Optical Imaging Platforms for Advanced Preclinical Modeling

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Advanced in-life visualization of cancer development and progression

CrownBio provides non-invasive 2D and 3D bioluminescence and fluorescence optical imaging, for real-time longitudinal monitoring of tumor growth, progression, metastasis, and gene expression.

Optical imaging is a powerful translational screening tool, for maximizing scientific output and supporting the 3Rs.

- Efficient and informative efficacy evaluation for different cancer stages, from primary lesions through to metastasis.
- Validation across a broad range of cancer types at relevant organ and metastatic sites.
- Applicable for both solid tumors and hematological cancers.
- Available for both xenograft models in immuno-deficient mice and syngeneic models in immuno-competent mice.
- In-life imaging of multiple mice at multiple time points.
- Bespoke bioluminescent model development available for unique cell lines.

Assess cancer progression through 3D optical imaging

Orthotopic models to mimic primary lesions

Spontaneous metastasis models to mimic disease progression

Experimental metastasis models to mimic late stage disease

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Optical Imaging Platform Key Facts

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CrownBio provides clinically relevant optical imaging models enabling in-life visualization of tumor growth and disease progression:

- Delivering efficient and informative efficacy evaluation for different cancer stages, through *in vivo* imaging of:
  - orthotopic models to mimic primary lesions
  - spontaneous metastasis models to evaluate dissemination and disease progression
  - experimental metastasis models to replicate late stage disease
  - systemic models to replicate hematological disease.
- Imaging of a range of cancer types, including xenografts in immunodeficient mice and syngeneic models in immuno-competent mice.
- Evaluation of multiple mice simultaneously using the highly sensitive *in vivo* imaging system IVIS® Spectrum CT.
- Generation of high quality 2D and 3D quantitative bioluminescence and fluorescence representations (400–840nm) with fast and low dose CT imaging.
- Available techniques include 3D diffuse tomography, including Fluorescent Imaging Tomography (FLIT) and Diffuse Luminescence Imaging Tomography (DLIT).
- Bespoke bioluminescent model development available for unique cell lines.

Optical Imaging in Preclinical Oncology Research

Bioimaging is a widely used technique in preclinical cancer drug discovery, including strategies such as optical imaging e.g. fluorescent or bioluminescent imaging (BLI), as well as ultrasound, MRI, PET, etc.

Optical imaging provides high throughput real time *in vivo* tumor detection, monitoring, and follow-up. These features offer the availability of more clinically relevant models through imaging, allowing a variety of tumor associated-properties to be dynamically visualized in living animal models[1]. Optical imaging also allows increased scientific benefit by providing more data from the same animal.

CrownBio Provides an Optical Imaging Panel of Bioluminescent Xenograft and Syngeneic Models

CrownBio has developed in-house a comprehensive optical imaging panel of bioluminescent orthotopic and metastatic cell line derived xenograft and syngeneic models. Our panel of bioluminescent xenograft models are shown in **Table 1** (on the next page), with cell lines available for development in **Table 2**. Syngeneic models are detailed in our dedicated syngeneic section, in **Table 4** on page 7.

All of our models are developed from key peer-reviewed cell lines; any cell lines or models of interest which are not detailed within this factsheet can be established upon request.

BLI of our models can be used to provide valuable disease information at different cancer stages, through orthotopic models to mimic primary lesions, to the imaging of both spontaneous and experimental metastases to replicate progression to late stage disease (**Figure 1**). Our imaging of cancer progression is performed using the *in vivo* imaging system IVIS Spectrum CT, which is the industry's most sensitive detection technology[2].

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Table 1: CrownBio Bioluminescent Cell Line Derived Xenograft Panel
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Tissue	Cell Line	Model Type
Solid Tumor Xenograft Models		
Brain, GBM	U-87 MG	Orthotopic
Breast	MCF7	Mammary fat pad with metastasis
	MDA-MB-231	Mammary fat pad with metastasis Experimental metastatic (intracardiac injection with bone metastases, treatment with Taxotere® drives CNS metastases)
	MDA-MB-468	Orthotopic (ongoing)
	SK-BR-3	Orthotopic (ongoing)
	T47D	Orthotopic (ongoing)
	ZR-75-1	Orthotopic Experimental metastatic (intracardiac injection with bone metastases)
Liver, HCC	Hep 3B	Orthotopic
Lung, NSCLC	A549	Orthotopic with metastasis
	NCI-H2228	Orthotopic Experimental metastatic (stereotactic intracranial implantation with CNS progression)
Ovarian	A2780	Peritoneal ascites
Prostate	PC-3M	Orthotopic with metastasis
Hemopoietic Xenograft Models		
AML	MV-4-11	Systemic

Table 2: Banked Bioluminescent Cell Lines Available for Development

Tissue	Cell Line
Bladder	T24
Brain, GBM	U-251G
Breast	BT-474
Lung, LCLC	NCI-H460
Lung, SCLC	DMS-114
Ovarian	OVCAR-3
	SK-OV-3 (Herceptin® resistant)
Pancreatic	BxPC3
Prostate	DU145, LNCaP

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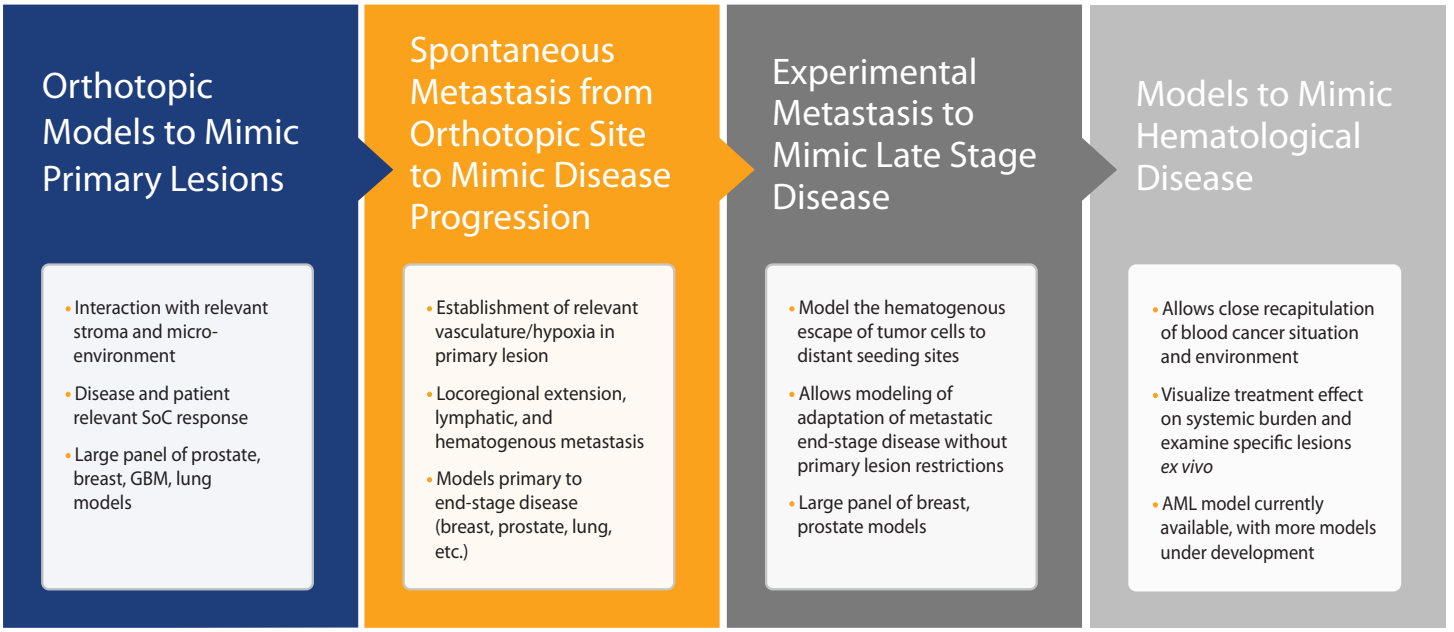
Figure 1: Imaging of Cancer Stages and Progression



Orthotopic Xenograft Models

CrownBio bioluminescent orthotopic models are shown in **Table 1**. These xenografts are used to mimic more patient-relevant primary lesions, and establish models with more relevant vasculature. Implantation is confirmed via DLIT, and progressive in-life tumor growth is monitored with BLI. Palpable tumors in the mammary fat pad (MFP) can be monitored using electronic calipers to correlate tumor volume with concurrent BLI results. Following tumor treatment, endpoints include monitoring tumor burden (*in vivo* and *ex vivo*), tumor growth, and survival analyses.

Example data is shown in **Figure 2** for the Hep 3B model. HCC develops following direct intrahepatic injection of Hep 3B–lux cells into nude mice. Tumor establishment and growth are monitored weekly via BLI, with the SoC treatment sorafenib confirmed to decrease tumor volume. Terminal *ex vivo* analysis using both BLI and macroscopic evaluation confirm the tumor burden in the liver.

CrownBio also provides models for routes other than orthotopic use e.g. the A2780 ovarian model is injected into the peritoneal cavity. Similar to our orthotopic models, tumor growth and establishment can be monitored weekly by BLI, with *ex vivo* analysis consisting of both BLI and macroscopic evaluation confirming tumor mass in the liver, lymph nodes, ovaries, and uterus.

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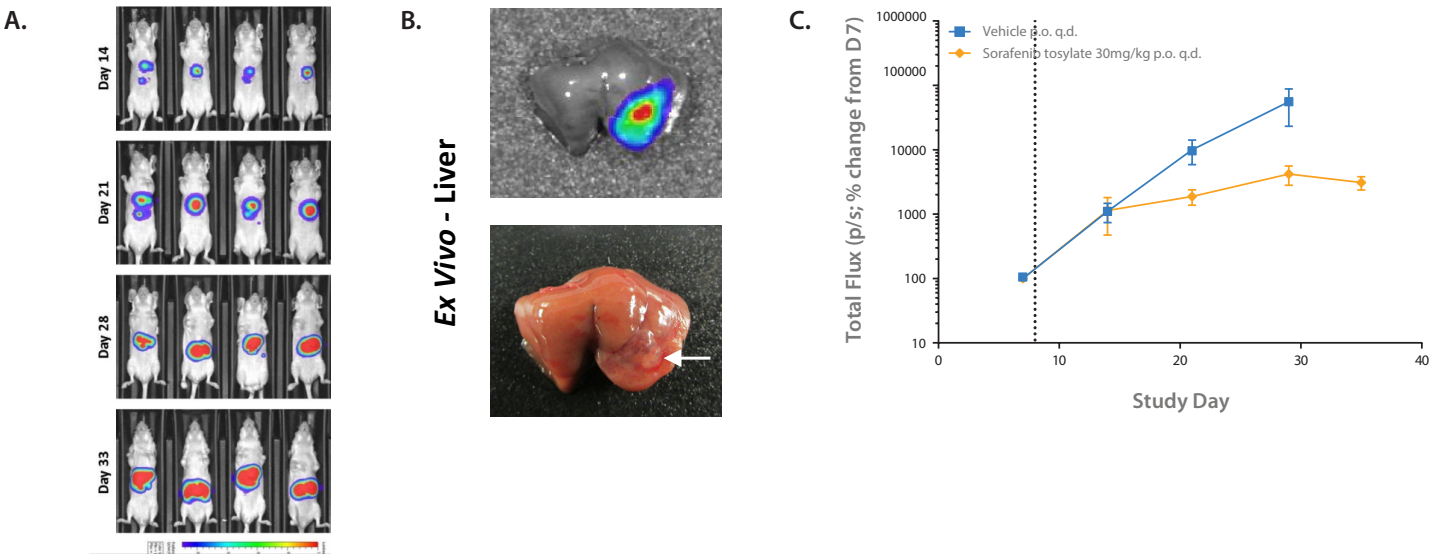
Orthotopic Xenograft Models

Figure 2: Hep 3B HCC Orthotopic Tumor Growth and Treatment Monitored by BLI
A: In-life tumor establishment and growth. B: *Ex vivo* tumor visualization. C: Tumor size assessed by bioluminescent imaging +/- 50mg/kg sorafenib p.o., q.d. treatment.



Spontaneous Metastasis Xenograft Models

Spontaneous metastasis is observed for a variety of our orthotopic models including cancers of the lung, prostate, and breast, allowing models to be used to mimic and monitor disease progression (**Table 3**). Similar to the human disease, common metastatic sites include local lymph nodes, lung, liver, and bone.

Endpoint readouts following treatment include primary tumor growth inhibition, tumor burden, terminal tumor weight, with *ex vivo* imaging of primary tumors and metastatic sites supported by macroscopic and histological evaluations.

Table 3: Summary of Bioluminescent Spontaneous Metastasis Models

Tissue	Breast		Lung, NSCLC	Prostate
Cell Line	MCF7	MDA-MB-231	A459	PC-3M
Implantation Site	Mammary fat pad	Mammary fat pad	Left lung	Prostate
SoC Tested	Primary tumor responds to tamoxifen citrate	Primary tumor responds to docetaxel	--	Primary tumor responds to docetaxel
Spontaneous Metastasis Sites	Inguinal lymph node (LN), lumbar LN, lung, forelimb, hindlimb	Brachial/axillary LN, inguinal LN, lumbar LN, lung, liver, bone, brain (typically late-stage)	Bilateral lung, local LN, liver, kidneys, bone, brain	Lumbar LN, lung, liver, forelimb, hindlimb

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Experimental Metastasis Xenograft Models

CrownBio provides a range of experimental bioluminescent metastasis models to mimic late stage disease, allowing modeling of adaptation of metastatic end-stage disease without primary lesion restrictions. Our models include:

- MDA-MB-231 triple negative breast cancer model which metastasizes to bone
- The above model treated with Taxotere to limit long bone lesion progression, which then develops CNS lesions (both detailed in CrownBio's Triple Negative Breast Cancer Application Note available from our website **www.crownbio.com/publications/application-notes/**)
- ZR-75-1 breast cancer model which also metastasizes to bone
- NCI-H2228 NSCLC EML4-ALK mutant cell line which is an experimental metastasis model for CNS progression
- Lung metastasis models using breast cancer cell lines such as MDA-MB-231 are also being developed.

Metastases can be monitored in real time, with endpoint read outs similar to our other models.

Brief example data are shown in **Figures 3** and **4** for the MDA-MB-231 model. Following intracardiac injection, cells rapidly lodge in the end plates of the long bones, jaw, ribs, and spine and lytic lesions form. The metastases are monitored in real-time, and lytic lesions can be detected and quantified post-mortem by imaging individual limbs for bioluminescence or x-ray. The SoC for bone metastases, pamidronate, has been successfully utilized as a positive control comparison in this model, and significantly reduces lytic lesions ($p<0.001$, one-way ANOVA).

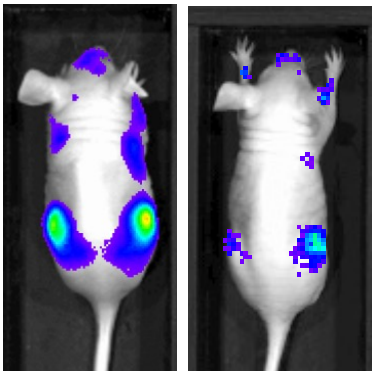
This model can be further utilized to mimic the clinical presentation of brain metastases following successful treatment of initial disease, with the addition of Taxotere treatment. This treatment limits the progression of long-bone lesions, reducing a loss in clinical condition, which no longer drives a model endpoint after ~3 to 4 weeks.

However, due to the inability of Taxotere to cross the BBB, CNS lesions developing in the brain escape treatment. These lesions can be detected by bioluminescent imaging (shown in **Figure 4**) and 3D DLIT co-registered with microCT allows confirmation of their exact site. This model then allows evaluation of novel agents for this aggressive metastasis type.

Figure 3: MDA-MB-231 Experimental Bone Metastasis Formation and Treatment Monitored by BLI

A: BLI of lytic lesions. B: Photon emission over time following pamidronate treatment.

A.



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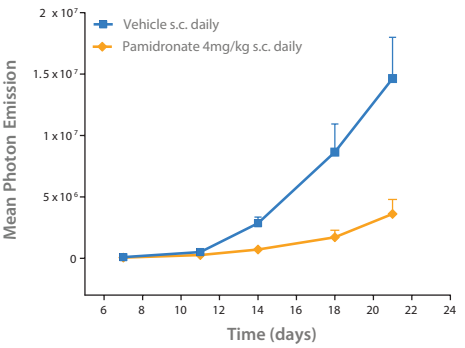
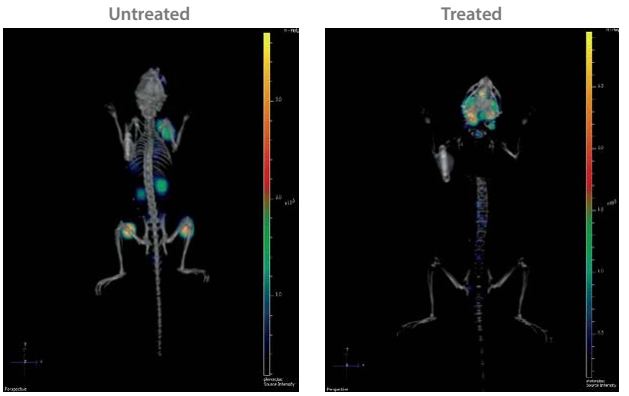


Figure 4: MDA-MB-231 Experimental Brain Metastases Model Monitored by BLI



Bioluminescent Syngeneic Models

CrownBio has validated a large panel of syngeneic models for immuno-therapy research (fully detailed within our Syngeneic FactSheet available from our website at **www.crownbio.com/publications/factsheets/**). Included within our syngeneic collection are bioluminescent models detailed within **Table 4**. These syngeneics include metastatic models allowing the study of clinically relevant metastatic invasion, metastatic lesions in secondary organs, and the evaluation of immunotherapeutic agents to target this metastasis.

Table 4: CrownBio Bioluminescent Syngeneic Panel
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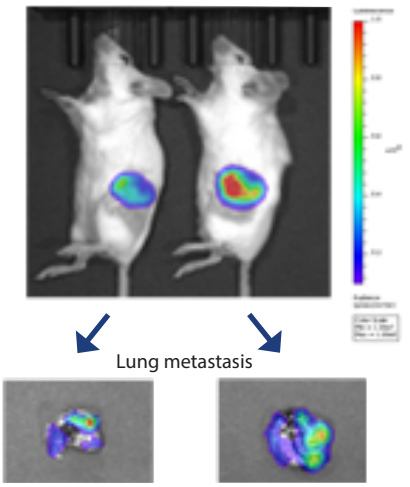
Tissue	Cell Line	Model Type
Mouse bladder	MBT-2	Orthotopic (in development)
Mouse breast	4T1	Mammary fat pad with metastasis
	EMT6	Mammary fat pad
Mouse liver	H22	Orthotopic
	Hepa 1-6	Orthotopic
Mouse melanoma	B16-F10	Metastatic (intracardiac injection with bone metastasis, i.v. injection with lung metastasis), under refinement
Mouse pancreas	Pan02	Orthotopic
Mouse prostate	RM-1	Orthotopic (in development)

Example data is shown in **Figure 5** for the 4T1 bioluminescent syngeneic model. The breast carcinoma cell line metastasizes from the MFP to the lung, lymph nodes, liver, and brain, and from a subcutaneous site to the lung. Metastasis can be assessed by terminal *ex vivo* imaging. Syngeneic models are highly useful for monitoring response to immunotherapeutics including immune checkpoint inhibitors, and 4T1 response to an anti-CTLA-4 antibody is also documented in Figure 5.

Figure 5: 4T1 Metastasis Monitored by BLI, and Immunotherapeutic Treatment
A: *Ex vivo* BLI of metastatic lung lesions from subcutaneous implantation.
B: TGI following treatment with anti-CTLA-4 antibody.

A.



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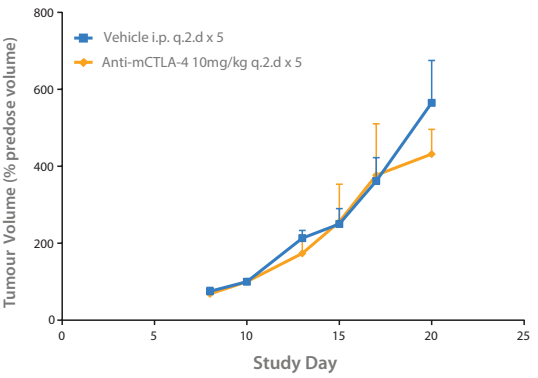

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Summary

Bioimaging techniques such as optical imaging provide multiple advantages over traditional preclinical oncology study methods including real time, non-invasive monitoring of disease, providing clinically relevant models which allow a variety of tumor-associated properties to be dynamically visualized in living animal models.

Optical imaging modalities provide sensitive, cost-effective, and visual high throughput *in vivo* tumor detection, monitoring, and follow-up.

BLI also enables rapid end-point assessment of tumor burden and multiple organ metastasis, providing a multitude of data from a single study animal. Syngeneic models allow evaluation of immunotherapeutics targeting both primary and metastatic disease.

CrownBio provides a panel of bioluminescent orthotopic and metastatic cell line derived xenograft and syngeneic models, which provide real time detail of compound efficacy either alone or in combination, both at the primary tumor site and metastatic lesions.

CrownBio are committed to furthering technologies for preclinical oncology drug development, and are continually evolving our panel of bioluminescent models. We can work with clients to develop cell lines and models of interest that are not currently within our collection.

References

[1]O'Neill K, Lyons SK, Gallagher VM *et al.* Bioluminescent imaging: a critical tool in pre-clinical oncology research. *The Journal of Pathology* 2010;220(3): 317-27.
[2]IVIS SpectrumCT Product Note. Pre-clinical in vivo imaging. Available from: https://www.perkinelmer.com/lab-solutions/resources/docs/BRO_010459C_01%20PRD_SpectrumCT.pdf