

Importance of Preclinical Imaging in Drug Discovery

Vinod Kaimal, Scientist; Patrick McConville, Director
Discovery and Imaging Services

Imaging in drug discovery: Where does it fit?

The process of discovering and bringing a drug to market consists of several stages, beginning with identification and validation of a drug target and continuing through lead identification by high-throughput screening, lead optimization, and profiling in relevant disease models. When a promising compound is found, a decision must be made on whether to take the drug into development. This long and expensive undertaking typically requires nearly \$800 million and takes about 12 years before an approved drug is brought to market¹.

Shortening the drug discovery and development process is critical to managing this cost and can be achieved by improving the characterization of compounds and their effects in early phases of testing. Imaging has the potential to dramatically increase the efficiency of lead candidate selection by providing earlier and more highly predictive data, compared with traditional methods. Imaging is also well suited to facilitating translation between preclinical testing and clinical evaluation of drugs. Imaging methods are also more easily applied than traditional methods in the newer, more realistic models of human disease that are becoming increasingly prevalent, such as models of invasive disease in the tissue of origin as well as transgenic mouse models.

The bridge between pre-clinical and clinical testing

Preclinical imaging enables the most powerful and clinically translatable methods for monitoring disease progression currently possible. In clinical trials, early image-based indicators are being increasingly used for effective enrollment of patients or efficient switching of treatment paradigms, leading to substantial time and financial savings for the pharmaceutical company. In the world of drug discovery, the efficiency benefits are analogous. One of the most important goals of a pharmaceutical company attempting to bring a product to market (as well as one of the most important mandates for a contract research organization) is to streamline the discovery and development phases of its products. The more efficient these processes, the quicker a company can invest resources into the most viable candidate without wasting them on less promising compounds. If soundly and strategically applied, preclinical imaging can help accomplish that goal.

Imaging tools can help better assess anatomy and disease morphology, physiological and functional parameters (e.g., blood flow and tissue oxygenation), and molecular and cellular processes (e.g., cellular proliferation, metabolism, and metabolite levels). The increasingly broad focus on imaging endpoints in clinical trials (e.g., MRI, PET, CT) further motivates the need for preclinical imaging as a tool for validating and optimizing imaging protocols used for a given agent or class of agents. By focusing on imaging earlier in a preclinical development program, the best-suited imaging biomarkers can be determined and validated, leading to increases in efficiency and cost savings in later stage clinical development.

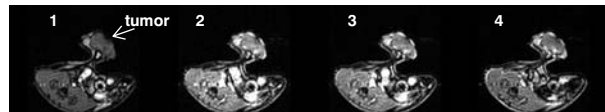
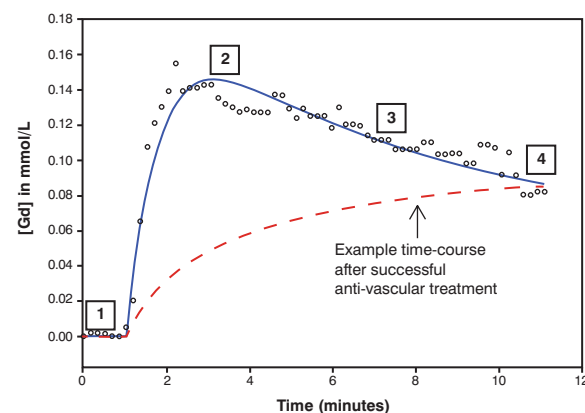


Figure 1: (Above) DCE MRI^{2, 4, 21, 22} in the MDA-MB-231 tumor line implanted subcutaneously in SCID mice. After acquiring a baseline scan, a bolus of gadolinium-based contrast agent is administered intravenously and continuous rapid MRIs are used to assess the gadolinium tracer concentration in the tumor over time.

(Below) The calculated tracer concentration time-course is fitted using a standard kinetic model to calculate quantitative measures of vascular permeability, vascular surface area, and blood flow. Successful inhibition of blood flow, vascular permeability, and/or angiogenesis results in a changing profile of the tracer uptake in the tumor (example treatment effect is shown by the dotted red line). The protocol as it is performed in mice is designed to reflect the standard clinical protocol as closely as possible to maximize clinical translation of results.



Imaging allows greater efficiency of predictive data generation

Preclinical imaging allows for the combination of what have traditionally required separate cohorts/analyses to gather more data from a single study. For example, imaging modalities can be combined in a single study, or a single modality can be leveraged to obtain multiple physiological or functional parameters and endpoints, in addition to anatomical or other traditional endpoints. Increasingly, manufacturers are combining traditionally separate imaging modalities, further facilitating this approach and optimizing the power and efficiency of imaging studies. For example, industry imaging labs are routinely using combined MRI (e.g., tumor vascularity) and PET (e.g., metabolism and cellular proliferation readouts) to obtain the most powerful and predictive data. The investigator benefits by obtaining a multi-faceted dataset from a single study that provides key information about the mechanism of action of a drug in addition to traditional information for efficacy readout (e.g., tumor growth rate). This enables earlier and more informed decisions in the drug development process and enables optimization of image-based biomarkers for use in concurrent or future clinical trials.

Application of various imaging modalities in preclinical testing

Magnetic Resonance Imaging (MRI):

MRI provides excellent soft-tissue contrast and high-resolution images and is unparalleled in its flexibility and broad applicability. It is widely used in the clinic to obtain anatomical and functional information.

MRI technologies have evolved beyond the ability to reveal anatomy alone and can detect tissue function and molecular changes. For instance, dynamic contrast-enhanced (DCE) MRI is the most clinically proven method for quantitatively measuring the vascular permeability of a tumor (**Figure 1**)²⁻⁴. This is an important capability given the prevalence of vascular targets in a variety of diseases, including the widespread focus on anti-angiogenic and vascular disrupting therapies in oncology. DCE MRI is filling the need for a clinically translatable method for quantitatively determining vascular response to therapy (see figure). Preclinically, alternative methods to accomplish this largely rely on sacrificing animals and invasive methods that can uncouple the readout from the disease property being measured. This can decrease the relevance of the data gleaned from the measurement.

MRI can also be used to determine the early response of a tumor at the cellular level to treatment. Diffusion MRI uses water diffusion as a biomarker for cellularity of tumors by measurement of the water apparent diffusion coefficient (ADC)⁵⁻⁹. Successful treatment of a tumor with a cytotoxic agent results in cell kill and therefore lower cellular density. This is reflected as an increase in the water ADC value. Imaging facilities such as those at Charles River routinely use

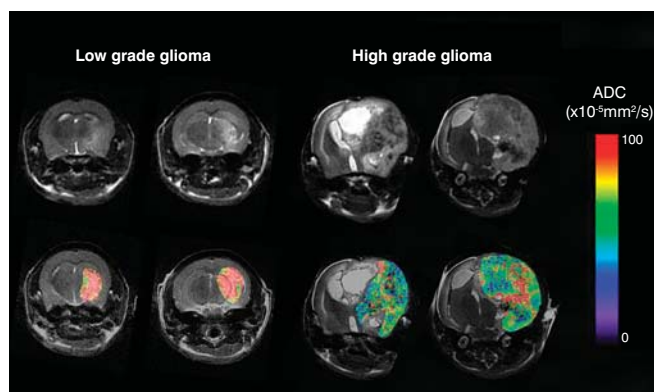


Figure 2: Diffusion MRI in the tv-a transgenic model of glioma in mice. The upper row shows T2-weighted anatomical scans, with the lower row including color ADC overlays. The ADC is a measure of cellularity of the tumor, with a higher value indicating decreased cellular density. In this case, ADC has been used to distinguish tumor grade. ADC has also been used clinically for early prediction of patient treatment outcome^{8, 9}, and has also been successfully applied in a variety of animal tumor models to quantify anticancer treatment response⁷⁻⁹.

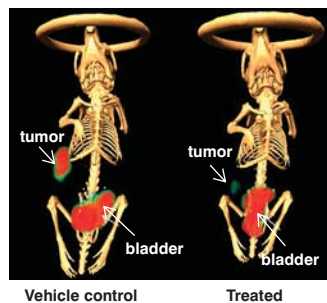


Figure 3: 18-FLT PET imaging in mouse tumor models. FLT is a PET tracer that is trapped in dividing cell DNA and can therefore be used to detect cellular proliferation. The example above shows FLT PET images in a subcutaneous tumor model, overlaid on surface-rendered CT data. The treated animal shows a decrease in FLT uptake, indicating inhibition of cell proliferation, without a decrease in the tumor size. Combined PET/CT imaging is now a clinical imaging mainstay and is also being used as such preclinically.

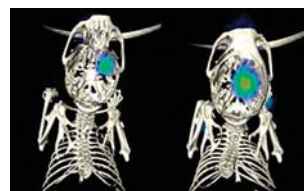


Figure 4: These example PET/CT images show FLT uptake in intracranial human tumors in mice, where the inability of FLT to cross the blood brain barrier provides very low normal tissue background levels.

diffusion MRI in oncology efficacy studies in animal models (**Figure 2**). Charles River is also exploring the use of magnetic resonance spectroscopy (MRS) in the evaluation of cancer therapeutics. MRS is a clinically translatable technique for measurement of metabolite concentrations in tissue. For example, elevated choline levels have been confirmed for several cancers including breast, prostate, colon, and brain. Choline levels can therefore be used as a biomarker for response to anticancer therapies¹⁰.

Positron Emission Tomography (PET):

PET is also becoming an increasing area of focus for drug discovery and development because of its unique capabilities. In oncology, the two most clinically relevant PET protocols involve use of fluorodeoxyglucose (FDG), for quantification of tissue metabolism¹¹ and inflammation¹², and fluorothymidine (FLT), for quantification of cellular proliferation¹³. These two protocols represent two of the most in demand and commonly used preclinical imaging protocols at Charles River (**Figures 3 and 4**). PET is also playing a key role in quantifying receptor occupancy for new targeted therapies in a variety of disease states. The near future will bring a broad suite of new commercially available designer PET tracers, each a biomarker for a critical disease process¹⁴. Many of these are already being used in clinical trials and preclinical studies. These tracers illustrate the ability of imaging to meet needs where traditional methods have not.

Bioluminescence Imaging:

An example of earlier deployment of imaging is *in vivo* biophotonic imaging (bioluminescence or fluorescence imaging). In this modality, a luminescent protein or enzyme can be transfected into diseased cells that are then implanted into an animal or expressed in a transgenic animal^{15, 16}. The light emitted from the implanted animal is then imaged and used to track disease progression and treatment response (**Figure 5**). Expression of the light-emitting reporter can also be tied to a conditional molecular process to enable imaging of the drug mechanism at the target level^{17, 18}. This modality also allows the use of exogenous reporters that enable quantification of a molecular or cellular process. An increasing spectrum of these “smart” optical probes is becoming commercially available. Biophotonic imaging enables a high level of throughput, offers results in less than a minute, and can therefore be more cost-effective compared with modalities like MRI and PET. Furthermore, bioluminescence imaging is readily translated *in vitro*. This allows screening assays so that more efficient judgments can be made before moving to the *in vivo* stage. This also furthers a responsible approach to research from a humane care point of view.

Computed Tomography (CT):

CT is well suited for anatomical imaging of the skeletal tissue in models of arthritis. For example, in a collagen-induced arthritis model, untreated animals undergo significant bone remodeling and deterioration in bone structure, while successfully treated animals (e.g., methotrexate) show inhibition of this disease progression (**Figure 6**). Manual scoring systems or software algorithms can be used to quantify, for example, the bone surface roughness as a measure of severity of the disease^{19, 20}.

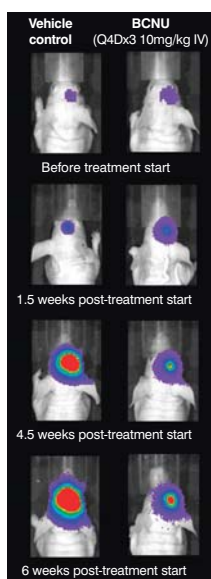
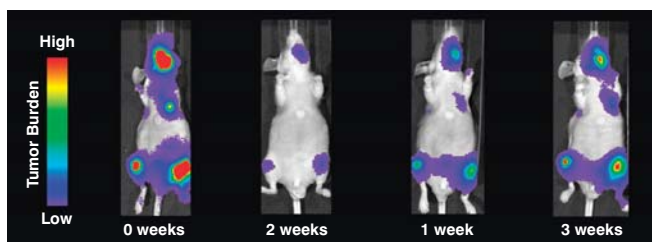


Figure 5: (Above and Left) Bioluminescence imaging in a luciferase-expressing D54-MG-luc intracranial human glioma model. The bioluminescence signal is directly proportional to the number of tumor cells. The first image time-course (see left) shows continued tumor progression (top to bottom) in vehicle control animals (left column), while by comparison, BCNU treatment results in significant tumor growth inhibition (right column). Bioluminescence imaging is an efficient tool for monitoring invasive, disseminated, metastatic, or deep tissue tumor growth and response to therapies. The second image time-course (Above) shows progression of tumor growth in an intra-cardiac injection model of bone metastasis using a luciferase-expressing human prostate tumor line, PC-3-luc. Imaging allows multiple individual metastases to be tracked and quantified simultaneously in a single study.



Figure 6: Surface rendering of CT images of rat ankles in a collagen-induced arthritis model. Severe disease in the vehicle-treated control causes progression of bone remodeling and osteolysis. Image scoring or software algorithms that compute bone surface roughness^{19, 20} can be used to assess the severity of disease and the efficacy of treatment.

technical sheet

Conclusion

In summary, the opportunity for the development and enhanced application of preclinical imaging is substantial because of its potential to increase the efficiency and accuracy of the drug discovery and development process. Technological advancements, such as the availability of multiple integrated modality imaging systems, will soon see preclinical imaging grow at unprecedented levels. Increasingly sophisticated and well-validated imaging technologies, probes, and biomarkers will further drive the uniqueness of imaging. Preclinical imaging is not only necessary to accelerate the drug development process, but also enables predictive endpoints that cannot be measured non-invasively in any other way.

The images shown in this paper were generated by Charles River Discovery and Imaging Services scientists at, Ann Arbor.

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