New Learnings:

Advancing Drug Discovery and Development through Non-Invasive Biodistribution Imaging

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Molecular Imaging™

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About Molecular Imaging™

Molecular Imaging, Inc. is a specialty contract research organization (CRO) located in Ann Arbor, Michigan. The company is the only CRO dedicated to providing pre-clinical, *in vivo* multi-modality imaging services -- anatomical, functional and molecular -- to the pharmaceutical, diagnostics and medical devices industries. The combined experience in pharmacology, imaging and disease models at Molecular Imaging, Inc. positions the organization as a valued partner for the life sciences industry.

Molecular Imaging, Inc. employs a wide array of imaging technologies to evaluate a new drug candidate's therapeutic potential, or determine the value of new diagnostics or medical devices. In the drug discovery and development process, these technologies are used to select potential drug candidates, evaluate novel targets, determine and optimize treatment regimens, and validate potential clinical trial imaging strategies. The current imaging modalities include small animal MRI, microPET, microCT, SPECT, FMT, optical imaging and X-ray based techniques.

Molecular Imaging, Inc. offers disease models in many therapeutic areas and actively seeks collaborations to further advance the use of imaging techniques in life sciences research and development efforts. In addition, Molecular Imaging's 20,000 sf facility, which includes a vivarium, is flexible and designed to meet the varied needs of the industry.

As a CRO with nearly a decade of experience, Molecular Imaging, Inc. has applied its technology to the evaluation of small molecules, biologics, antibodies, proteins and peptides, as well as gene therapies, vaccines, medical devices and novel imaging probes and reagents.

The staff works closely with clients to guide all aspects of the study design. A wealth of experience in pharmacology, disease models and imaging allows Molecular Imaging, Inc. to provide superior quality and timeliness, using the most relevant animal models and imaging technologies.

Introduction

Pre-clinical imaging is playing an increasingly important role in the evaluation of new drug candidates' therapeutic potential. With pre-clinical imaging, researchers can:

- Evaluate drug candidates using more clinically relevant and predictive disease models
- Track disease progression
- Increase decision-making confidence
- Quantify endpoints that are directly translatable to clinical practice

Drug discovery and development scientists can employ a wide array of imaging technologies, including MRI, micro-CT, micro-PET, SPECT, X-Ray and optical (two-dimensional and fluorescence molecular tomography) to quantitatively assess test agent performance at anatomical, functional and molecular levels. Molecular imaging technologies can also be utilized to conduct applications research to develop and optimize the use of imaging, to further advance imaging in the field of drug discovery.

This report, **New Learnings: Advancing Discovery and Development through Non-Invasive Biodistribution Imaging,** presents an overview of *in vivo* biodistribution imaging and the use of various imaging modalities in addressing the drug discovery and development challenges in the pharmaceutical industry. This paper provides an overview of currently available imaging strategies and services; identifies the key challenges and relevant opportunities to utilize this fast-growing technology; and demonstrates how utilization of imaging, along with deep *in vivo* imaging expertise, can deliver enhanced predictive power for drug discovery.

Molecular Imaging Provides Robust Data for Decision-Making

Drug discovery and development in the pharmaceutical industry has recently shifted towards biologic molecules and nanoparticle-based compounds that have promising diagnostic and therapeutic value. These molecules include antibodies, antibody fragments, antibody drug conjugates (ADCs), peptides, proteins and nanoparticles. One of the key success factors in bringing these molecules into clinical use is to characterize their optimal biodistribution in the subject. Important aspects of drug biodistribution include:

- Molecular targeting precision -- how well the molecule binds or targets specific cell types, tumors or diseased tissues
- Pharmacokinetics -- in the bloodstream and tissues
- Whole body distribution -- including clearance pathways and kinetics

In vivo Imaging	Traditional *ex vivo*
Longitudinal; non-invasive	Serial sacrifice; invasive
Paired data points	Unpaired data points
Fine kinetics in same animals	Limited by separate animal cohorts and limitations of blood sampling
Reduced group sizes and animal numbers (n=3 to 6)	Greater animal numbers required (n=10 to 12)
3-Dimensional data	2- or 1-Dimensional data
Inherent living tissue and sub-tissue resolution	Tissue sampling; subject to *ex vivo* deterioration

Figure 1

Comparison of in vivo imaging methods to traditional ex vivo methods for quantification of biodistribution in animal models.

The use of imaging-based biodistribution methods is advancing rapidly, both with industry demand and increased access to radiochemistry facilities and expertise. With the ability to provide three-dimensional quantitative data, PET, SPECT and Fluorescence Molecular Tomography (FMT) have become critical tools for biologics and nanoparticles characterization.

As seen in Figure 1, in comparison to traditional *ex vivo* techniques, *in vivo* pre-clinical imaging provides an opportunity to perform **measurements of molecule biodistribution, clearance and kinetics in a longitudinal and non-invasive way**. The traditional *ex vivo* methods are invasive and involve serial sacrifice. These methods also lead to un-paired data points, which is a less powerful channel for statistical analysis.

Additionally, *in vivo* imaging provides **access to fine kinetics** in the same animals. This is quite limited in traditional methods, in which separate animal cohorts and restricted blood sampling is the norm. *In vivo* imaging modalities also involve **high sensitivity and, therefore, allow reduced animal numbers** (n=3 to 6 per test group is commonly sufficient). In traditional methods, this number is two to three times larger, to accommodate sufficient time points (n=10 to 12 per test group).

In vivo imaging also provides **whole-body, 3-dimensional data** whereas, in traditional methods, researchers rely on 2- or even 1-dimensional data, depending on their tissue sampling techniques.

Finally, using *in vivo* imaging allows researchers to **interrogate living tissue at sub-tissue resolutions**. In contrast, *ex vivo* methods, including tissue sampling and tissue deterioration, can limit studies.

Molecular Imaging Offers Advanced Options for Drug Development and Clinical Monitoring

Currently, the three imaging modalities that are dominating the use of pre-clinical biodistribution imaging are Positron Emission Tomography (PET), Single Photon Emission Computed Tomography (SPECT) and Fluorescence Molecular Tomography (FMT). PET and SPECT are nuclear medicine imaging techniques that **generate 3D images** of an injected radiotracer in the body. These modalities enable 3D imaging of appropriately labeled molecules, including antibodies, antibody fragments, ADCs, proteins and peptides. Some key isotopes used in PET are ^{89}Zr, ^{124}I, ^{68}Ga and ^{64}Cu, whereas SPECT imaging relies commonly on ^{99M}Tc, ^{123}I, ^{125}I, ^{111}In, ^{177}Lu, ^{117}Sn, ^{201}Tl and ^{67}Ga.

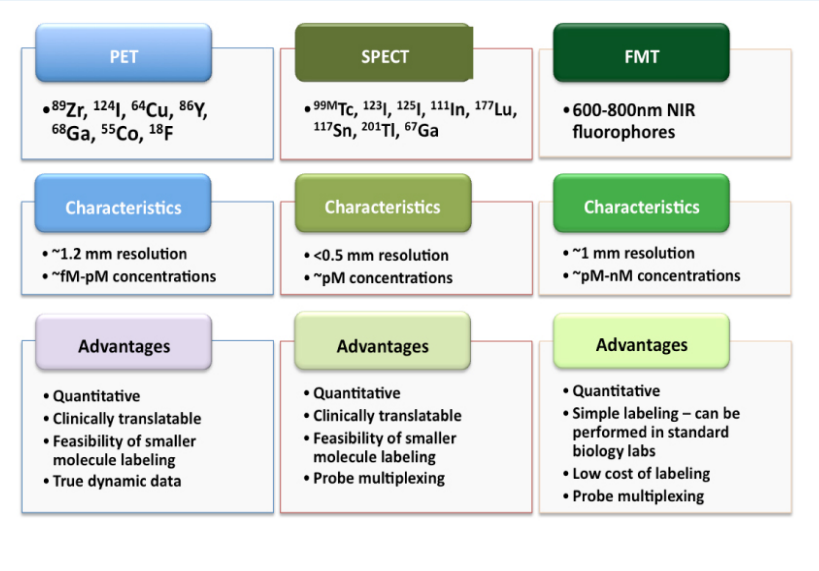


Figure 2

Comparison of in vivo imaging modalities' characteristics and advantages for quantification of biodistribution in animal models.

FMT is a non-depth limited, highly sensitive imaging technology that resolves and reports near infrared (NIR) fluorescence distribution in the whole body. This modality provides **a rapid and efficient means of characterizing biologic molecule behavior** *in vivo* and can facilitate a subsequent clinical path that incorporates more complex and/or expensive radiolabeling. In FMT, the availability of cost-effective labeling kits for NIR fluorophores in the 600-800 nm-range provides an opportunity to use quantitative *in vivo* biodistribution in early discovery.

The characteristic resolution and sensitivity of these modalities are compared in Figure 2. All three modalities allow for quantitative 3-D imaging;

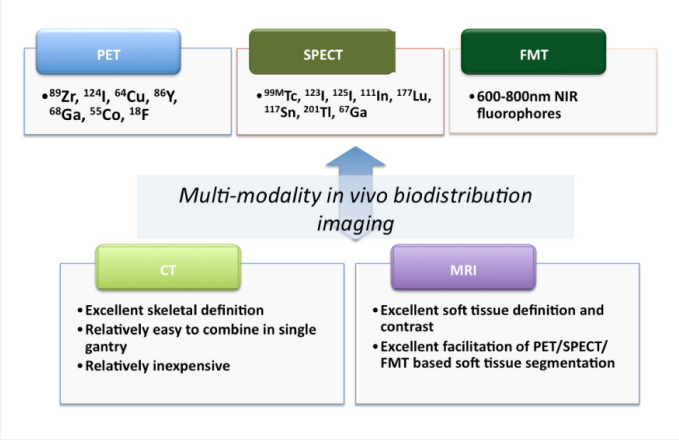


Figure 3

Multimodality imaging: CT and MRI are used to provide anatomical localization to PET, SPECT and FMT images. CT and MRI add skeletal and soft tissue definition, respectively.

however, **PET and SPECT are clinically translatable** and are more feasible for smaller molecule labeling (peptides, proteins). Between the two, PET provides true dynamic data, whereas SPECT allows for detecting multiple molecules, through differential labels at the same time.

FMT is associated with simple and low-cost labeling methods that lend themselves to **early risk assessment, through screening and lead selection** in early discovery. PET and SPECT are not only effective in early drug discovery phases, but are also translatable to later drug development phases and clinical trials.

Multimodality imaging is also critical in biodistribution imaging. For example, Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) provide an anatomical image overlay to PET, SPECT and FMT images. Both CT and MRI can enhance and improve the accuracy of data by adding **skeletal and soft tissue delineation and localization**, as outlined in Figure 3.

Molecular Imaging, Inc. has incorporated state-of-the-art animal handling technology into its biodistribution imaging program. Part of this has been a collaboration with ASI Instruments (Warren, MI), a developer and manufacturer of novel animal handling and positioning technology for multi-modality imaging (see Figure 4). This cost-effective and efficient throughput technology facilitates multi-modality imaging[1].

Applications in *In Vivo* Biodistribution Imaging

Pre-clinical imaging plays an increasingly important role in the evaluation of new drug candidates' therapeutic potential. Using pre-clinical imaging, researchers can evaluate drug candidates using realistic and predictive disease models, track disease progression, increase decision-making confidence and quantify endpoints that are directly translatable to clinical practice.

The following cases present further examples of *in vivo* biodistribution imaging, and the use of various imaging modalities, in addressing the drug discovery and development challenges in the pharmaceutical industry.

Antibody Tumor Targeting using ^{89}Zr PET Imaging

^{89}Zr PET was used to distinguish the targeting properties of an antibody. An antibody was used in animals with different tumor types. In Figure 5, the top row shows a tumor that is non-expressing of the target for this antibody. As shown with the arrow, there is minimal accumulation of the antibody in the tumor. Using the same protocol, a tumor expressing

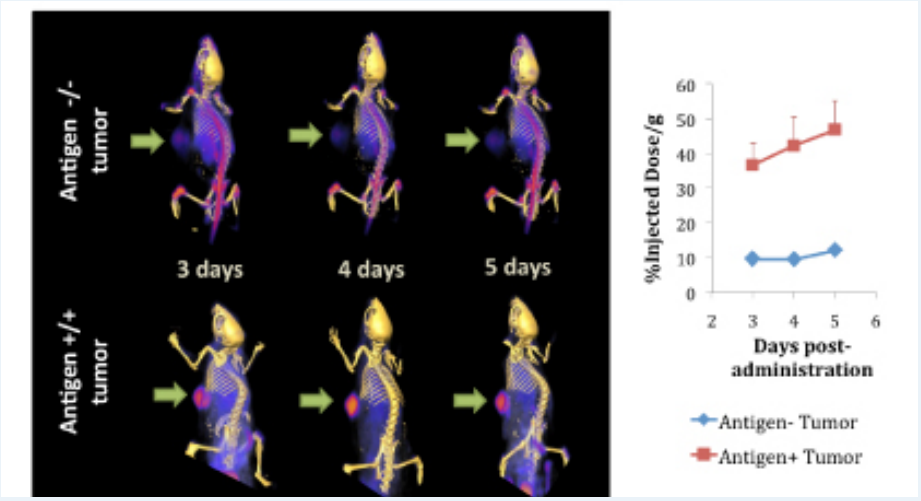
Figure 4

Enhancing in vivo biodistribution imaging using multi-modality animal handling technology: ASI Instruments provides cost-effective, multi-modality animal handling technology to improve throughput efficiency, without sacrificing imaging sensitivity and resolution.

1 McConville, Patrick. "Small Animal Preparation and Handling in MRI." Methods in Molecular Biology 771 (2011): 89-113. PubMed. Web. 9 Oct. 2013.

Figure 5

Antibody tumor targeting using ^{89}Zr: Top row shows a tumor under PET imaging that is non-expressing of this antibody. The arrow shows minimal accumulation of the antibody in the tumor. Using the same protocol in the bottom row, PET imaging shows a different tumor type in a different animal is clearing the expression of the antibody. The graph on the right confirms the percent accumulation of the antibody at 3 to 6 days after administration, in both animals.



the target in a different animal shows specific binding of the antibody and the related kinetics. In Figure 5, the graph on the right clearly confirms the percent accumulation of the antibody in the tumor at 3 to 6 days after administration.

Nanoparticle Tagging with Near-Infrared (NIR) Fluorophore

As seen in Figure 6, in this study, nanoparticles with different sizes were labeled with an NIR fluorophore and administered to test the targeting of a tumor and clearance through normal tissues.

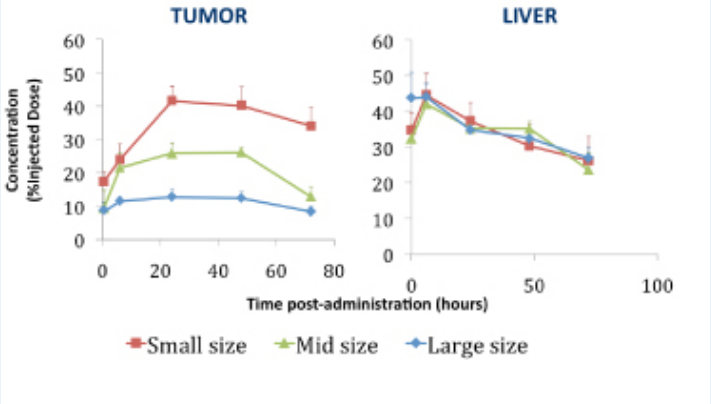


Figure 6

Nanoparticle tagging using Near-Infrared (NIR) fluorophore FMT imaging: The molecules were imaged to assess the most effective size of the nanoparticle for treatment of a tumor (on the left). Specific accumulations of these nanoparticles were detected using FMT imaging. FMT imaging shows that the small-size nanoparticle is most suitable to target this tumor type as it has the highest concentration in the tumor post administration. In comparison, all three nanoparticles are cleared from the liver (on the right), irrespective of their size.

The molecules were imaged to assess the effect of the size of the nanoparticle for this tumor type. Specific accumulation of this nanoparticle was detected in the tumor. As shown in Figure 6, the small-size nanoparticle showed the highest concentration in the tumor, followed by mid-size and large-size particles. In comparison, all three nanoparticles were cleared from the liver, with dynamics independent of their size. This demonstrates how FMT can clearly distinguish the specific kinetic and tissue-targeting properties of a nanoparticle, and then facilitate the selection of an optimal nanoparticle design for tumor therapies.

Conclusion

PET, SPECT and fluorescence labeling of biologics and nanoparticles provides quantitative *in vivo* targeting and biodistribution assessments, and facilitates early portfolio de-risking and decision-making in the drug discovery and development process.

Factors that commonly influence the success of *in vivo* biodistribution imaging studies are:

- Labeling chemistry, e.g., radiochemistry (choice of isotope and labeling protocol development)
- Labeled product QC and validation that ensures critical properties of the parent molecule are maintained in the labeled version
- Pharmacology: disease model used and disease progression kinetics
- *In vivo* imaging protocol design: modality choice, imaging timing and study group design

With recent developments, sophisticated imaging technologies are now an accessible and affordable reality for pharmaceutical companies through sub-contracting. Commercial-grade radiochemistry capabilities are also increasingly available by contracting, and are creating an efficient and cost-effective way to perform molecular radiolabeling, and can readily be used in conjunction with imaging modalities.

For a successful *in vivo* imaging study, all modalities should ideally be available in a single core facility and be used in an integrated fashion, along with the appropriate animal disease models.

External partners and contract research organizations (CROs) such as Molecular Imaging, Inc. can provide animal models, related pharmacology and radiochemistry provision to facilitate these studies.

Background for this Report

Founded in 2003, Molecular Imaging, Inc. employs a wide array of imaging technologies, including MRI, micro-CT, micro-PET, SPECT, X-Ray and optical (two-dimensional and fluorescence molecular tomography) to quantitatively assess test agent performance at anatomical, functional and molecular levels. Molecular Imaging also conducts applications research to develop and optimize the use of imaging, and actively seeks collaborations to further advance imaging in the field of drug discovery.

This report is supported by a recent webinar hosted by Molecular Imaging, Inc., titled **New Learnings: Advancing Discovery and Development through Non-Invasive Biodistribution Imaging,** which presented an overview of *in vivo* biodistribution imaging and the use of various imaging modalities in addressing the drug discovery and development challenges in the pharmaceutical industry. The opportunity is for an enhanced utilization of various molecular imaging technologies to be able to provide more robust data for decision making in the biotech and pharmaceutical industry.

To view the webinar, please visit: http://bit.ly/17cNFZk

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