CDAS Discovery Alliances & Services (CDAS) in vivo compound evaluation programs are designed to quickly and reliably determine and characterize drug activity in a wide range of animal models. Our strategy is to provide our clients novel products, services, and applications to identify new, effective drugs while minimizing the risk of adverse effects associated with those drugs.

**Introduction**

CDAS in vivo drug development programs offer highly specialized and customized studies for non-invasive optical imaging, drug safety/efficacy evaluation, and biodistribution studies. Client studies rely on in-house, well-established oncology animal models and/or on animal disease models commercially available or supplied by our clients. Our programs are designed to facilitate the identification and validation of therapeutic compounds, and the early detection of potential side effects associated with such compounds.
Programs Overview

Our complete in vivo custom assay services are tailored to each client’s specific study needs. Our scientific team will assist you at every step of the study; from experimental plan design through the data analysis and preparation of a detailed scientific study report. The different components of our in vivo programs can be used individually, to answer a specific question such as drug efficacy or combined for a complete in vivo drug characterization with multiple outputs.

Evaluation of Drug Properties

To maximize the outcome of drug evaluation in our oncology animal models, we help our clients design specific, cost-effective studies in order to determine conventional parameters such as maximum tolerated dose, pharmacokinetic profile, and chronic toxicity that are essential to determine dosing concentration and frequency.

Drug Efficacy Studies

Our comprehensive in vivo drug efficacy programs will allow you to assess the therapeutic efficacy of lead compounds and to uncover potential side effects caused by the administration of such compounds.

Biodistribution Studies

CDAS has developed a strong expertise in biodistribution studies using nanoparticles, antibodies, recombinant viruses, and lipid-based formulations.

Complementary Tissue Analysis Services

We have extensive experience in performing immunohistochemistry (IHC), immunofluorescence (IF), fluorescent in situ hybridization (FISH), tissue microarrays (TMAs), as well as a broad array of tissue stains that are all fully complementing our in vivo programs.

Optical Imaging Technology

In Vivo Bioluminescent/Fluorescent Imaging Platform

In addition to being inherently low throughput, traditional animal modeling techniques are marginally predictive of success in human clinical trials, and thus, a high failure rate in drug development persists. New technologies such as our proprietary non-invasive IVIS imaging instrument have been developed to overcome the bottlenecks in animal testing.

The IVIS imaging system utilizes the light emitted by a bioluminescent or fluorescent molecule expressed in a live organism, and analyzes the source and strength of that bioluminescent or fluorescent signal, allowing extensive longitudinal modeling in the same live animal. Using the IVIS platform, CDAS researchers can view an entire animal or focus on one organ or system for added detail and sensitivity. This real-time in vivo imaging instrument enables us to identify disease pathways, study mechanisms of action, and evaluate and monitor the effects of drug compounds on disease progression in live animals. Real-time in vivo imaging offers the advantage of quantifying tumor burden in whole mouse, non-invasively tracking the progression of cellular growth and/or monitoring the expression of a reporter gene when challenged in a disease model or with a compound.
Benefits of IVIS Imaging Technology

- Higher throughput
- Higher data content and quality
- More predictive animal models
- Ideal for small animal imaging
- In vivo tracking and monitoring of tumor cells, stem cells, bacteria
- Quantitative – light output is proportional to number of labeled cells

Evaluation of Drug Properties/Drug Efficacy Studies

We have established numerous oncology animal models to evaluate drug properties and assess anti-cancer therapies of the drug over the course of treatment in vivo. Our models include a wide range of spontaneous, syngeneic, and xenogeneic oncology models particularly useful for cost-effective, rapid, and reliable in vivo drug screening and drug efficacy confirmation studies.

The following parameters are determined prior to drug efficacy studies in order to evaluate the optimum drug testing conditions:

- Acute toxicology
- Pharmacokinetic/Pharmacodynamic profile
- Chronic toxicology and tolerability

The IVIS in vivo imaging system offers exceptional sensitivity through detecting fluorescent and bioluminescent signals emitted by only a few cells in a live animal. IVIS-based imaging studies provide high quality data and more accurate and clinically relevant predictions earlier in a preclinical drug development process. 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.

Our oncology drug efficacy research program is fully modular and can be tailored to meet investigators’ needs.

Typical efficacy study parameters considered are:

- Choice of animal model
- Drug treatment protocol including route of administration
- Imaging schedule
- Physical measurements

Figure 1. The IVIS Imaging System and Living Image software controls image acquisition and data analysis for biophotonic imaging.

Figure 2. Same group of anesthetized test animals at each time point of an experiment uses far fewer animals than current methodology. Using the same set of animals at each time point yields improved statistical relevance.

Figure 3. Comprehensive compound evaluation in rodents.
Oncology Animal Models

CDAS has established a wide range of animal models in oncology for preclinical cancer research studies. These models are used to assess anti-cancer therapies over the course of treatment in vivo. All our studies are highly customizable with protocols optimized to meet our clients’ study requirements (i.e. route of administration, treatment protocol, imaging schedule...).

To conduct in vivo anti-cancer drug efficacy studies, CDAS uses relevant bioluminescent (light-emitting) cancer cell lines implanted into immunocompromised or immunocompetent animals. Tumor growth/metastasis and drug efficacy are monitored throughout the duration of the study using our proprietary real-time in vivo imaging IVIS platform.

The non-invasive optical bioluminescence/fluorescence IVIS technology increases the throughput of in vivo efficacy studies since it requires fewer test animals and shorter timelines as compared to conventional animal testing paradigms. The combination of bioluminescent cancer cell lines and the IVIS platform enables us to perform longitudinal evaluation of tumor development and to monitor the efficacy of drug compounds on tumor growth while offering more and higher quality data. As such, more accurate and clinically relevant predictions can be made earlier in a preclinical drug development process.

The recombinant luciferase cell lines used at CDAS to establish oncology animal models have various origins. Clients may provide cell lines, choose from the broad portfolio of Bioware® bioluminescent cell lines or arrange for the custom creation of recombinant bioluminescent cell lines developed by CDAS.

CDAS has established spontaneous, syngeneic, and xenogeneic oncology animal models allowing for specific drug efficacy studies.

<table>
<thead>
<tr>
<th>Spontaneous Model:</th>
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<tbody>
<tr>
<td>The spontaneous pancreatic OncoMouse tumor model developed by CDAS, EL1-luc/EL1-SV40 T-antigen transgenic, offers a non-invasive approach for monitoring pancreatic tumor development and provides a more relevant biological picture of cancer progression.</td>
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<th>Syngeneic Models:</th>
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<tr>
<td>Studies are performed in C57BL6 and BALBc immunocompetent animals using 16F10 and 4T1 cell lines. Primary research applications are in cancer vaccine development and basic research.</td>
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</table>

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<th>Xenogeneic Models:</th>
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<tr>
<td>Studies are performed in NIH-Ilnu/bg (nude/beige) mutant immunocompromised animals with deficiencies in both innate and adaptive immune systems. CDAS offers a wide range of xenogeneic oncology models as indicated in Table 1.</td>
</tr>
</tbody>
</table>

![Figure 4. Bioware Ultra 4T1-luc2 Tumor Model.](image)

![Figure 5. Tumor progression of orthotopically xenografted MCF-7-luc-F5 cells](image)
Table 1. Xenogeneic models.

<table>
<thead>
<tr>
<th>Models</th>
<th>Main Application</th>
<th>Assessments</th>
<th>Additional Options</th>
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<tbody>
<tr>
<td><strong>Subcutaneous Tumor Models</strong></td>
<td>Rapid screening of <em>in vivo</em> activity of lead compounds</td>
<td>– Physical measurements (tumor size and volume) – <em>In vivo</em> optical imaging (bioluminescent signal analysis) – Animal survival</td>
<td>– <em>Ex vivo</em> tumor qualitative and quantitative analysis – Blood samples – Histology, IHC, IF – Gene expression – Cytokine analysis</td>
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<tr>
<td><strong>Orthotopic Tumor Models</strong></td>
<td>Drug efficacy assessment against both primary and metastatic tumors. Cells are grafted according to tissue origin</td>
<td>– Physical measurements at the termination of the study (tumor size and volume) – <em>In vivo</em> optical imaging (bioluminescent signal analysis) – Animal survival</td>
<td>– Number, size and localization of tumor metastasis – <em>Ex vivo</em> tumor qualitative and quantitative analysis – Blood samples – Histology, IHC, IF – Gene expression – Cytokine analysis</td>
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<tr>
<td><strong>Intravenous Tumor Models</strong></td>
<td>Drug efficacy assessment against metastatic tumors and determination of anti-metastatic effects of the drug</td>
<td>– Physical measurements (tumor size and volume) – <em>In vivo</em> optical imaging (bioluminescent signal analysis) – Animal survival</td>
<td>– Number, size and localization of tumor metastasis – <em>Ex vivo</em> tumor qualitative and quantitative analysis – Blood samples – Histology, IHC, IF – Gene expression – Cytokine analysis</td>
</tr>
</tbody>
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Vehicle treated mitomycin C at 2 mg/kg

1x10⁶ PC-3M-luc cells injected s.c. into nu/nu mice

Day 14 Day 21 Day 28 Day 14 Day 21 Day 28

5x10⁶ PC-3M-luc cells were injected into prostate lobes of male nu/nu mice

Day 7 Day 21 Day 28 Day 7 Day 21 Day 28

Data from Pfizer® (Sugen) – Murray et. al., 2003

Vehicle treated 5-Fluorouracil at 100 mg/kg

SU11248 at 80 mg/kg
Table 2 lists our oncology animal models. CDAS is fully capable of developing virtually any custom oncology model using our growing Bioware® portfolio of over 60 bioluminescent and fluorescent recombinant cell lines or client provided cell lines. Our custom recombinant bioluminescent or fluorescent cell line creation services are designed to develop your cell line of interest. CDAS uses a well-established lentivirus-based approach and provides complete characterization of the newly created line versus the parental cell line.

<table>
<thead>
<tr>
<th>Models</th>
<th>Model/Application</th>
<th>Animal Host Options</th>
<th>Tumor Graft</th>
<th>Cell Lines</th>
<th>Drug Injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xenogeneic</td>
<td>Anticancer Activity</td>
<td>NIH-III nu/bg (nude/beige)</td>
<td><strong>Subcutaneous</strong></td>
<td>HT-29 NCI-H460 MDA-MB-231 MCF-7 (ER+) MB-231 (ER-) LNCaP</td>
<td>i.v., i.p.</td>
</tr>
<tr>
<td></td>
<td>Colon Cancer</td>
<td>NIH-III nu/bg (nude/beige)</td>
<td><strong>Orthotopic/Primary</strong> Colon Lung Breast Breast</td>
<td>HT-29 NCI-H460 MDA-MB-231 MCF-7 (ER+) MB-231 (ER-)</td>
<td>i.v., i.p.</td>
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<td>Lung Cancer</td>
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<td>Breast Cancer</td>
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<tr>
<td>Xenogeneic</td>
<td></td>
<td>NIH-III nu/bg (nude/beige)</td>
<td><strong>Intravenous Metastasis</strong> organs/tissues with metastasis</td>
<td>HT-29 (colon) H460 (lung)</td>
<td>i.v.</td>
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<tr>
<td>Syngeneic</td>
<td>Immunotox</td>
<td>C57BL6 BALBc</td>
<td><strong>Orthotopic/Primary</strong> Skin Breast</td>
<td>B16F10 4T1</td>
<td>i.v., i.p.</td>
</tr>
</tbody>
</table>
Biodistribution Studies

In vivo drug biodistribution studies are pivotal in improving drug efficacy and limiting potential side effects. The possibility to monitor in real-time the accumulation of a formulation in a specific organ or tissue in a high throughput and cost-effective manner, allows scientists to optimize drug formulations for their biodistribution properties. A more targeted in vivo drug delivery approach can potentially reduce or eliminate significant drug side effects by i) limiting the drug exposure to targeted organs/tissues and/or ii) significantly reducing therapeutic drug concentration for treatment.

At CDAS, we are fully capable of studying the therapeutic effects of labeled large molecules (e.g., antibodies or other proteins), nanoparticles, recombinant viruses and lipid-based formulations in our animal disease models. We have established validated study protocols to attach fluorescent labels to the large molecule drugs of interest. This approach enables researchers to measure the uptake of the therapeutic agent and to have a dynamic view of its in vivo biodistribution, bioavailability, and efficacy using our proprietary IVIS imaging system.

Complementary Tissue Analysis Services

It is critical for researchers to extract meaningful data from intact tissue in order to study the expression level of multiple target proteins simultaneously within cells and in their native microenvironments. In translational oncology and pharmaceutical drug development this is very critical for preclinical drug evaluation, target validation, and drug safety/efficacy studies and provides faster, more clinically relevant information.

Our comprehensive offering in the area of multispectral tissue analysis services strongly complements our in vivo compound evaluation programs. Our extensive tissue analysis platform offers high quality multiplex biomarker analysis tools including immuno-histochemistry (IHC) and immunofluorescence (IF) assays suitable for drug efficacy/safety, biomarker discovery/analysis in addition to multiplex fluorescent in situ hybridization (FISH) assays for genetic characterization studies. We also offer a wide variety of fully optimized tissue microarray (TMA) and biomarker services suitable for oncology studies, along with a broad array of tissue stains.

Figure 6. IHC assay showing the expression of P53 in NIH16 xenograft tissue.

Figure 8. IF of HER2/ER/DAPI in human breast cancer tissue.

Figure 7. ER/HER2 expression in human breast cancer TMAs.

Figure 9. FISH analysis on FFP showing HER2/CEN17 in human breast cancer tissue.