REACTION BIOLOGY

White Paper

The Hollow Fiber Model

In Vivo Screening Assay to Speed Up Your Drug Discovery Process

Efficacy testing with confidence

Use the Hollow Fiber Model to select the best drug candidates and tumor models for subsequent xenograft tumor testing with higher predictability than conventional assays.

Get your results faster

The Hollow Fiber Model is ideal for researchers who need to screen drug candidates with a fast turnaround in a matter of just two weeks.

Speed up the entire drug discovery process

The Hollow Fiber Model can be performed early in the drug discovery process with simultaneous evaluation of pharmacokinetic and pharmacodynamic parameters.

Let's discover together.

The Hollow Fiber Model:

In Vivo Screening Assay to Speed Up Your Drug Discovery Process.

SUMMARY

When a drug discovery project enters the phase of in vivo testing many questions arise: *"How reliable is our in vitro data? Did we choose the right lead compounds? Which is the best tumor model to use for in vivo efficacy testing?"*

The Hollow Fiber Model is an in vivo drug screening assay bridging the gap between in vitro and in vivo testing of of anticancer agents allowing for rapid answers regarding drug efficacy with simultaneous evaluation of pharmacokinetic and pharmacodynamic parameters.

In brief, tumor cells grow in fibers that consist of a semi-permeable membrane retaining the cells and allowing for the influx of nutrients and drugs. Three fibers, each loaded with one tumor cell line, get implanted into mice for treatment between one to six weeks.

Including the Hollow Fiber Model in the drug discovery process allows for lead candidate identification and lead optimization before entering the phase of time consuming human xenograft tumor testing. In addition, the efficacy of combination therapies, dosing schedules or formulations can be investigated. With the use of the Hollow Fiber Model, better drugs can be made faster and more cost effective.

The Fibers

Encapsulating tumor cells with semi-permeable membranes for testing of cancer therapeutics in vivo was established by scientists of the National Cancer Institute (NCI) and subsequently implemented in their drug discovery routine1,2.

The hollow fibers consist of polyvinylidene fluoride (PVDF) and are straw like in shape with one millimeter diameter. They can be filled with cell suspension and thereafter separated in two-centimeter segments by heat-sealing. The pores of the fibers have a 500kDa cut-off which limits the passage of cells, but allows nutrients, oxygen, and drugs to travel freely. Small molecule compounds, antibodies, and antibody-drug conjugates, as well as natural drugs, can be assessed in the Hollow Fiber Model3. Three fibers, each loaded with another tumor cell line, get implanted under the skin and in the peritoneum of mice as described in figure 1.

Discover Better Drugs

Lead compounds are identified and selected in biochemical and cellular assays in iterative cycles. The in vivo effectivity is largely neglected in this phase because of time restrictions, low compound amounts, and cost. At the end of the early drug discovery process, attention is paid to in vivo activity with an unpredictable outcome. Not only is the potency an important parameter, but other factors like in vivo half-life, access of the target cells, and the target itself play fundamental roles. Why not implement an in vivo assay early in the iterative cycle

process of lead identification and optimization? The Hollow Fiber Model is a simplified xenograft tumor model that allows for rapid answers of the drug efficacy with inclusion of pharmacokinetic processes4 or measurement of pharmacodynamic effects via Western blot5, cell cycle analysis, or gene expression5,6.

Hollow Fibers: Where 3D Tumor Spheroids & Xenograft Tumors Meet

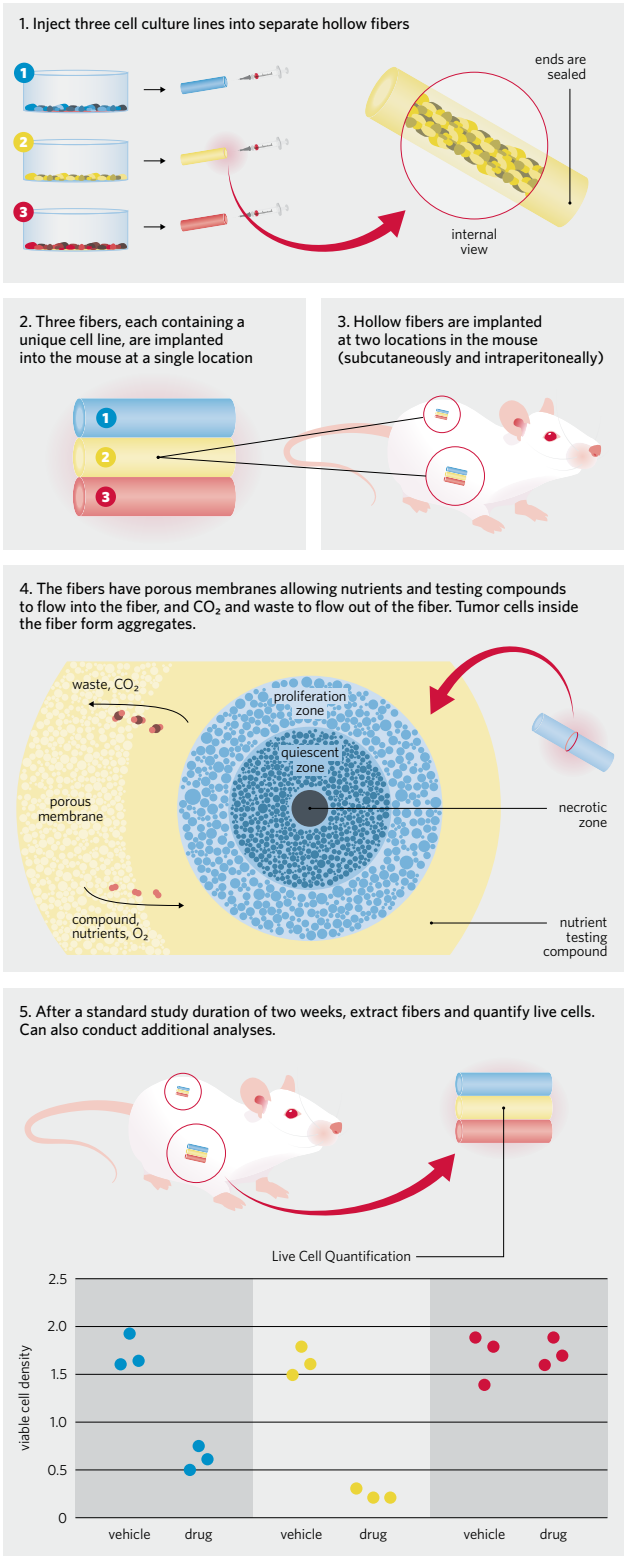
Drug screening in 2D cell cultures often disappoints in regard to predictability of efficacy in treating cancer in both mice and humans. Unlike proliferating cells, tumors contain cell populations in different cell cycle stages with various cell signaling mechanisms and gene expression patterns which may cause resistance to treatment. In the last decade, 3D tumor models were heavily investigated because they mimic the tumor architecture more closely in regard to cell morphology, proliferation, and signal transduction7. Tumor cells in hollow fibers grow in a similar fashion as in 3D tumor models: the proliferation of cells inside the fibers results in areas with different cell densities, a gradient of oxygen and nutrients with areas of viable cells in nutrient-rich areas, quiescent cells in hypoxic areas, and necrotic areas8,9. Furthermore, the three-dimensional growth of the cells results in barriers for the drug to reach the inner areas of the tumor cell aggregates.

The predictive value of the Hollow Fiber Model supports lead compound identification

The predictable value of the Hollow Fiber Model for drug efficacy in xenograft studies has been shown by several independent labs. The NCI investigated the efficacy of 690 compounds that were tested in both the Hollow Fiber Model and in xenograft studies. Of the 93 compounds which showed high activity in the Hollow Fiber Model, 55% also showed activity in at least one xenograft model2. A study performed in Korea10 with 20 standard tumor cell lines showed a predictive value of 92%.

In a case study, we compared the performance of the drug Crizotinib, which is a small molecule compound developed for the treatment of non-small cell lung cancer11, in the Hollow Fiber Model and xenograft tumor models. Of the nine cell lines tested, two cell lines could be identified as drug-sensitive in the Hollow Fiber Model. The same two cell lines also responded to the drug when grown as tumors in mice while the non-responding cell lines also were proven to be insensitive to treatment with Crizotinib when grown as xenograft tumors.

Figure 1. Procedure of the In Vivo Hollow Fiber Model



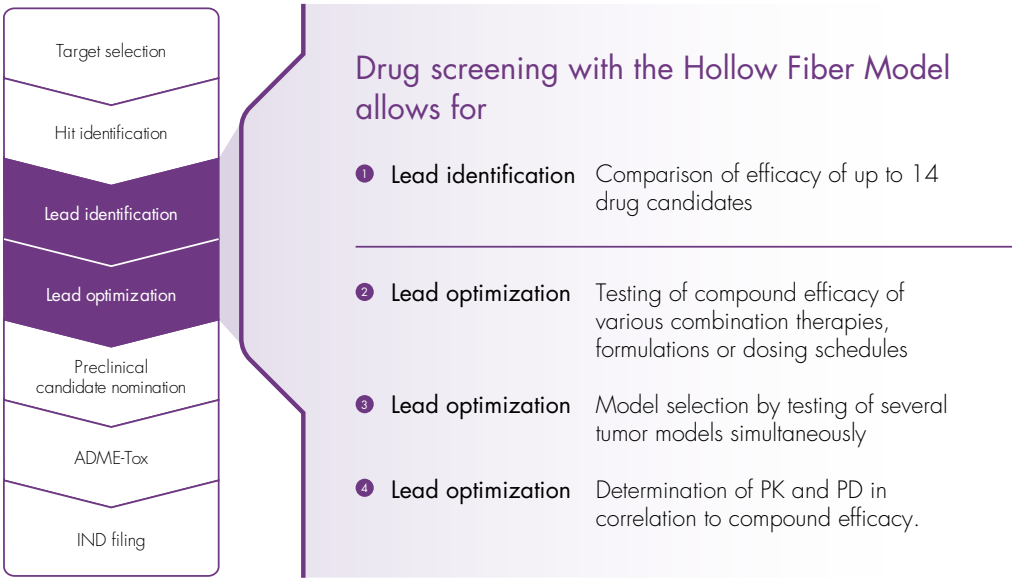


Figure 2:

The Hollow Fiber Model supports the drug discovery process in the phases of lead identification and lead optimization.

PK – pharmacokinetics,
PD – pharmacodynamics,
ADME – absorption, distribution, metabolism, and excretion,
IND – investigational new drug

Antibodies can be tested in the Hollow Fiber Model

The hollow fiber membrane has a cut off of 500kDa - plenty of room for the diffusion of small molecules, but how about larger molecules such as antibodies or antibody-drug conjugates (ADC)?

To investigate the efficacy of antibodies in the Hollow Fiber Model, Trastuzumab and Kadcyla®, an ADC composed of Trastuzumab and the chemotherapeutic emtansine, were tested on the breast cancer cell lines SK-BR3, JIMT-1, and MDA-MB-468. These cell lines express the receptor HER2, the target of Trastuzumab, in the order SK-BR-3 > JIMT-1 > MDA-MB-468, whereas MDA-MB-468 cells were referred to as the negative control in the past publications[12]. A study with a total of 12 mice was performed. Fibers were loaded with JIMT-1 cells, SK-BR-3 cells, or MDA-MB-468 cells. For each cell line, one

	2D Cell Culture	3D Cell Culture	Hollow Fiber Model	Tumor Study
Cell Growth	Monolayer of proliferating cells in normoxic conditions	proliferating, quiescent and dying cells coexist in normoxic, hypoxic or necrotic zone	proliferating, quiescent and dying cells coexist in normoxic, hypoxic or necrotic zone	proliferating, quiescent and dying cells coexist in normoxic, hypoxic or necrotic zone
Drug resistance, Predictability	Poor	Moderate	High	High
Media	Cell culture media	Cell culture media	Mouse body fluids	Mouse body fluids
Biodistribution/ Pharmacokinetics	No	No	Accounted for	Accounted for
Compound Administration	In cell media	In cell media	Any route of administration	Any route of administration
Tumor/Stroma Interaction	No	In co-culture with selected stroma cells	In co-culture with selected stroma cells	yes
Investment	Inexpensive	Moderate	Moderate	Expensive
Turnaround Time	Weeks	Weeks	Weeks	Months

Table 1:

Difference in the cell growth in 2D and 3D cell culture versus Hollow Fiber Model and xenograft studies.

Unlike any cellular assay, the Hollow Fiber Model is performed in the mouse. After administration, the drug encounters high serum concentrations or other potentially inhibiting factors in the mouse blood. Biodistribution, pharmacokinetic and pharmacodynamic characteristics of the drugs will play a role in the performance of killing tumor cells in the hollow fibers as well. There is no limitation to the type of cell that can be investigated using the Hollow Fiber Model. The cells do not need matrices to help them to get in contact with each other. Therefore, all tumor cell lines can be investigated.

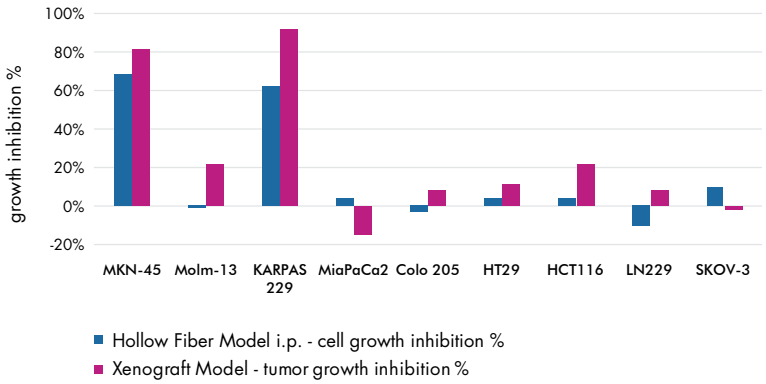
Taken together, the growth of cells in a tumor-like architecture, as well as the accounting of mouse specific factors, ensures a high translatability of the results of the Hollow Fiber Model for subsequent xenograft studies.

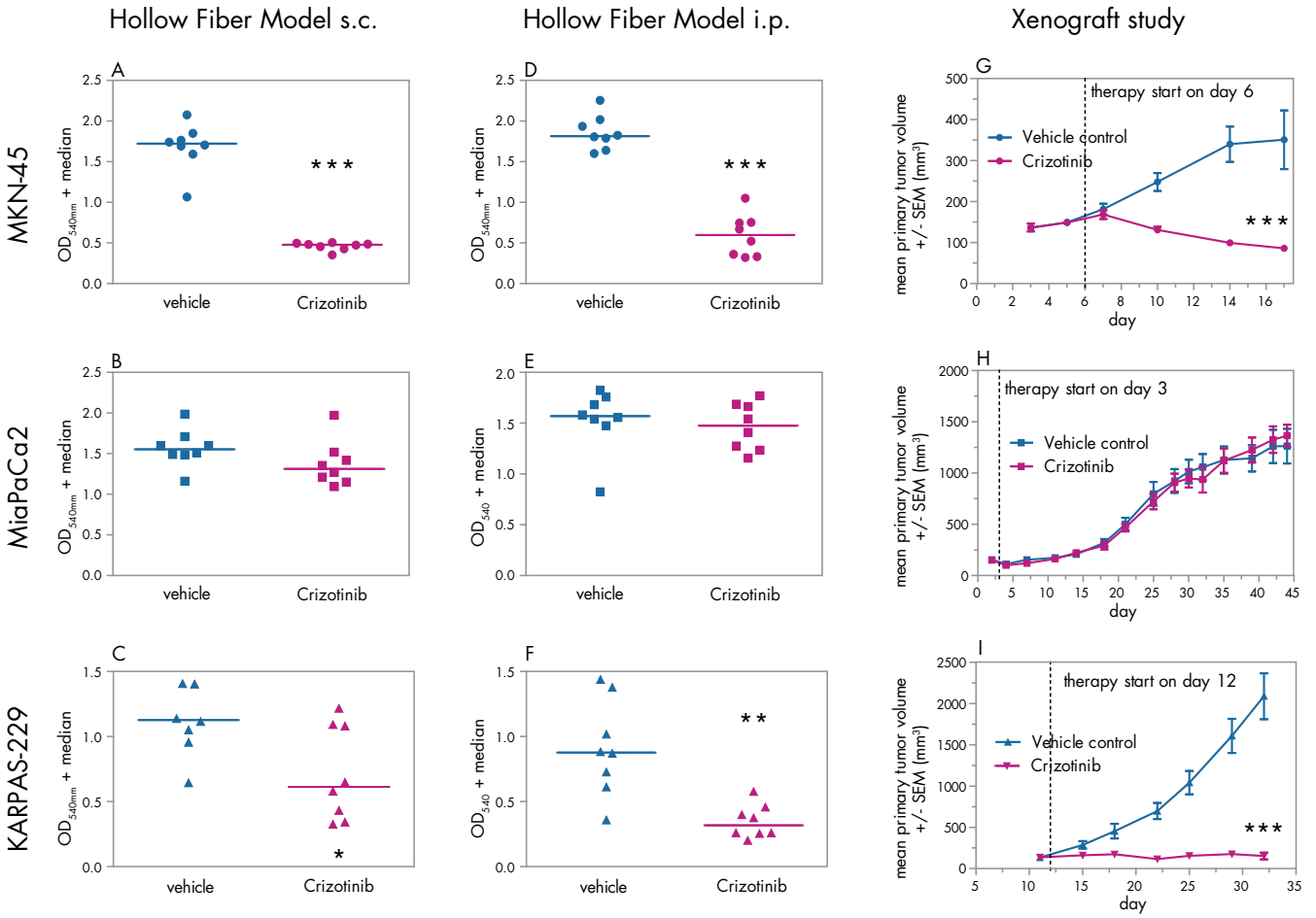
fiber was implanted subcutaneously and one was implanted intraperitoneally into NMRI nude mice. The vehicle, Trastuzumab or Kadcyla®, was administered to the animals intravenously twice weekly for two weeks at doses of 20mg/kg and 10mg/kg, respectively.The analysis reveals that none of the selected cell lines are inhibited by the treatment with Trastuzumab. JIMT-1 tumor growth inhibition by Trastuzumab is reported to occur via antibody-dependent cell-mediated cytotoxicity (ADCC)13, which is prevented in the Hollow Fiber Model because immune cells cannot enter the fibers. In contrast, Kadcyla® acts directly on the cells via the cytotoxic agent bound to Trastuzumab. In our study, Kadcyla® shows efficacy, highest on SK-BR3 cells followed by JIMT-1 and not significant but noticeable for MDA-MB-468. This study shows that the Hollow Fiber Model can be used to investigate antibody-drug conjugates such as Kadcyla®.

Figure 3:

Comparison of efficacy of kinase inhibitor Crizotinib in Hollow Fiber Model and xenograft model.

Hollow fibers were loaded with cells and implanted in mice. Crizotinib was administered daily at 50 mg/kg per oral gavage for two weeks. Hollow fibers were than isolated and viable cells quantified. For xenograft studies, tumor cells were implanted subcutaneuosly. When tumors reached about 100 mm3 mice were treated with Crizotinib as described above until study termination.





Shown is a summary of the data for all nine tumor cell lines. The mean of intra-peritoneally implanted hollow fibers are shown as ratio of treated versus untreated group. Xenograft tumor data are depicted as tumor growth inhibition. Detailed results of cell lines MKN-45, MiaPaCa2 and KAPRAS-229 are shown in the figures below. s.c. – subcutaneous, i.p. – intra-peritoneal, * $p<0.05$, ** $p<0,01$, *** $p<0.001$ unpaired T-test.

Both, scientists and mice have an advantage

Speed up the drug discovery process

The Hollow Fiber Model is ideal for researchers who need to screen a number of drugs with a high turnaround in a matter of weeks.

Save money

The Hollow Fiber allows for confident shortlisting of drugs to be taken into expensive xenograft studies without the fear of losing valuable candidates. Xenograft studies can be started more confidently with the tumor model that was proven to be killed by the anticancer agent in the in vivo setting. Because of the simultaneous investigation of three tumor cell lines, each mouse in the Hollow Fiber Model delivers 6 endpoint data instead of just 1 endpoint data. Because the treatment period is shorter and the number of mice per group is lower when compared to a xenograft study, less drug is needed.

Save mice

The tumor cell growth is contained by the fiber membrane; therefore, mice suffer less than mice bearing tumors. The number of mice can be dramatically reduced because less xenograft studies are needed, and the study period is shorter for each mouse. This model is in concordance to the ethical 3Rs rule (reduce, refine, replace).

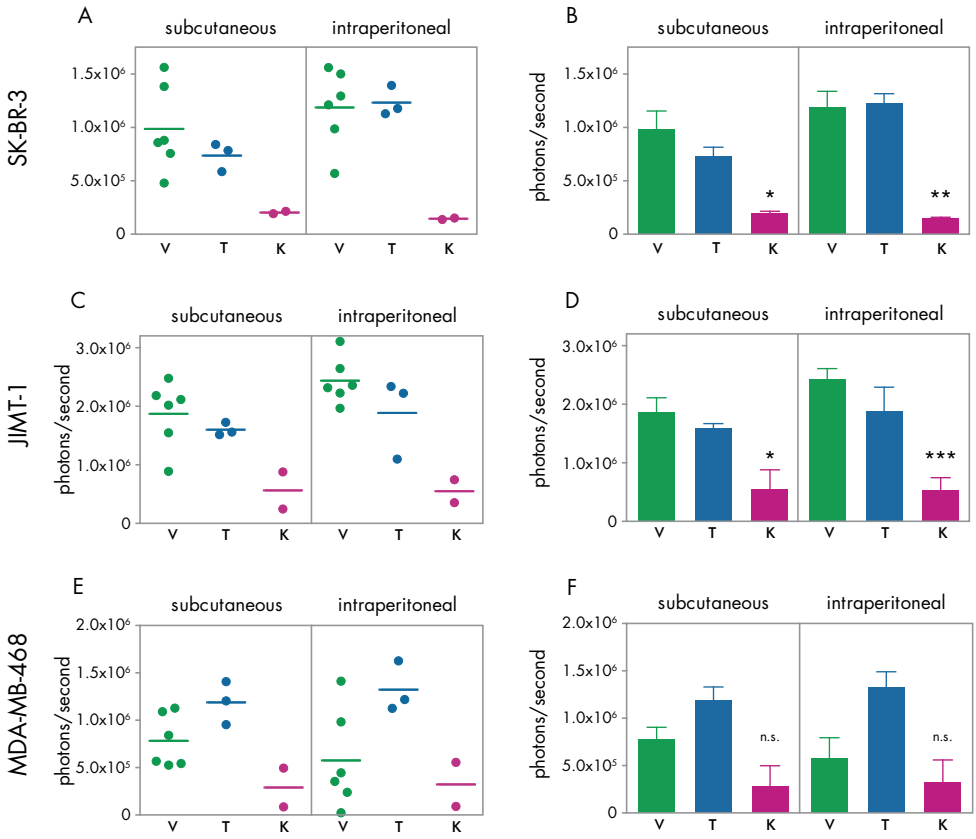


Figure 4:

Efficacy of antibody-drug conjugate Kadcyla® on three breast cancer cell lines with the Hollow Fiber Model.

Hollow fibers loaded with SK-BR-3, JIMT-1 or MDA-MB-468 cells were implanted in mice subcutaneously and intraperitoneally. The mice were treated with either vehicle (V) Trastuzumab (T) or the Trastuzumab-Emantasine andibody-drug conjugate Kadcyla® (K) for two weeks. Hollow fibers were isolated and viable cells were quantified at end point of study. Data shown as single points (A, C, E with mean) or bar graphs (B, D, F with mean + SEM).

ANOVA test was used to determine statistical significant results.
* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, n.s. not significant

Game changer

The Hollow Fiber Model has the potential to change the way drug discovery is done today. The NCI, for example, incorporated the Hollow Fiber Model into their drug discovery routine: "the hollow fiber assay is […] being utilized as the initial in vivo experience for agents found to have reproducible activity in the in vitro anticancer drug screen." ProQinase makes the Hollow Fiber Model available for every lab in academic institutions, biotechnology or pharmaceutical industry to test new anticancer drugs to substantially speed up the process and increase the chances of successful drug discovery.

What Our Animal Testing Experts Say To The Hollow Fiber Model:

Dr. Cynthia Obodozie, head of animal facility at ProQinase, is particularly intrigued by the Hollow Fiber Model: "First, from an ethical point of view, mice do not suffer the usual burden of bearing tumors because the tumor growth is restricted by the fiber membrane. Second, the assay runs with such a low variability that group sizes of three to six mice are sufficient."

Holger Weber, head of the animal testing unit at ProQinase, says "the Hollow Fiber Model development has not reached its end. Co-culture investigations are underway which will circumvent current limitations and increase the impact on future drug development."

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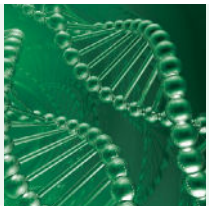
OUR SERVICES

Recombinant Proteins



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Phosphatases • PARPs • Substrates
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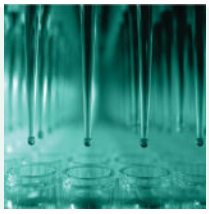
Target-specific Assays



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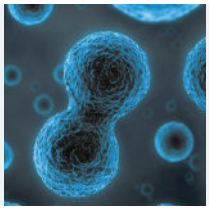
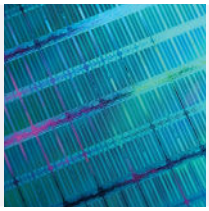


Kinase and Epigenetic Assays • Cell Proliferation Assays
Soft Agar Assays • Drug Combination Testing
3D Tumor Spheroid Assays • Migration and Invasion Assays • Angiogenesis Assay • Custom-Assay Development

Biophysical Assays



Surface Plasmon Resonance • Thermal Shift
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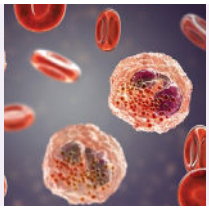
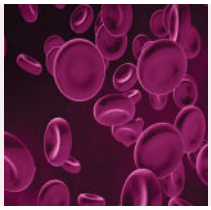
In Vivo Pharmacology



In Vivo Hollow Fiber Model
Xenograft Models • Syngeneic Models
Orthotopic Models • Metastasis Models
Proprietary Models • Flow Cytometry

ADME & Safety



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Let's discover together.

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