integrating and interpreting data sets produced by different experimental methods such as non-invasive imaging, high-throughput genotyping, DNA microarrays, protein arrays, and high-volume clinical data.

In this context, the most urgent challenge for the immediate future is to standardize imaging procedures for a better qualification of multiple biomarkers. There is now a real need to dedicate worldwide networks to develop consensus recommendations and progress in this key area. The Pharmacodynamic/Pharmacokinetic Technologies Advisory Committee of Cancer Research UK recommend the development of non-invasive methods that measure common biological processes - particularly proliferation, cell cycle status, apoptosis, invasion, and angiogenesis - affected by many different drug classes and considered as more cost-effective than those that measure a specific molecular target (28).

Translational research is a multidisciplinary field based on teams rather than individuals. The challenge is to build efficient consortia with individuals coming from different entities such as academia, big pharmas, biotechs and CROs and having different scientific backgrounds. In this context, Oncodesign, dedicated to the preclinical evaluation of cancer therapies, has developed in-house skills for small animal imaging and established partnerships with the Laboratory for Preclinical Imaging and Imaging Technology of the Werner Siemens-Foundation (Tuebingen, Germany), dedicated to bridge the gap between in vitro biomedical research and in vivo imaging; and with PHARMIMAGE, a pharmaco-imaging platform in Dijon (France). Many technological platforms have been built in the past five years to help drug manufacturers with the development of biomarkers in parallel to the development of therapeutic drugs. Today, a large panel of imaging technologies and imaging biomarkers are being developed and identified as surrogate endpoints of drug efficacy with different mechanisms of action in preclinical studies. The real validation will be achieved by integrating more data from clinical trials incorporating these noninvasive imaging biomarkers, which will need to be correlated with other classical biomarkers and patient survival.

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