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How to Improve the Predictive Value of Preclinical Oncology Studies: Imaging Orthotopic Models

White Paper

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The Challenge: How Can We Improve the Predictive Value of Current Preclinical Oncology Models?

There are concerns that the lack of an organ-specific tumor microenvironment (TME) limits the predictive value of current subcutaneous preclinical oncology models. Tumor growth within an organ, the PK/PD effects of therapeutic agents, and resulting drug sensitivity are all affected by an organ-specific TME, as well as other related features including:

- relevant vasculature
- stromal interactions
- appropriate environmental physical pressures
- various immune cell populations.

> "Orthotopic, metastatic models provide many advantages over traditional subcutaneous models, which are maximized by tumor imaging"

These issues highlight the need for relevant models more faithfully recapitulating the primary TME than standard available systems. These improved models should also allow invasion, enabling the metastatic niche to be appropriately targeted by new therapeutics.

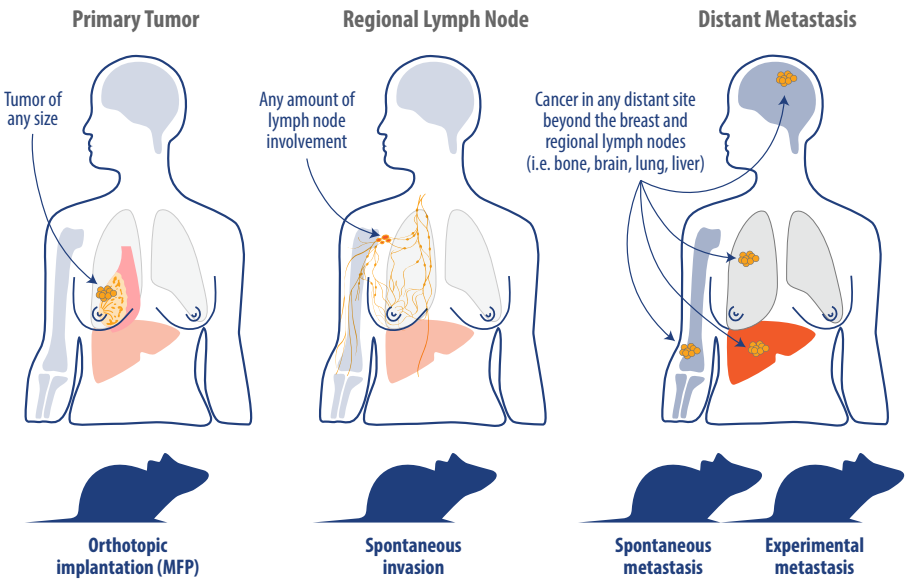
This White Paper presents the use of orthotopic models which allow establishment of a relevant organ-specific TME, tumor metastasis, and provide a more translatable and predictive response to oncology agents. We'll also discuss combining orthotopic models with optical imaging, which enables real time, longitudinal monitoring of disease progression and allows you to maximize orthotopic and metastatic model benefits in the preclinical setting.

Orthotopic Tumor Model Background and Metastatic Model Types

Orthotopic tumor models are generated by seeding tumor cells at the original organ site of tumor origin. This mimics the primary lesion, and enables establishment of a clinically relevant organ-specific TME, including vasculature, hypoxia, and stromal infiltration. Both syngeneic and xenograft orthotopic tumor models can be developed.

A primary advantage of some orthotopic models is spontaneous progression to later stage disease, and the ability to metastasize, which is rarely seen for subcutaneous implantation (shown in Figure 1 for breast cancer). The orthotopic tumors can start to invade local, surrounding tissue, followed by lymphatic migration and hematogenous metastasis. Such preclinical modeling of primary to late stage disease progression, including metastasis, is highly relevant to human disease. It allows investigation of the mechanisms of tumorigenesis, as well as agent evaluation for different disease stages, including relapse.

Figure 1: Example Orthotopic Tumor Disease Progression – Breast Cancer



It should be noted that not all orthotopic models spontaneously metastasize, with the ability dependent on the draining lymph nodes, site of implantation, cell type, and model duration.

Alongside spontaneous metastasis, there are occasions where experimental metastasis models are more appropriate. These are required when primary disease progression is extremely rapid, leaving insufficient time for tumor metastasis to establish within a specific mouse, or when spontaneous metastasis incidence is low. Instead, metastasis can be modeled experimentally, essentially reflecting the adaptation and growth of metastatic end-stage disease. Experimental metastasis models are important for monitoring treatment response or resistance in a patient-relevant metastatic niche without the burden of the upper primary lesion.

Experimental metastasis models are developed by directly seeding tumor cells into different metastatic sites (e.g. brain, bone, or lung) or by releasing cells into the circulation. This includes intracardiac seeding for bone metastasis, i.v. for lung metastasis, or intrasplenic implantation for liver metastasis. Systemic tumor models are established either through i.v. inoculation or i.p. inoculation for peritoneal ascites.

> “Spontaneous and experimental metastasis models allow late stage disease modeling, and evaluation of agents targeting common and hard-to-treat metastatic sites”

Benefits and Challenges of Orthotopic/Metastatic Modeling

The main benefits and challenges of orthotopic modeling are shown in Table 1.

Table 1: Orthotopic Model Benefits and Challenges

Benefits	Limitations
Establishment of a relevant organ-specific tumor microenvironment	Specific surgical skills required to establish the model, achieve reproducibility, and maintain animal welfare
More translatable response to therapy	Tracking tumor progression requires frequent monitoring of clinical signs which can be subjective, and the establishment of humane endpoints
Involvement of significant immune cell populations	Quantification typically carried out at end stage by histology, PCR, or other assays which can take many weeks to perform

> Through the establishment of an organ-specific TME, orthotopic models provide a more translatable preclinical response

As discussed above, orthotopic models allow the establishment of an organ-specific TME including relevant stroma, fibrous tissue, and immune cells, enabling a more clinically-relevant, translational response to new therapeutics.

Specialized surgical skills are needed for specific orthotopic implantation, to ensure that cells are delivered to the correct site reproducibly and to maintain the welfare of all animals used. The other main challenges relate to tracking tumor progression and tumor quantification, which are more complicated for orthotopic models where the tumor is not necessarily visible and cannot be measured by calipers. These challenges can be overcome through sufficient resourcing and training in surgical procedures for orthotopic implantation.

The full benefits of orthotopic metastatic modeling can only be realized if the disease is tracked and monitored within the animal in real time. This is now being enabled by preclinical tumor imaging, which is revolutionizing how we use orthotopic and metastatic models.

Leveraging Imaging for Orthotopic and Metastatic Modeling

Imaging is routinely used in clinical trials, for patient recruitment and monitoring/switching treatment regimens saving both time and resources. Many clinical imaging modalities have now been adapted for preclinical use, driven by a need for enhanced orthotopic models better recapitulating human disease.

Preclinical imaging has helped refine and improve studies, while also reducing animal use and associated costs. It enables animals to be more accurately randomized to treatment arms, and tumor tracking can be performed non-invasively without animal termination.

Imaging is particularly relevant to orthotopic models, where tumors are found in deep lying organs. It can also be applied to both orthotopic and subcutaneous tumors to track physiological molecular processes, monitor hypoxia, or follow

compound distribution. The key application of imaging which helps enhance orthotopic model use is the ability to perform real time, longitudinal monitoring of disease progression. Imaging also allows evaluation of physiological and functional changes, and the tracking of molecular and cellular processes.

Optical Imaging of Orthotopic Models

Many different imaging modalities are available to preclinical researchers, a review of which is outside the remit of this White Paper. Optical imaging is commonly used for preclinical studies, encompassing bioluminescence and fluorescence techniques. There are limited clinical applications of optical imaging due to the need to transduce bioluminscence into the cells. Therefore, while other imaging modalities have been adapted to small animals by scaling down clinical technologies, optical imaging has evolved from microscopic and cellular imaging.

Table 2 shows the related key advantages and limitations of optical imaging. With regards to imaging orthotopic models (and oncology studies in general) the main benefits are non-invasive tumor monitoring and tracking tumor metastasis, which can be fast and higher throughput than other modalities, using bioluminescent tools which have a strong signal, good signal to noise ratio, and are broadly available. Optical imaging is also user friendly compared with other imaging modalities, and more affordable, providing an increased cost/benefit due to the many different uses in a study e.g. randomization, large amounts of data from each animal guiding treatment decisions mid study.

> “Optical imaging key benefits for orthotopic models include non-invasive tumor monitoring and metastasis tracking”

Table 2: Optical Imaging Advantages and Limitations

Advantages	Limitations
Non-invasive, real time tumor progression monitoring, providing longitudinal data	Animals must be immobilized and anesthetized for best optical imaging quality
Allows tracking of metastasis	Signal interference from the primary lesion can affect results
Ex vivo tumor burden quantification	Time consuming and semi-quantitative process
Efficient for larger studies, fast and high throughput imaging: up to 5 animals imaged at a time, 2D images acquired in seconds, user friendly software	Analysis of large datasets can be time consuming and require expertise, image acquisition is slower for 3D imaging (one animal at a time)
Cost efficient with large amounts of data acquired from each animal, optimal randomization, and better-informed treatment decisions	Surgical procedures, multiple imaging sessions, and maintenance of specialized equipment can add costs
Bioluminescent/fluorescent tools are broadly available	Generating and optimizing the right construct can be time consuming. Some tissues cannot be transduced e.g. PDX
Bioluminescence has a strong signal	Signal can be weaker in deep lying organs
Bioluminescence has a good signal:noise ratio: emission wavelength around 620-680nm where tissue autofluorescence is minimal, allowing signal amplification and straightforward tumor tracking	Bioluminescence signal production requires cellular ATP and oxygen. Scarcity of these in larger tumors can complicate data analysis vs controls
Cellular and molecular imaging can be performed using reporter based systems and injectable fluorescent probes	Limited to fluorophores emitting in the far red wavelengths due to insufficient penetration of tissue and autofluorescence. Insufficient tumor penetration by injectable probes

To demonstrate the varied utilities for combining orthotopic/metastatic models with optical imaging, we present here 3 case studies which show how:

- Spontaneous metastasis models are used to evaluate novel agents for advanced disease
- Experimental metastasis models can help identify suitable combination treatments
- Experimental brain metastasis models are used to discover new therapeutics with enhanced BBB penetration.

> "Spontaneously metastatic models recapitulate clinical disease, offering more predictive preclinical studies"

Case Study 1

Evaluating Novel Therapeutic Options for Late Stage Advanced Disease with Spontaneous Metastasis Models

Late stage breast cancer is an area of high unmet need. Up to 30% of patients with resected primary tumors develop metastasis, and metastatic breast cancer is currently incurable with limited treatment options. It is generally poorly understood when this metastasis will occur, and whether it is caused by dormant cells which have escaped early from treatment, or surviving residual tumor cells which have become more aggressive.

In order to develop new agents against late stage disease, more predictive preclinical models are needed to mimic advanced/metastatic breast cancer. Orthotopic models with spontaneous metastasis provide a preclinical setting more closely recapitulating late stage disease features.

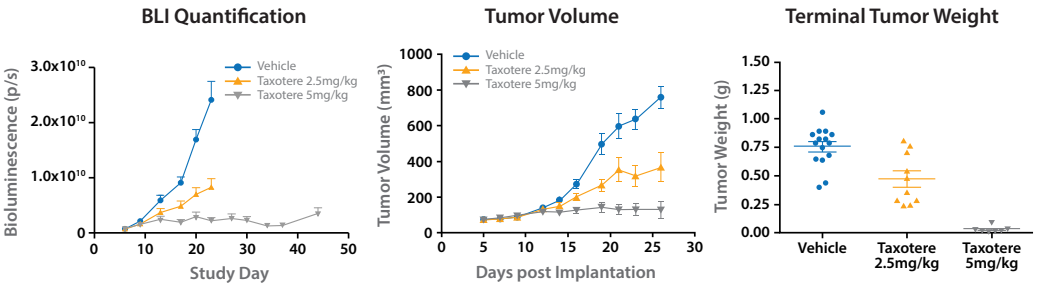
Spontaneous Metastasis of the MDA-MB-231-luc Orthotopic Model

The MDA-MB-231-luc triple negative breast cancer model provides a highly reproducible orthotopic model with a strong, stable signal from the primary tumor. The model is fast growing (faster than the parental line), due to clonal selection for model brightness, and is also more invasive.

To mimic the primary tumor, MDA-MB-231-luc cells are inoculated into the fourth mammary fat pad (MFP), close to the inguinal lymph nodes. Bioluminescent imaging (BLI) is performed twice weekly, and effectively quantifies both tumor burden and treatment response, as compared and correlated with caliper-measured tumor volume or final tumor weight (Figure 2). Model duration is typically three to four weeks, driven by the primary MFP lesion reaching size limits. Significant response to standard of care docetaxel can be seen at the MFP site, extending the survival of mice in a dose dependent manner.

Figure 2: MDA-MB-231 Treatment Response Quantified by Bioluminescent Imaging

Licensing from MD Anderson is required to access MDA-MB-231 models at CrownBio.



The MDA-MB-231-luc model spontaneously metastasizes to various organs, such as liver, lung, bone, and brain (Figure 3). The incidence of metastasis is quantified at end stage by *ex vivo* terminal assessment of tumor associated bioluminescence (TABL). However, by shielding the metastatic sites from the primary tumor in the fourth MFP (located in the lower half of the animal), the extent of tumor spread to the lung and brain can be quantified by BLI in real time, *in vivo* (Figure 4).

Figure 3: Instance and Location of MDA-MB-231-luc Model Spontaneous Metastases

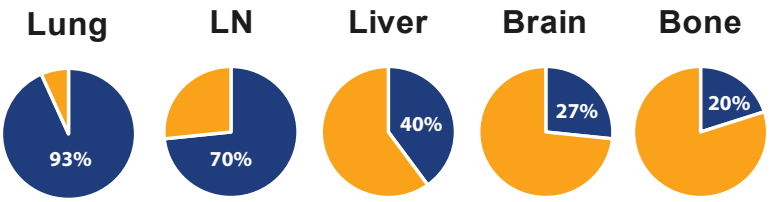
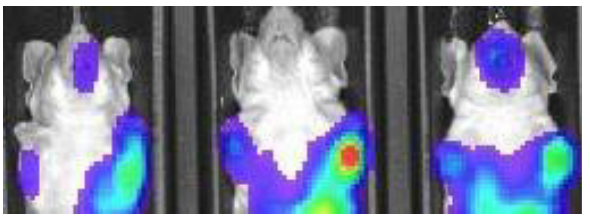
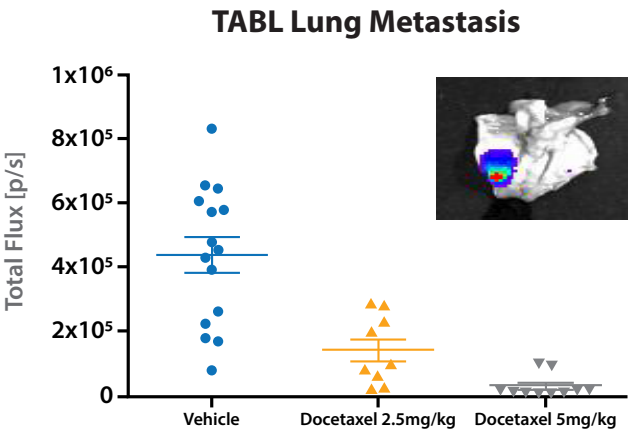


Figure 4: Metastasis Detected in Real Time by BLI



Standard of care treatment response can be assessed for metastatic lesions, including the lung and lymph nodes. For this model, treatment response correlates with that of the primary lesion (Figure 5). By studying metastatic lesion data, and through characterizing model growth, optimal study group sizes can also be determined for this model. For example, n of 15 is quite typical; however, to study metastatic lesions the n number may need to be increased appropriately based on study objectives.

Figure 5: MDA-MB-231-luc Lung Metastasis Treatment Response



Case Study 1 Conclusions

The orthotopic implantation of MDA-MB-231-luc cells allows modeling of late stage metastatic breast cancer, and the assessment of treatments targeting these metastases. Orthotopic implantation of cancer cells allows the establishment of a relevant tumor niche, as well as spontaneous metastasis. BLI is utilized to follow tumor growth, metastatic spread, and response to treatment in real time provided that challenges with primary tumor interference are overcome.

Case Study 2

Experimental Models to Assess Bone Metastasis Targeting Agents and Combinations

Metastatic breast cancer has a high clinical incidence of spreading to the bone, with the resulting bone lesions proving difficult to treat. To allow development of new therapies, including combination regimens, relevant models which metastasize to the bone are needed.

> “Experimental metastasis models fill a need for modeling metastatic sites occurring at low incidence spontaneously”

The MDA-MB-231 spontaneous metastasis breast cancer model (discussed in case study 1) has low incidence of bone metastasis, therefore there is a need to establish experimental metastasis models.

Experimental Bone Metastasis of the MDA-MB-231-luc Model

An experimental bone metastasis model is generated by directly injecting MDA-MB-231 cells into the bone environment or by intracardiac (i.c.) injection. Following i.c. injection into the left ventricle, the tumor cells enter the circulation and lodge in the long bones, jaw, ribs, and spine, with lytic lesions then developing. Model duration is approximately 3-4 weeks, with endpoint driven by long bone lesions in the hind/fore limb and deterioration in clinical condition - reduced mobility, abnormal gait, and subdued behaviour.

As there is no interference from a primary tumor in this model, lytic lesions can be effectively imaged as early as 5 days post injection to verify the success of model development. Once detected, the lesion can be tracked and quantified by BLI in real time to evaluate new treatment efficacy, with response to standard of care pamidronate shown in Figure 6. Imaging using a range of other modalities (Figure 7) demonstrates that BLI is effective at detecting bone lesions in this model and when compared to MRI and x-ray technologies provides a higher throughput alternative.

Figure 6: MDA-MB-231-luc Experimental Bone Metastasis Model Treatment Response

Licensing from MD Anderson is required to access MDA-MB-231 models at CrownBio.

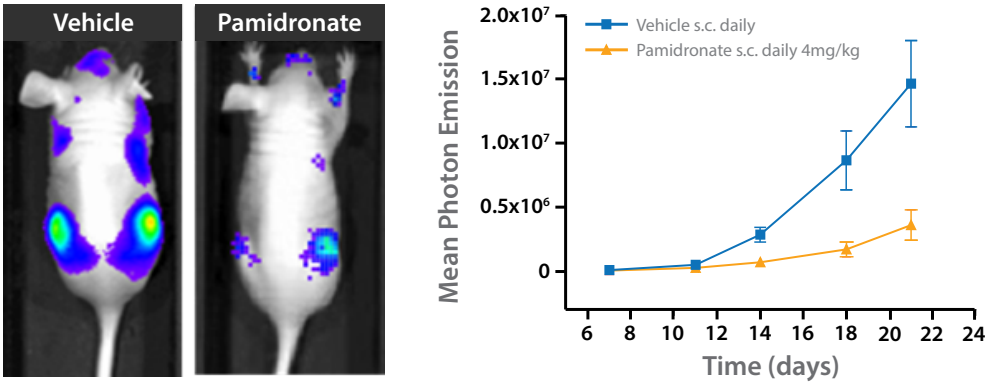
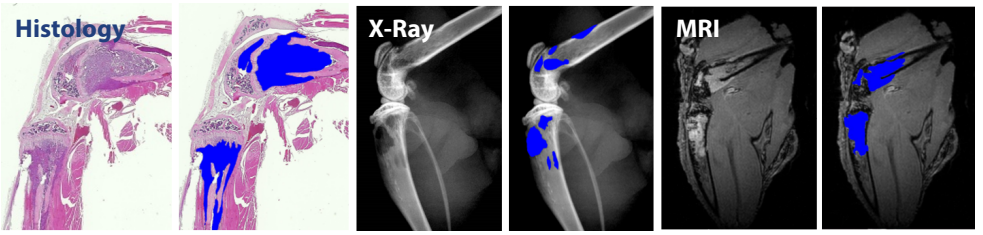


Figure 7: Bone Metastases Imaged Across a Range of Modalities



Case Study 2 Conclusions

The i.c. injection of MDA-MB-231-luc cells allows experimental modeling of bone metastases, which only occur with a low incidence spontaneously. The experimental metastasis model is highly effective for the evaluation of new agents and novel combination strategies to tackle late stage, metastatic bone disease.

Case Study 3

Experimental Brain Metastasis Models to Discover New Therapeutics with Enhanced BBB Penetration

Breast and lung cancer, along with other cancer types, metastasize to the brain in late stage disease often many years after treatment of the primary tumor. Brain metastases pose a significant challenge for therapeutic delivery, and are therefore difficult to treat, as many agents do not cross the blood brain barrier (BBB).

In vivo models of brain metastasis play crucial roles in facilitating the discovery of new therapeutics and optimization of existing drugs with enhanced BBB penetration. As spontaneous models of brain metastasis are limited, new experimental models are required.

Experimental Brain Metastasis of the MDA-MB-231-luc Model

An experimental brain metastasis model is developed by i.c. injection of MDA-MB-231-luc cells (as described in case study 2). Bone metastases develop, however, the administration of Taxotere® limits progression of long bone lesions which no longer drive a terminal endpoint (Figure 8, with lesions co-registered with microCT to confirm the exact site).

> “Experimental brain metastasis models provide the opportunity to evaluate and optimize agents crossing the BBB”

Due to limited BBB penetration by Taxotere, CNS lesions of the spine and brain continue to develop, mimicking clinical progression following Taxotere treatment, which drive the model endpoint at approximately 8 weeks. The model allows the evaluation of combination regimens including Taxotere, to concurrently treat both bone lesions and brain metastases.

Figure 8: Development of a MDA-MB-231-luc Brain Metastasis Model

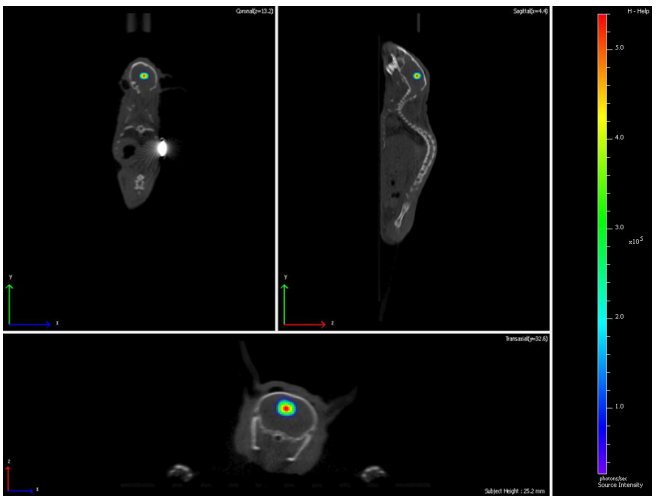
Licensing from MD Anderson is required to access MDA-MB-231 models at CrownBio.

Stereotactic Intracranial Implantation of the NCI-H2228-luc Model

Due to the limited brain metastasis of most tumor models, stereotactic intracranial implantation can also be performed, with diffuse luminescence imaging tomography (DLIT) used to confirm implantation at the correct site. Environmental pressures should be considered when modeling brain lesions, as they can be very different from other sites, with loss of clinical condition even for small tumors. Imaging can be used to select the correct size tumors to enable chosen animals to continue on study.

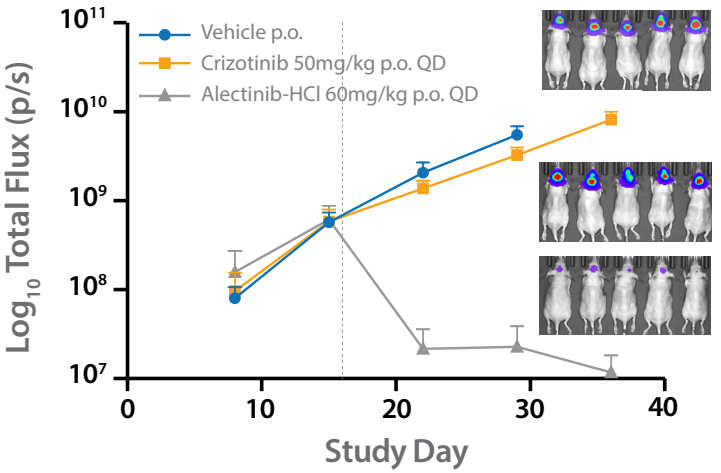
Brain lesions can be mimicked by the implantation of NCI-H2228-luc NSCLC cells harboring EML4-ALK fusion, which occurs in approximately 3-5% of patients (Figure 9). Tumors with these gene fusions respond well to ALK inhibitors, with better outcomes than for NSCLC standard first line treatment, before progression and brain metastasis occurs for a subgroup of patients.

Figure 9: NCI-H2228-luc Brain Implantation Confirmed by DLIT



Treatment of the model with crizotinib, an ALK inhibitor with low BBB penetration, stabilized body weight and clinical signs compared with vehicle, but progressive brain tumor growth drives the model humane endpoint. In comparison, treatment with alectinib, an ALK inhibitor with improved BBB penetration results in continuous tumor regression, confirming the retention of the blood brain barrier for the model duration and the selective penetration of targeted agents (Figure 10).

Figure 10: Treatment of Brain Lesions with ALK Inhibitors with Variable BBB Penetrance



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Case Study 3 Conclusions

Late stage disease, such as brain metastasis, is challenging to mimic in the preclinical setting, requiring experimental models. Development of brain metastasis under Taxotere treatment in the intracardiac model or direct implantation of lung cancer cells into the site of metastasis can provide the models needed. These experimental models provide the ideal setting to evaluate novel agents which cross the BBB.

Summary

Orthotopic *in vivo* tumor models fulfil a need for more relevant models, recapitulating the organ-specific TME and allowing clinically-relevant disease metastasis. Combined with optical imaging, spontaneous metastasis models provide a platform for evaluating agents targeting late stage disease, with BLI providing real time data on metastatic spread and treatment response.

For metastatic sites which occur at a low incidence spontaneously (e.g. bone, brain), experimental models offer an alternative approach for investigating aggressive disease stages and novel agent development. These models paired with imaging allow progression of drug development programs for challenging metastatic targets within preclinical oncology.

> “Imaging orthotopic and metastatic models provides clinically-relevant real time data on disease progression, and a predictive platform for preclinical evaluation of new treatment modalities”