Applications in Oncology

Introduction

Caliper Life Sciences reagents in oncology offer unique opportunities to measure tumor growth and metastasis in a variety of models. In vivo bioluminescent imaging allows the non-invasive detection and quantification of orthotopic, metastatic and spontaneous tumors in the whole mouse. Caliper’s biophotonic imaging technology has been optimized for high sensitivity, such that micrometastases can be detected that would otherwise require histopathology to identify.

Recently Caliper Life Sciences introduced its newest set of light producing tumor cell lines using the luc2 vector. These cell lines are significantly brighter than the ones created with traditional methods, allowing for more sensitive non-invasive detection of cells in vivo. The figure to the left shows tumor development from five 4T1-luc2 cells. The brightness of these cell lines for the first time allows researchers to detect down to a single cell in vivo non-invasively.

Caliper’s oncology models can be used to assess anti-cancer therapies over the course of treatment in vivo. Non-invasive, bioluminescent imaging of tumor growth and metastasis allows longitudinal evaluation of tumor development before, during and after treatment, offering an excellent preclinical strategy to assess tumor response and recurrence.

Unlike other non-invasive imaging modalities such as PET or MRI, Caliper’s imaging technology is rapid, easy to perform and amenable to high throughput. The equipment can be used inside of a barrier facility, and thus can be employed for routine drug screening. Furthermore, the proprietary image analysis software enables accurate quantification for ease of data analysis.

Xenograft models by our cell lines offer the ability to:

- Quantify tumor burden in the whole mouse
- Detect micrometastases with high sensitivity
- Visualize metastases spontaneously generated from a primary tumor
- Follow responses to therapeutic treatments non-invasively in longitudinal studies

All Caliper Life Sciences cell lines meet the highest standards and are confirmed to be pathogen free.
Bioware Oncology Cells – Selected Models

Prostate Carcinoma: PC-3M-luc-C6

PC-3M-luc-C6 is a luciferase-expressing cell line that was derived from PC-3M metastatic human adenocarcinoma cells by stable transfection of the North American Firefly Luciferase gene expressed from the SV-40 promoter. This cell line can be used to establish:
- Subcutaneous tumor models (Figure 1, page 2)
- Metastatic tumor models (Figure 2, page 2)
- Orthotopic tumor models (Figure 3, page 3)

PC-3M-luc-C6 Subcutaneous Tumor Model

PC-3M-luc-C6 can be used to measure growth of subcutaneous (s.c.) tumors and monitor responses to potential chemotherapeutic agents.

Figure 1. Dorsal views of a s.c. PC-3M-luc-C6 tumor model. Images of tumor growth over time are illustrated and quantified relative to tumor volume. 5 x 10^6 cells were implanted in male athymic mice and tumor growth was monitored using the IVIS Imaging System and caliper measurements over 5 weeks.

PC-3M-luc-C6 Bone Metastasis Model

The power of the imaging technology to detect metastatic tumors can be demonstrated with the PC-3M-luc-C6 cells. Intracardiac (i.c.) or intravenous (i.v.) injection of cells can be used to generate experimental metastatic models. In the i.c. model illustrated in Figure 2, the metastatic signals began to appear after one week. By week 3, all mice developed metastatic tumors in bones such as mandible, maxilla, and/or femur/tibia. These metastases were confirmed by ex vivo imaging or histopathology.

Figure 2. PC-3M-luc-C6 cells (3x10^6) were injected into the left ventricle of male athymic mice (n=6). Images were taken weekly for 4 weeks. Ventral images are shown for a representative mouse. Selected tissues were imaged ex vivo to confirm in vivo signals.

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In addition, researchers performing in vivo bioluminescent imaging must do one of the following: (1) use luminescent assay reagents purchased from Promega or Caliper for all determinations of luminescent activity resulting from the research use of this product and its derivatives; or (2) contact Promega to obtain a license for the use of the product and its derivatives in conjunction with luminescent assay reagents not purchased from Promega or Caliper. No reach-through payments shall be owed to Promega relating to an organization's commercialization of products that are discoveries resulting from the research use of this product or its derivatives, provided that such products of the organization do not fall within the valid claims of any issued patents assigned to or licensed by Promega, or that such commercialization would not be a violation of the terms of this label license.

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Bioware Ultra

Brighter cell lines using the enhanced luc2 vector

Bioware Ultra cell lines are the only commercial lines with sensitivity to detect a single cell non-invasively in vivo. The image shows detection of a single 4T1-luc2 cell in a nude mouse using an IVIS Spectrum.

Increasing the brightness of tumor producing cell lines significantly improves researchers' ability to monitor early tumor behavior, detect micrometastases sooner and follow tumor development from a very small number of cells. These cells are bright enough to measure tumor growth way before the tumors are palpable, allowing a researcher to start collecting data at time points impossible with traditional caliper measurements. Bioware Ultra cell lines are optimal for studying a variety of xenograft models including orthotopic models in mice.

With these cell lines, one can also follow responses to therapeutic treatments non-invasively in longitudinal studies, expeditiously and cost effectively.

Figure 7 shows the Subcutaneous tumor growth from an initial injection of 5x10^5 4T1-luc2 cells in a nude mouse. As seen in the figure, one can monitor tumor development right after implantation till the end of the study non-invasively and in real time. With Bioware Ultra you can start collecting data literally from Day 0. As seen in the graph, with caliper measurements you will have to wait for at least 28 days, when the tumor is first palpable.

Our cell lines in conjunction with an IVIS system allows you to monitor tumor development right from the onset and collect data throughout tumor development.

Bioware Ultra Cell Lines

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<tr>
<th>Model</th>
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</tr>
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<td>MDA-MB-231-luc2</td>
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</tr>
<tr>
<td>PC-3M-luc2</td>
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</tr>
<tr>
<td>LnCap-luc2</td>
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<td>B16-F10-luc2</td>
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<td>HCT 116-luc2</td>
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<td>HT-29-luc2</td>
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<td>U-87 MG-luc2</td>
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<td>NCI-H460-luc2</td>
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</tr>
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<td>MOLT-4-luc2</td>
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<td>ACHN-luc2</td>
<td>Yes</td>
</tr>
<tr>
<td>BxP3-luc2</td>
<td>Yes</td>
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• All Bioware Ultra cell lines from Caliper Life Sciences meet the highest standards and are confirmed to be pathogen free. The in vivo growth rate of these cell lines is similar to their parental cell lines and the stability of luciferase expression is tested for a minimum of 4 weeks.

• Luciferase expressed from the human UBC promoter.

• luc2 pG4A vector licensed from Promega Corporation, Madison, WI.

Contact Information:
If you have any questions regarding these cell lines please contact Caliper at 508.497.6592 or e-mail: reagents@caliper5.com

PC-3M-luc-C6 Orthotopic Prostate Model

PC-3M-luc-C6 cells can also be used to establish an orthotopic prostate model as shown in Figure 3. In vivo imaging demonstrated the progression of PC-3M-luc-C6 tumors in the prostate of male athymic mice. All mice developed tumors and the mean tumor bioluminescence correlated well with mean tumor weight at week 5.

B16-F10-luc Melanoma

The B16-F10-luc-G5 mouse melanoma cell line was derived by stable transfection of the North American Firefly Luciferase gene expressed from the SV-40 promoter into B16-F10 cells. These cells can be used to establish subcutaneous or experimental metastatic models. Injected i.v., cells colonize the lung and form lesions that can be imaged non-invasively. Figure 4 illustrates a representative mouse from a lung metastasis experiment. Imaging results were compared to lesions counted on the surface of the lungs. A strong statistical association between mean bioluminescence and mean lung lesions was found (R^2=0.9833).
MDA-MB-231-luc Breast Cancer

MDA-MB-231-luc is a luciferase expressing cell line that was derived from MDA-MD-231 human adenocarcinoma cells by stable transfection of the North American Firefly Luciferase gene expressed from the SV40 promoter. MDA-MB-231-D3H1 cells are derived from a primary orthotopic tumor of MDA-MB-231-luc-D3 cells. This cell line can be used in vivo to establish experimental metastasis model (intravenous and intracardiac) and orthotopic mammary fat pad model with metastasis. Figure 5 illustrates an experimental metastasis model in a nude mouse with intracardiac injection of these cells.

**Intracardiac Injection: Experimental Metastasis in Nude Mice (Harlan)**

![Figure 5. MDA-MB-231-luc-D3H1 cells (1x10⁵) are injected into the left ventricle of female nude mice (n=5). As shown, mice were imaged weekly from dorsal and ventral views for 10 weeks. Selected tissues are imaged ex vivo to confirm in vivo signals. Metastatic signals begin to appear after five weeks. By week 10, metastases are detected ex vivo in 80% of mice (4/5).](image)

**Lung and Colon Carcinoma Bioware Cell Line Models**

Additional cell lines offered by Caliper include A549-luc-C8 human lung carcinoma, HT-29-luc-D6 human colon and Hela-luc human cervical carcinoma models. A549-luc-C8 and HT-29-luc-D6 can be used to establish subcutaneous or metastatic tumor models. Hela-luc cells can be used of subcutaneous and intravenous lung colonization tumor models.

For a complete list of Bioware cell lines, please contact a Caliper representative for more details.

**Signal Transduction/ Mechanism-Based Reporters**

Caliper’s Bioware cell line models are also available that link luciferase expression to signal transduction pathways that are key to the oncogenic process. These models offer the ability to track activation of factors that signal cell death or survival (p53-RE-luc), or angiogenesis (hVEGF-luc).

**Human VEGF (hVEGF)-luc/PC-3M Model**

Angiogenic signaling can be imaged using the hVEGF1-luc/PC-3M cell line, as illustrated in Figure 6. The human VEGF promoter (2.3kb) was fused to the North American Firefly gene and stably introduced into PC-3M cells. Subcutaneous tumors were established and imaging was used to monitor the expression of hVEGF-luc during the course of tumor growth as compared to SV-40-luc. In vivo imaging revealed activation of hVEGF1-luc relative to SV-40-luc during early stages of tumor development. This up-regulation of VEGF may function to signal development of blood vessels in newly forming tumors.

![Figure 6. Male SCID mice were implanted s.c. with 3 x 10⁶ hVEGF1-luc/PC-3M cells on the left and SV-40-luc/PC-3M cells on the right flank. Growth was monitored for 17 days. hVEGF-luc expression increased rapidly during the first week after cells were implanted, as compared to the constitutive SV-40-luc. The graph illustrates Fold expression relative to Day 0.](image)
MDA-MB-231-luc Breast Cancer

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Intracardiac Injection: Experimental Metastasis in Nude Mice (Harlan)

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4
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Our cell lines in conjunction with an IVIS system allows you to monitor tumor development right from the onset and collect data throughout tumor development.

Caliper Life Sciences currently offers seven Bioware Ultra cell lines covering a range of cancer models including Breast, Colorectal, Prostate, Lung as well as Lymphoma. Four additional cell lines including one for Brain cancer will be available in the very near future.

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<tr>
<td>BxPC3-luc2</td>
<td>Pancreatic Cancer</td>
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• Luciferase expressed from the human UBC promoter.

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Contact Information:
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**PC-3M-luc-C6 Orthotopic Prostate Model**

PC-3M-luc-C6 cells can also be used to establish an orthotopic prostate model as shown in Figure 3, page 3. In vivo imaging demonstrated the progression of PC-3M-luc-C6 tumors in the prostate of male athymic mice. All mice developed tumors and the mean tumor bioluminescence correlated well with mean tumor weight at week 5.

**Figure 3.** PC-3M-luc-C6 cells (5x10⁵) were injected into the prostate of male athymic mice. Mice were imaged weekly for 5 weeks to monitor tumor growth. At the end, mice were euthanized, and tumors excised and weighed.

**B16-F10-luc Melanoma**

The B16-F10-luc-G5 mouse melanoma cell line was derived by stable transfection of the North American Firefly Luciferase gene expressed from the SV-40 promoter into B16-F10 cells. These cells can be used to establish subcutaneous or experimental metastatic models. Injected i.v., cells colonize the lung and form lesions that can be imaged non-invasively. Figure 4 illustrates a representative mouse from a lung metastasis experiment. Imaging results were compared to lesions counted on the surface of the lungs. A strong statistical association between mean bioluminescence and mean lung lesions was found (R²=0.9833).

**Figure 4.** B16-F10-luc-G5 (5x10⁵) cells were injected i.v. to colonize the lungs of male athymic mice (n=60). Total in vivo photon counts were compared to macroscopic surface lung lesions to examine the correlation between bioluminescence and traditional methods of determining tumor burden in the lung. Bioluminescence was quantified twice a week from the in vivo signals emitted from dorsal plus ventral views prior to sacrifice (day 14-16).
BioWare Oncology Cells – Selected Models

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3. Attempt to alter, modify or re-engineer the Materials in any way, or extract or transfer any genetic material from the product to another organism; or
4. Sublicense the rights granted herein.

Buyer is not prohibited from using the Materials to discover or develop products that it intends to sell for consideration as long as those products do not contain any Materials. If Buyer has purchased breeding rights to an animal model, Buyer may have the animal model bred by a third party, provided that such third party (i) does not use the animal model for any purpose other than breeding for the benefit of Buyer, (ii) destroys or returns the animals upon conclusion of the breeding services, and (iii) is otherwise legally bound by the terms of this label license.

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Xenograft models by our cell lines offer the ability to:

- Quantify tumor burden in the whole mouse
- Detect micrometastases with high sensitivity
- Visualize metastases spontaneously generated from a primary tumor
- Follow responses to therapeutic treatments non-invasively in longitudinal studies

All Caliper Life Sciences cell lines meet the highest standards and are confirmed to be pathogen free.