



Efficacy Evaluation of Cancer Drugs

From In Vitro Kinase Assays to Xenografts

From designing and performing animal studies to histopathology analysis and follow-up molecular biology and biochemistry experiments, our experienced staff works together with our clients to ensure that we provide the in vivo services they need. Our modern, accredited 12,000-square-foot animal facility includes suites dedicated to housing athymic mice, separate surgical suites, a complete pathology laboratory, state-of-the-art intravital and confocal microscopes for analysis, and an irradiator facility for evaluating the effect of test agents on the response of tumors to radiation therapy.

SRI offers a variety of tumor models to assess the cytostatic or cytotoxic characteristics of a potential therapeutic agent. Key capabilities include in vitro cancer cell biology studies, standard xenograft and syngeneic models, the Z-chamber model, metastasis studies, and radiation protection and enhancement studies.

In Vitro Studies

SRI offers a variety of in vitro assays to evaluate a test article's effects on tumor cell biology, including proliferation, cytotoxicity, apoptosis, and cell cycle analysis. We also perform functional, cell receptor binding, and activity assays and can examine a compound's effects on migration and invasion (Matrigel® and fibrin gel). We offer several assays to evaluate a compound's effects on angiogenesis, including endothelial proliferation, migration, and tube formation studies as well as the ex ovo chick chorioallantoic membrane (CAM) assay.

Xenograft and Syngeneic Tumor Implants

Human xenograft implants in nude athymic mice or syngeneic transplants in appropriate rodent models are the industry standard for assessing the success of an anti-cancer agent, whether the compound works by directly affecting the growth of the tumor or by controlling processes such as angiogenesis. Tumor cell cultures or tumor fragments are implanted either ectopically or orthotopically and the tumors grown to a specified size. The animals are



Bridging the drug development gap



randomized, placed into groups, and the test groups are treated with a prescribed drug regimen over the test period. Typically, tumor sizes are measured twice a week and body weights once a week. The animals are sacrificed at the end of the test period and tissues are removed for pathological evaluation.

There are a number of sources for obtaining cancer cell lines (e.g., the American Type Culture Collection). Whenever possible, we like the client to supply the cell line so that the in vivo studies are performed from the same cell line source as the initial in vitro studies. Some human tumor lines that we commonly use in xenograft studies are:

- Breast (MDA-MB231, MCF-7)
- Lung (A549)
- Colon (HT25)
- Prostate (DU-145, PC-3)
- Ovary (Ovcar-3, SKOV-3)
- Brain (U87)
- Pancreas
- Fibrosarcoma
- Leukemia

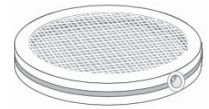
Examples of syngeneic cell lines are:

- Mouse leukemia (L1210, P388)
- Mouse melanoma (B16)
- Rat gliosarcoma (9L)
- Rat mammary adenocarcinoma (R230)

In addition, we are experienced in performing carcinogen-induced lung, colon, bladder, and breast tumor studies.

We are also experienced at performing less-common xenograft studies. Based in part on the growth rate, primary tumor formation, metastatic rate, and tumor pathology, we can determine whether the cell line is suitable for use in a xenograft or syngeneic model. When cancer cell line cultures do not form consistent primary tumors in animals, we can harvest the primary tumors that do form, fragment the tumors, and implant the fragments in a second group of animals. Animal-to-animal passage is performed until enough tumor material is generated for a desired study. Highly metastatic cell lines cannot be used in traditional xenograft studies because multiple tumor formation makes growth measurements impossible, but we offer a set of metastasis models for these cell lines. Very slow growing tumors are very costly to evaluate in xenograft/syngeneic models due to extended treatment regimens and animal care costs, but other animal models, such as the Z-chamber, may be useful when cell culture growth rates are slow.

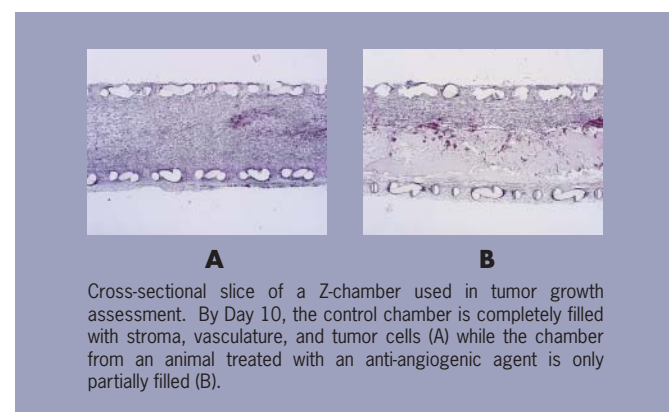
Z-chamber Model



The Z-chamber is an innovative way to study the effects of test compounds on tumor growth and angiogenesis without causing undue stress and morbidity to the animals involved. A Z-chamber's simple design consists of a flat Plexiglas cylinder with 180 μm pore size nylon mesh top and bottom and an injection port in the side of the chamber for the introduction of matrix, tumor cells, and test compounds. Chambers are available for both mouse (5 mm ID) and rat (10 mm ID).

For cancer studies, fibrinogen and a catalytic amount of thrombin are introduced through the injection port of a Z-chamber. Tumor cells are then added through the port, becoming entrapped in the fibrin clot as it forms. Even though the nylon mesh size is large enough to allow test agents, nutrients, proteins, and even cells to enter and leave the chamber, the fibrin environment is natural to the tumor cells and they tend to remain in the chamber. In a typical experiment, four chambers are implanted in the subcutaneous space of the animals by using small, 1 cm incisions that can be closed with single sutures. Depending on the number of cancer cells introduced into the chamber and the cells' normal growth rate, only 6 to 12 days are needed before removing and analyzing the chamber's contents.

The tissue recovered from the chamber can be analyzed in several ways. The tissue can be sliced, fixed, and stained using basic histology stains. Tissue volumes can be measured and compared to volumes obtained from controls. Isolated tissues can be examined using molecular biology techniques, cell sorting, and biochemical assays.

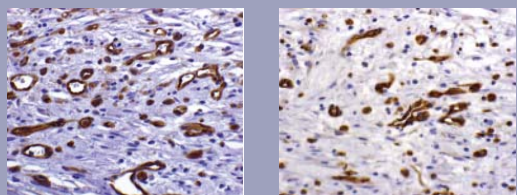


Using the Z-chamber model can circumvent some of the problems that occur in xenograft/syngeneic models. The chambers are manufactured with precision, having a consistent volume and end cap porosity. This

leads to consistent tumor colony growth rates and high reproducibility from animal to animal. When exploring poorly characterized cell lines, only a small group of animals is needed to establish the time required for the chamber to be filled with the tumor colony and support tissue. Having the ability to control the number of cells introduced into the chambers allows the study of both fast- and slow-growing cell lines in similar time frames.

Additional advantages of the Z-chamber design include:

- Minimal animal handling and surgical expertise required
- Weight loss and stress are not observed in animals
- Choice of chamber or systemic administration of test agent
- Test agent effects on wound healing and tumor biology can be studied simultaneously
- Four chambers per animal increases the statistical test number, n, while minimizing the number of animals
- Ability to customize matrix components to assess tumor-matrix interactions



Histology slices from tumor Z-chamber study. Tissue from treated animal (B) had diminished vascular density as compared to control (A).

IN VIVO HIGH-THROUGHPUT SCREENING

The Z-chamber can be used as an efficient, cost-effective primary in vivo screen for potential anti-tumor agents. Treating three animals per group (n = 12), a 100-animal study can be used to evaluate as many as 30 compounds in a 12-day period. A typical xenograft study would require over 5 times as many animals, over 15 times the animal care costs, and may require months to evaluate 30 compounds. Similarly, a test agent could be evaluated against a wide variety of tumor cell lines.

Irradiation Studies

Irradiation is the preferred approach to treating many cancers. For years, researchers have sought compounds that can selectively enhance a tumor's sensitivity to radiation. More recently, researchers have been investigating

compounds that can be used to protect healthy tissues and possibly allow radiologists to increase the radiation dose or exposure time to the affected areas.

SRI performs evaluations of the ability of a drug to sensitize tissue to or protect tissue from radiation exposure. We also perform GLP and non-GLP radiation toxicology studies, either as stand-alone services or as part of a comprehensive development package. Our irradiation facilities have both a Pantak HF320 X-ray source and a Mark 1-68A 137Cs gamma emission source. These facilities are used for both in vitro and in vivo studies. In addition to whole body animal irradiation, focal irradiation is accomplished by using specially designed jigs. Human xenograft, syngeneic, and Z-chamber models are compatible with radiation administration and can be used to evaluate compounds as radiosensitizers, radioprotectants, and for use in combination therapy.

Because irradiation sensitization and protection studies are highly experimental in nature, we work closely with our clients to design studies that will give the highest return on their investment. Variables, including the dosing regimen of the test compound, the sequence of exposure to radiation, irradiation source, irradiation dose, and length of monitoring period, are jointly defined before the final protocol is submitted to the IACUC. While general health, body weight, and survival are key statistics for radiation studies using animals, pathology is crucial to understanding the differential effects of drugs on healthy and diseased tissues. SRI has expertise in both animal and human pathology and offers a wide variety of follow-on study capabilities.

Having an on-site irradiator source allows us to work with clients to develop novel models for evaluating a test article's effects against leukemia or on stem cell proliferation. In these experiments, the animal is normally subjected to whole body irradiation (3.5 to 4 Gg) to suppress immune and stem cell proliferation. Leukemia cells (or stem cells) are introduced into the animal 24 hours after the initial irradiation. The animal is kept in a sterile environment and treated with the test article under a defined protocol. The animals are sacrificed at the end of the experiment and the tissues recovered for evaluation.



Pathology and Follow-On Studies

Once the tissues have been collected, a variety of follow-on studies can be performed. We have exceptional expertise in human and animal pathology. Our capabilities include

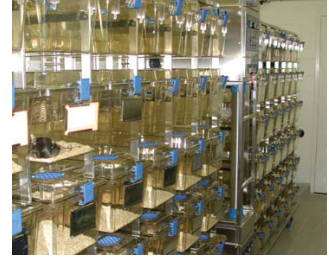
- Basic histology (H&E stain):
- Use of special stains (e.g., Masson Trichrome)
- Proliferation and apoptosis immunostains
- Immunohistochemistry for both monoclonal and polyclonal antibodies
- Fluorescent immunohistochemistry
- Cellular hypoxia measurements
- Blood vessel density measurements
- Biochemical assays for target effectiveness on treated animal tissues

Molecular biology is an integral tool for analyzing treated tissues, building molecular probes, and developing new animal models. Our expertise includes:

- DNA, RNA, and protein extraction from cell and tissue
- Western, Northern, and Southern blot analysis, immunoprecipitation, kinase assays, and ELISA
- Gene construction, transfection, and expression
- DNA sequencing and mutation detection
- cDNA synthesis, primer design, PCR, and RT-PCR
- Genotyping, DNA enzymology, and plasmid DNA sub-cloning
- Developing knockout mouse and transgenic mice
- FACS analysis
- Differential gene expression

ANIMAL CARE FACILITIES

SRI's animal care facilities have been AAALAC accredited since 1974. Animal care is closely monitored by SRI's Laboratory Animal Medicine Department (LAMD). All procedures for animal care and housing are performed



in accordance with the NRC Guide for the Care and Use of Laboratory Animals (1996) and The Animal Welfare Act as amended and standards incorporated in 9 CFR Part 3, 1991. Standard Operating Procedures (SOPs) for all studies are submitted to SRI's Institutional Animal Care and Use Committee (IACUC) for approval prior to the initiation of any study.

You Make the Call

For further information, contact our Client Services Team:

Toll Free: (866) 451-5998

Phone: (650) 859-3000

Fax: (650) 859-3041

Email: biosciences@sri.com

<http://www.sri.com/biosciences>