

The Role of *In Vivo* Imaging in Drug Discovery and Development



Advances in non-invasive *in vivo* imaging techniques have raised the use of animal models in preclinical drug discovery and development to a new level, enabling quick and efficient drug efficacy screening.

During the preclinical stage of research in drug discovery and development, *in vitro* and *in vivo* tests are conducted to ensure the drug candidate is safe to test in humans prior to the initiation of clinical trials. Preclinical research employs animal models to facilitate the investigation of human disease and to aid in the development of new therapeutics. The biological relevance of an animal model is critical to the predictive value of clinical outcome. Traditionally, data obtained from animal studies is derived from *in vitro* analyses of tissue and fluid samples collected from cohorts of treatment groups. However, the development of non-invasive, *in vivo* imaging techniques has vastly advanced the sophistication of animal models. Non-invasive, live animal imaging delivers fast, longitudinal, accurate, real-time and quantitative assessments of drug efficacy. Consequently, one gains more data points from fewer animals; which is in adherence with the 3R's principle of the animal research regulatory effort to **Reduce, Replace and Refine**. Furthermore, the implementation of preclinical *in vivo* imaging enables translational medicine, de-risks compound candidates, significantly shortens 'time to the clinic', and lowers cost while maximizing biological understanding.

Preclinical Imaging in Drug Discovery and Development

The primary goal of preclinical imaging is to improve the odds of clinical success and reduce drug discovery and development time and costs. Currently, only about one in 10 drugs of New Molecular Entities (NMEs) make it to the market at an estimated R&D cost of 2.5 billion USD in 2017 per NME¹. Secondarily, imaging can facilitate personalized medicine, tailoring diagnosis and treatment to the individual patient's genetic make-up.

As imaging can be implemented at various stages of the drug development process, it is important to incorporate it into the initial experimental master plan. *In vivo* imaging is a biology-driven practice, in which the disease dictates the animal model and the imaging modality. Today, it is possible to carry the same biomarker from *in vitro* testing all the way through clinical trials, and potentially use the same biomarker as companion diagnostic or prognostic test. Translation from bench to bedside and subsequent back-translation to the bench is a powerful cycle of information gathering in drug development.

Careful quantitative modeling and de-risking of each NME throughout development optimizes pipeline management and facilitates decision-making towards abrogating or accelerating trials. Part of this modeling helps to ensure that smart choices in animal models, reporter and imaging modalities are used at the earliest preclinical stages possible reducing time and cost in each phase of development.

Optical Imaging

Optical Imaging is increasingly being utilized as a standard method in modern preclinical drug discovery and development. This modality provides a virtual “window” into the animal and makes it possible to track biological activity at the molecular level in real time. This technology is user-friendly, radioactivity free, high throughput and relatively economical with excellent sensitivity. Non-invasive, whole body *in vivo* optical imaging enables monitoring and assessing disease, drug biodistribution and molecular events in small animals through labeling with light emitting reporters. For example, tumor cells, stem cells, immunological (e.g. CAR-T) cells, gene therapy, viruses or bacteria can be genetically tagged with a luminescent (luciferase) or fluorescent protein. Biodistribution of drug delivery nanoparticles and biologicals (e.g. antibodies) can be monitored by means of labeling the moiety with a fluorescent dye. Light emission from within the animal is detected by means of a super cooled charged-coupled device camera, with the sensitivity to detect just a few luciferase expressing cells. By measuring and analyzing the light emission, *in vivo* optical imaging can help researchers monitor biodistribution, cellular or genetic activity and use those results to track drugs, gene expression, spread of a disease, or effects of a new drug candidate.

Multimodality Imaging

While optical imaging can monitor disease progression and evaluate effects of drug candidates with extremely high sensitivity, the ability to visualize biological events in anatomical context can provide valuable information. As such, optical imaging is commonly complemented with anatomical imaging modalities including MRI, 3D micro-computed tomography (microCT), or 2D X-ray to help provide anatomical localization and staging of disease. X-ray is the most familiar and least expensive imaging modality which offers skeletal reference as tissue absorbs photons when exposed to an X-ray beam. Having greater density, bones absorb more photons than lean tissue. The number of photons passing through the body are captured by the detector and the resulting images are a two-dimensional projection of a three-dimensional structure. While MRI has historically been the best technique for anatomical resolution and 3D rendering, the longer acquisition times, technical skill and high magnetic field strength requirement for imaging small animals make this modality relatively costly. MicroCT offers an excellent alternative imaging modality for studying structure and morphology of subjects, providing valuable anatomical and functional information. However due to the potential cumulative effect of radiation exposure, careful consideration must be made; especially in longitudinal studies, where total radiation dose may reach therapeutic levels compromising results and causing toxicity. Novel high-speed, low-dose, high-resolution microCT scanners such as the Quantum GX2 microCT (PerkinElmer) and IVIS SpectrumCT integrated optical/microCT (PerkinElmer) are now commercially available that mitigate many of these risks. These microCT systems enable sensitive, quantitative, longitudinal imaging of animal models beyond bone disease. Short acquisition times permit the use of (clinical) contrast agents, facilitating pulmonary, cardiovascular and other soft tissue imaging.

Accelerating Drug Discovery and Development with Innovative *In Vivo* Imaging Systems

PerkinElmer offers a wide range of molecular and anatomical imaging platforms to improve the efficiency of preclinical, *in vivo* drug discovery and development research. Below are just a few examples including integrated and standalone modalities as well as high throughput systems.

- The IVIS® SpectrumCT 2D and 3D optical imaging system is uniquely equipped with a transillumination fluorescence imaging module and advanced 3D optical reconstruction software algorithms facilitating *in situ* tomographic reconstruction and absolute quantification of the optical signal. Anatomical context of the optical signal is quickly rendered by co-registration with a built-in microCT scanner. (Figure 1 and Figure 2).
- The IVIS Lumina X5 2D optical imaging system is equipped with a microfocus X-ray source and geometric magnification, achieving industry leading X-ray resolution in a 2D optical/X-ray. Both the IVIS Lumina S5 and X5 uniquely feature a high-throughput imaging solution that includes an expanded field of view (FOV) allowing simultaneous imaging of five mice and ‘Smart’ animal handling accessories designed to streamline imaging workflow and accelerate drug discovery and development. Smart loading trays allow users to pose animals on the benchtop before placing the tray into the IVIS. Using fiducials built into the tray, the software can automatically recognize and draw subject regions of interest (ROIs) providing automated animal identification. (Figure 3).
- All IVIS® optical imaging instruments (PerkinElmer) provide National Institute of Standards (NIST) calibrated data. This calibration allows for data measurements of the photon emission from the subject in absolute physical light units of surface radiance. Software such as Living Image® (PerkinElmer) allow for image analysis of multiple time points within longitudinal studies, side by side using the same scale. Furthermore, this software facilitates tomographic imaging, providing: 1) anatomical localization of the source(s) in XYZ, 2) absolute intensity, and 3) extrapolation of number of cells or number of dye molecules. With the Living Image® 3D Multimodality Tools plugin and the mouse imaging shuttle (MIS) you can transfer animals from the Quantum GX2 microCT to the IVIS Spectrum without disrupting the animal’s position. The MIS features built in fiducial markers that allow for automated volume registration. (Figure 4).
- The Quantum GX2 is a standalone microCT instrument with a large dynamic range, facilitating the imaging from zebrafish to rabbits. Fast, low dose scans allow for longitudinal, quantitative *in vivo* imaging as well as soft tissue imaging with contrast agents. Co-registration with the IVIS Spectrum 3D optical imager is streamlined via the mouse imaging shuttle (MIS). Applications span from bone to cardiovascular, pulmonary, adipose, soft tissue and birth defect imaging. (Figure 4).

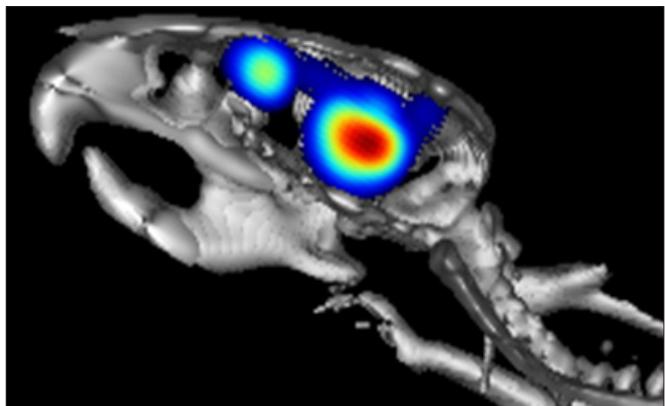


Figure 1. 3D tomographic reconstruction of a brain tumor model: orthotopic U87 MG-Red-FLuc human glioblastoma cells (PerkinElmer) expressing red shifted luciferase (Red-FLuc) gene from the firefly *Luciola Italica*. Subject i.p. injection using Xenolight® D-luciferin (PerkinElmer) enables visualization and tracking of primary tumor and metastatic burden. Cranial anatomical reference was obtained via co-registration with microCT imaging.

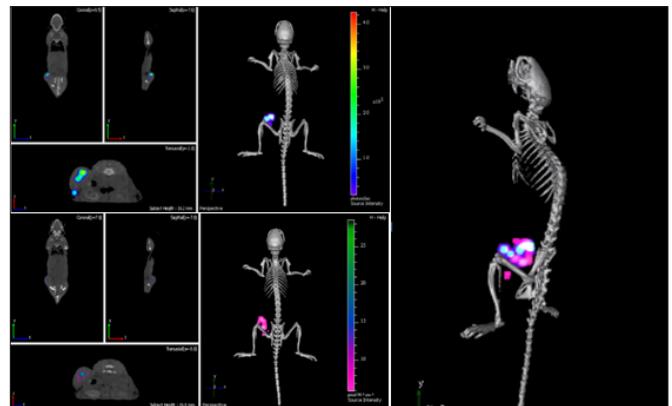


Figure 2. Co-registered image on the IVIS SpectrumCT highlights 3D tomographic imaging of targeted delivery. The tumor in the thigh of the mouse is luciferase expressing (pseudo-colored in blue) and liposomal nanoparticles are fluorescently labeled (pseudo-colored in pink). Skeletal anatomical reference is obtained via microCT. Multimodal imaging offers the unique ability to visualize and follow disease progression and pin-point specificity of targeted delivery³.

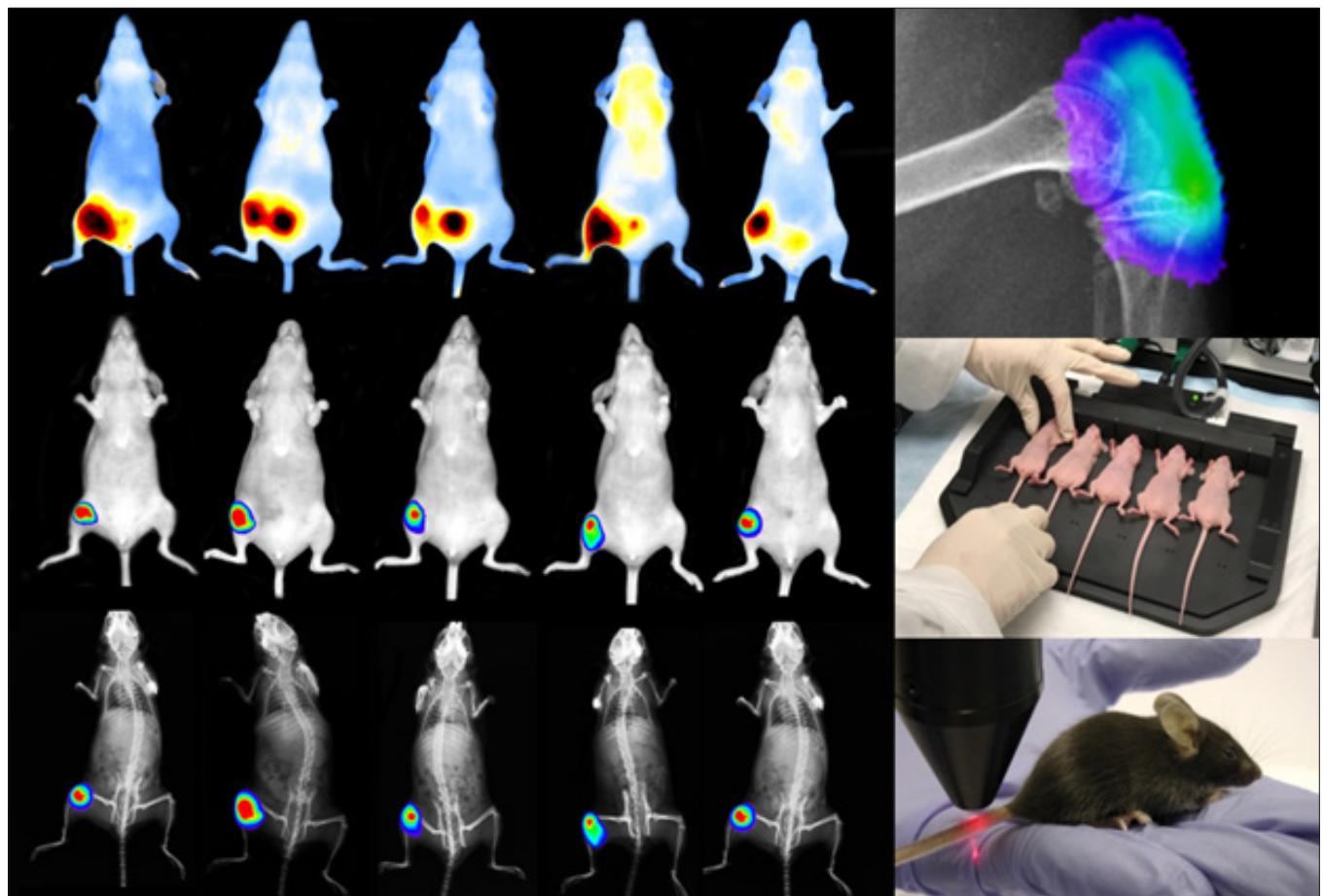


Figure 3. High throughput imaging: fluorescence, bioluminescence, and high-resolution X-ray overlay imaging using IVIS Lumina X5 system. Greater field of view (FOV) for simultaneous imaging of 5 mice, high-throughput accessories: posing tray and anesthesia docking station; software compatibility with PHARMASEQ p-Chip for automated animal recognition.

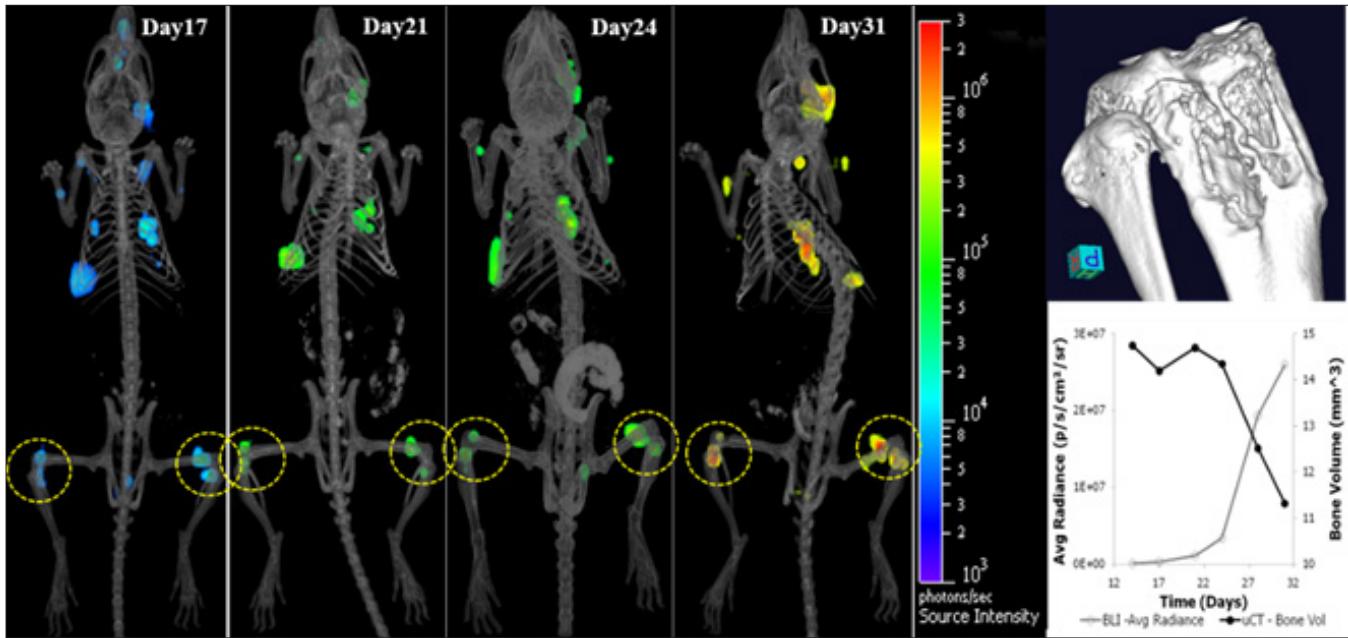


Figure 4. Data Quantification: IVIS Spectrum longitudinal bioluminescence imaging of tumor burden and quantification in average radiance ($\text{photons/sec}/\text{cm}^2/\text{sr}$) over time in the knee joint. Analyzed using Living Image® Software (PerkinElmer). Co-registration using Quantum GX2 microCT as anatomical reference and bone erosion (bone volume mm^3) quantification in the knee joint.

Translational Imaging

The translational relevance of preclinical tumor bioluminescence imaging (BLI) has been advocated by Dr. Cathy Zhang from Pfizer's Department of Cancer Biology: "Cancer metastasis presents an ongoing challenge for modern drug discovery. Recent advances in optical imaging technology provide great opportunities for researchers developing animal disease models that recapitulate the complexities of human cancer and metastatic progression. BLI allows quantitative assessment of the anti-tumor and antimetastatic efficacy. BLI is a sensitive, non-invasive tool for longitudinal assessment of tumorigenesis, metastasis, and therapeutic intervention in animal models. By using optimized BLI technique, clinically relevant disease models can be developed for efficient evaluation of drug effects, thereby allowing scientists to gain deeper knowledge of the underlying biology during disease progression and to accelerate cancer drug discovery" ².

Even though bioluminescence (BLI) and fluorescence (FLI) imaging have direct translational limits, it's clear that their implementation provides an insightful technology for fast, efficacious and cost-effective use and characterization of animal disease models and drug discovery validation. BLI and FLI represent an excellent alternative and/or complementary validation mechanism for more invasive techniques such as histopathology and biochemical assays. Through the combination of various imaging modalities, molecular, anatomical and functional data can accurately and quantitatively de-risk preclinical NME discovery efforts and facilitate Go/No-Go decisions of drug candidates in the pipeline faster in the development process.

Example of How *in vivo* Imaging Can Be Used in Immuno-oncology Drug Discovery and Development

Healing the body from cancer through potentiating the immune system is a challenging proposition on the verge of breakthrough. Novel immuno-onco therapeutic strategies may consist of antibodies and/or CAR-T cells. An *in vivo* imaging model used to facilitate efficacy studies in the development phase of these therapeutics may consist of using immuno-compromised mice which have been inoculated with a human tumor cell line expressing renilla luciferase (e.g. RediFect™ Green-RenLuc-Puromycin (PerkinElmer), and the associated substrate RediInject™ Coelenterazine H (PerkinElmer) to track tumor growth and metastasis. The adoptively transferred human CAR-T cells would be expressing firefly luciferase (e.g. RediFect Red-FLuc-Puromycin, and the respective substrate Xenolight® D-Luciferin, PerkinElmer). The injected therapeutic antibody would be tagged with a NIR fluorophore (e.g. VivoTag™ 680XL Protein Labeling Kit, PerkinElmer).

This *in vivo* test paradigm would assess the following parameters: tumor growth, regression and metastasis; immune cell biodistribution and tumor targeting; antibody biodistribution and tumor targeting; and treatment efficacy.

Summary

The increasing adoption of preclinical imaging technologies has become key to bridging the gap between *in vitro* assays and *in vivo* results, enabling the translation of those results into cures for disease. *In vivo* imaging not only impacts cancer and infectious disease research, but neuroscience and neurodegeneration, gene therapy, inflammation, diabetes, stem cell therapy, immunotherapy, osteoporosis, asthma, and a variety of other conditions are all beneficiaries of this technology. The vast body of published literature using PerkinElmer's imaging platforms (>15,000) underscores its utility in enhancing our biological understanding of disease, facilitating therapeutic discovery and development as well as personalized treatment.

References

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