In Vivo Pharmacology

Reaction Biology has established a large panel of subcutaneous, subQperior™, orthotopic and metastasis tumor models (including bioluminescence read-out) for testing the efficacy of novel therapeutic agents on primary tumors and metastases in animals. Moreover, standard and proprietary syngeneic tumor models are available, which enables efficacy testing of test compounds in immune competent animals and the investigation of the immune-modulatory effect (e.g. by flow cytometry). Tumor angiogenesis can be investigated in In vivo Angiogenesis Models. Reaction Biology's In Vivo Hollow Fiber Model allows to screen the most suitable cell line or the most promising antitumoral effective lead test compound in a cost and time reduced manner in mice. The service portfolio is supplemented by other study types (e.g. tolerability, PK, PD). In addition, new models can be established upon request.

Let’s discover together.

In Vivo Hollow Fiber Model

Field of Application

The In Vivo Hollow Fiber Model is a fast and economical in vivo screening approach which can be used for two different purposes:

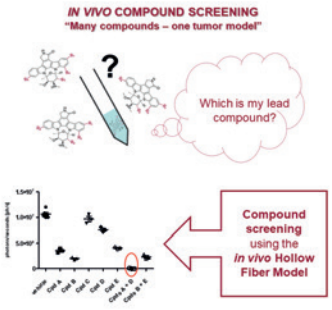
- Selection of a compound with the best in vivo activity against a tumor cell line of interest ("compound screening")
- Identification of the most suitable cell line for an in vivo efficacy study with a selected test compound ("tumor model screening")

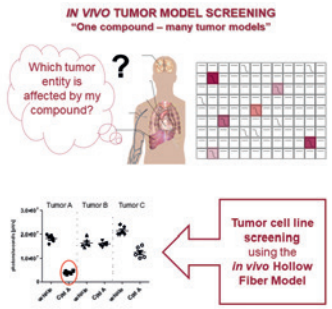
The method allows simultaneous evaluation of test items against up to three different tumor cell lines in two different compartments (s.c. & i.p. implantation) within the same mouse. Due to the low variability of the assay, studies can be performed with three mice per group. These characteristics predestine the Hollow Fiber Model for drug or tumor model screening in vivo.

Our Service

Typical Examples of Custom-Tailored Projects:

Objective	Short Description of a Standard Study
In vivo compound screening	Testing of up to 14 compounds in one study. Cell culturing; encapsulation of tumor cell line of interest in hollow fibers; subcutaneous & intraperitoneal implantation of hollow fibers; in vivo study with treatment (study duration of 14 days); hollow fiber harvesting; measurement of tumor cell viability by CellTiter-Glo; protocol & report
In vivo tumor model screening	Testing of up to 12 tumor cell lines in one study. Cell culturing; encapsulation of three different cell types in hollow fibers; subcutaneous & intraperitoneal implantation of hollow fibers; in vivo study with treatment (study duration of 14 days); hollow fiber harvesting; measurement of tumor cell viability by CellTiter-Glo; protocol & report





The Test Model	The key element of the assay are semi-permeable fibers which allow access of test items (<500 kDa: small molecules, antibodies etc.) to the encapsulated tumor cells
First Description in the Literature	Hollingshead et al (1995), Life Sciences 57, pp. 131 - 141
Available Cell Lines	Cell lines can be selected from Reaction Biology's cell lines or from our in vivo panel of established subcutaneous or orthotopic tumor models
Customer Cell Lines	Studies can also be performed with cell lines provided by our customers
Readout	Level of tumor cells in the hollow fibers are quantified by CellTiter-Glo

Subcutaneous and SubQperior™ Mouse Tumor Models

Field of Application

Subcutaneous mouse tumor models are widely used in preclinical drug development for measurement of antitumoral efficacy in a standardized and cost-effective manner.

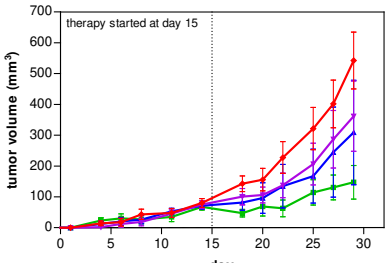
SubQperior™ tumor models are an innovative alternative overcoming many of the drawbacks of tumor models based on subcutaneous tumor cell implantation. Standard implantation via subcutaneous injection causes frequent ulceration resulting in early abrogation of studies, leaving researchers with too short treatment windows and high heterogenicity with poor statistical value of study results. SubQperior™ tumor models are based on tumor cell implantation into the mammary fat pad.

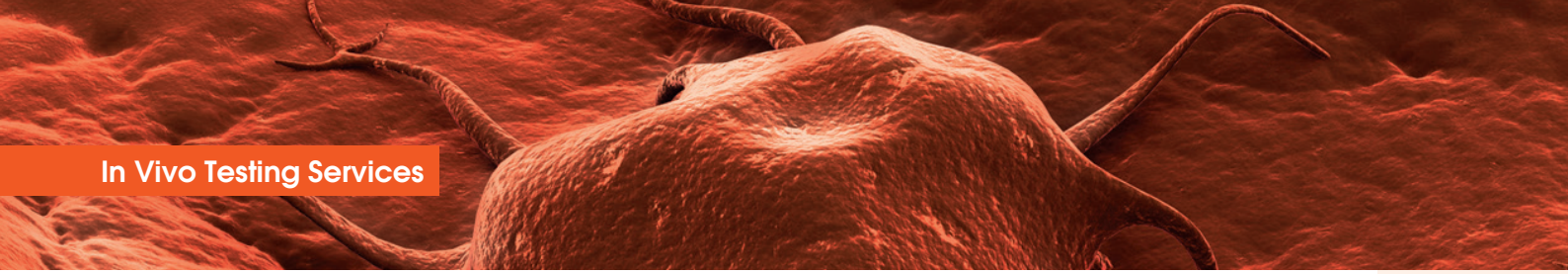
Advantages of SubQperior™ tumor models:

- Implantation results in larger tumors and longer treatment windows
- Homogeneous and reliable growth yields study outcomes with outstanding statistical value
- Tumors are measured via caliper, making the handling as easy and inexpensive as for subcutaneous models

Our Service

Established Cell Lines	Xenograft models (human cells); syngeneic models (murine cells)
Customer Cell Lines	Studies can be performed with cell lines provided by our customers
Reference Cpd	Available for most established cell lines
Standard Study	Comprises among other things: (i) cell culturing & cell implantation; (ii) measurement of animal weight (up to 3x/week); (iii) determination of tumor size (2x/week); (iv) treatment (1x/day; 5x/week); treatment period depending on model; (v) protocol & report
Optional Services	(i) blood sampling; (ii) paraffin embedding of tumor tissue or organs; (iii) histology & pathology; (iv) MRT; (v) Flow cytometry





Our Service

Human

Tumor Cell Line	Tissue Origin	Route of Application
Ramos	Ascites	metastasis (i.v.)
UM-UC-3	Bladder	orthotopic
HG-3	Blood	subcutaneous
HL-60		subcutaneous
KARPAS 299		subcutaneous
LP-1		orthotopic
MOLM-13		subcutaneous; metastasis (i.v.)
MOLT-4		subcutaneous
MV4-11		subcutaneous; metastasis (i.v.)
RPMI-8226		orthotopic
SU-DHL-6		subcutaneous
THP-1		subcutaneous
SJSA-1	Bone	subcutaneous
NCI-H929	Bone Marrow	subcutaneous; orthotopic
Raji		subcutaneous; metastasis (i.v.)
A172	Brain	orthotopic
LN-229		subcutaneous; orthotopic
U-118 MG		subcutaneous
U-87 MG		subcutaneous; orthotopic
BT-474	Breast	subcutaneous; orthotopic
HCC1187		subcutaneous
HCC1569		orthotopic
JIMT1		orthotopic; metastasis (i.c.)
MCF7		subcutaneous; orthotopic
MDA-MB-231		subcutaneous; orthotopic; metastasis (i.c.); metastasis (i.v.)
MDA-MB-468		orthotopic
COLO 201	Colon	subcutaneous
COLO 205		subcutaneous
COLO 320DM		subcutaneous
DLD-1		subcutaneous
GP2d		subcutaneous
HCT 116		subcutaneous; orthotopic; metastasis
HT29		subcutaneous
LoVo		metastasis
LS 174T		subcutaneous
RKO		subcutaneous
SW480		subcutaneous
SW620		subcutaneous/subQperior; orthotopic
WiDr		subcutaneous
HT1080	Connective Tissue	subQperior
HuTu80	Duodenum	subcutaneous
786-O	Kidney	subcutaneous; orthotopic
ACHN		subcutaneous
Hep3B2.1-7	Liver	subcutaneous; orthotopic
HepG2		orthotopic
Huh-7		subcutaneous; metastasis (i.v.); metastasis (i.c.)
PLC/PRF/5		subcutaneous; orthotopic
SNU-182		subcutaneous

Tumor Cell Line	Tissue Origin	Route of Application
A549	Lung	subcutaneous; metastasis (i.v.)
Calu-3		subcutaneous
Calu-6		subcutaneous
EBC1		subcutaneous
LK-2		subcutaneous
NCI-H1373		subcutaneous
NCI-H1437		subcutaneous
NCI-H226		subcutaneous
NCI-H292		subcutaneous
NCI-H441		subcutaneous/subQperior
NCI-H460		subcutaneous; metastasis (i.c.)
PC-9		subcutaneous
CAL-27	Oral	subcutaneous
A2780	Ovary	subcutaneous; orthotopic
Hey		subcutaneous
OVCAR-3		subcutaneous; orthotopic
SKOV-3		subcutaneous; orthotopic; orthotopic ascites
AsPC1	Pancreas	subcutaneous; subcutaneous/ subQperior; orthotopic
BxPC-3		subcutaneous; orthotopic
HuP-T4		subcutaneous
L3.6pL		orthotopic
MiAPaCa2		subcutaneous; orthotopic
PANC-1		subcutaneous; orthotopic
Stew		subcutaneous
Detroit 562	Pharynx	subcutaneous
PC-3	Prostate	subcutaneous; orthotopic
PC3-M		subcutaneous
A2058	Skin	subcutaneous
A375		subcutaneous
A431		subcutaneous; subcutaneous/ subQperior
C32		subcutaneous
C8161		subcutaneous; orthotopic
C8161.9		orthotopic
G361		orthotopic
HT144		subcutaneous
UACC-257		subcutaneous
Hs746T	Stomach	subcutaneous
MKN-1		subcutaneous
MKN-45		subcutaneous
HeLa	Uterus, Cervix	subcutaneous
SK-LMS-1	Vulva	subcutaneous

Mouse

Tumor Cell Line	Tissue Origin	Route of Application
MB-49	Bladder	subcutaneous; orthotopic; metastasis (i.c.); metastasis (i.v.)
MBT-2		subcutaneous
A20	Blood	subcutaneous; subQperior
C1498		subcutaneous; metastasis (i.v.)
E.G7-OVA		subcutaneous
EL4		subcutaneous
GL261	Brain	orthotopic; subQperior
4T1	Breast	subcutaneous; orthotopic; subQperior_orthotopic; metastasis (orthotopic); metastasis (i.v.)
EMT6		subcutaneous; orthotopic; subQperior_orthotopic; metastasis
EO771		orthotopic
HC11-NeuT		orthotopic
C26	Colon	subcutaneous
CT26wt		subcutaneous; subQperior; orthotopic
MC38		subcutaneous
MC38-CEA#		subcutaneous; subQperior; orthotopic
RENCA	Kidney	subcutaneous; subQperior; orthotopic
Hepa1-6	Liver	subcutaneous; subQperior; orthotopic
3LL	Lung	subcutaneous
AB12		subcutaneous; subQperior
LL/2 (LLC1)		subcutaneous, subQperior; orthotopic; metastasis
M109		subcutaneous
TC-1		subcutaneous
KPC-2838	Pancreas	subcutaneous
KPC-6419		subcutaneous
Pan02		subcutaneous, subQperior; orthotopic
B16-F0	Skin	subcutaneous; subQperior; orthotopic
B16-F10		subcutaneous; subQperior; orthotopic; metastasis (i.c.); metastasis (i.v.)
Clone M3/Cloudman S91		subcutaneous, subQperior

Orthotopic Mouse Tumor Models

Field of Application

In orthotopic tumor models, tumor cells are inoculated into the organ of their origin. This implantation site allows organo-typical interaction between tumor cells and surrounding stroma affecting growth, differentiation, and drug sensitivity of tumor cells. For human tumor cell lines immune-compromised mice are used with the advantage to study classical antitumoral test compounds. In contrast, murine tumor cell lines can be grown in immune-competent mice (syngeneic). These tumor models combine the advantage of tumor stroma interaction with a functional developed immune system to assess novel immunotherapeutic approaches.

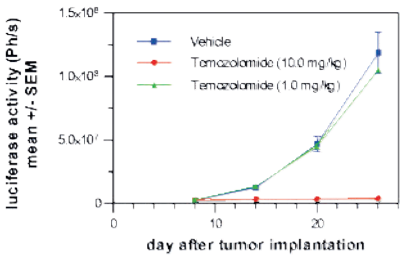
Our Service

#	Tumor Cell Line	Tissue Origin	Species
1	MB-49	Bladder	Mouse
2	A172	Brain	Human
3	GL261		Mouse
4	LN-229		Human
5	U-87 MG		Human
6	4T1	Breast	Mouse
7	BT-474		Human
8	EMT6		Mouse
9	EO771		Mouse
10	HCC-1569		Human
11	JIMT-1		Human
12	MCF7		Human
13	MDA-MB-231 (Z1)*		Human
14	MDA-MB-468		Human
15	CT26 wt	Colon	Mouse
16	MC38-CEA		Mouse
17	HCT-116		Human
18	SW620		Human
19	RENCA	Kidney	Mouse
20	786-O		Human

#	Tumor Cell Line	Tissue Origin	Species
21	Hep 3B2.1-7	Liver	Human
22	HepG2		Human
23	Hepa1-6		Mouse
24	PLC/PRF/5		Human
25	LL/2	Lung	Mouse
26	A2780	Ovary	Human
27	OVCAR-3		Human
28	SKOV-3		Human
29	AsPC1	Pancreas	Human
30	BxPC-3		Human
31	L3.6pL		Human
32	MiA-PaCa2		Human
33	Pan02		Mouse
34	PANC-1		Human
35	PC-3	Prostate	Human
36	B16-F10	Skin	Mouse
37	C8161		Human
38	C8161.9		Human

* in vivo selected subpopulation of the corresponding parental cell line

Customer Cell Lines	Orthotopic tumor models can be established with cell lines provided by our customers
Analysis of Tumor Size	Via bioluminescence imaging (BLI) using luciferase-transduced cell lines or calipering of skin and breast tumors
Reference Cpd	Available for most established cell lines
Standard Study	Comprises among other things: (i) cell culturing & cell implantation; (ii) measurement of animal weight (up to 3x/week); (iii) determination of tumor size via BLI (1x/week) / calipering (2x/week); (v) treatment (1x/day; 5x/week); (vi) treatment period depending on model (vi) protocol & report
Optional Services	(i) Blood sampling; (ii) paraffin embedding of tumor tissue or organs; (iii) histology & pathology (iv) MRT (v) flow cytometry



Study Example

Effect of Temozolomide (high dose = red, low dose = green) on orthotopic tumor growth of U-87 MG cells (vehicle control = blue) determined by in vivo bioluminescence imaging

Metastasis Mouse Tumor Models

Field of Application

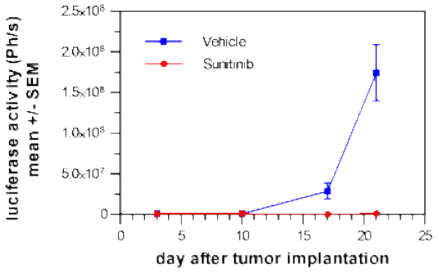
In metastatic tumor models, tumor spreading originates either from a primary tumor or is artificially induced by intravenous or intracardial tumor cell injection. The metastatic pattern is dependent on the tumor cell line with a preference for lung metastasis in the case of intravenous injection. For human tumor cell lines immune-compromised mice are used with the advantage to study classical antitumoral test compounds. In contrast, murine tumor cell lines can be grown in immune-competent mice (syngeneic), providing a functional immune system to assess novel immunotherapeutic approaches.

Our Service

#	Tumor Cell Line	Tissue Origin	Route of application	Species
1	Ramos	Ascites	i.v.	Human
2	MB-49	Bladder	i.c., i.v.	Mouse
3	C1498	Blood / Leukemia	i.v.	Mouse
4	MOLM-13		i.v.	Human
5	MV4-11		i.v.	Human
6	Raji	Bone Marrow	i.v.	Human
7	4T1 (M3)*	Breast	orthotopic	Mouse
8	4T1		i.v.	Mouse
9	EMT6		i.v.	Mouse
10	JIMT-1		i.c.	Human
11	MDA-MB-231 (Z1)*		i.c., i.v.	Human
12	HCT 116	Colon	orthotopic	Human
13	LoVo		orthotopic	Human
14	Huh-7	Liver	i.c., i.v.	Human
15	A549	Lung	i.v.	Human
16	LL/2		intra-splenic	Mouse
17	NCI-H460		i.c.	Human
18	B16-F10	Skin	i.c., i.v.	Mouse

* in vivo selected subpopulation of the corresponding parental cell line

Customer Cell Lines	Metastatic tumor models can be established with cell lines provided by our customers
Analysis of Metastasis	Via bioluminescence imaging (BLI) using luciferase-transduced cell lines
Reference Cpd	Available for most established cell lines
Standard Study	Comprises among other things: (i) cell culturing & cell implantation; (ii) measurement of animal weight (up to 3x/week); (iii) determination of tumor burden via BLI (1x/week); (v) treatment (1x/day; 5x/week); treatment period depending on model; (vi) protocol & report
Optional Services	(i) Blood sampling; (ii) paraffin embedding of organs; (iii) histology & pathology (iv) MRT (v) flow cytometry (vi) ex vivo analysis of organs



Immuno-Oncology Platform

Field of Application

Our immuno-oncology platform supports the discovery of immune modulatory drugs as well as for the investigation of additional effects of conventional drugs on the immune system.

SubQperior™

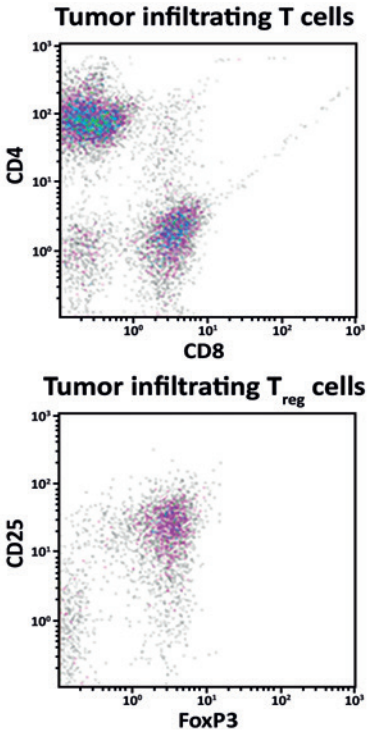
Tumor cell implantation into the mammary fat pad of mice results in superior tumor model performance over subcutaneous implantation.

SubQperior™ tumor models substitute almost all of our conventional syngeneic subcutaneous tumor models eliminating ulceration issues that resulted in short treatment windows and small tumor sizes in the past.

SubQperior™ tumor models allow reproducible and reliable testing of immune-modulating drugs.

Immunophenotyping

1. Flow cytometry is a perfect method to investigate the frequency of immune cells in tumor tissue and evaluate the immune-modulating effects of drugs. Our scientists can multiplex up to 20 markers for both, cell analysis and cell sorting. Off-the-shelf flow panels include the NK/T Cell Panel, T Cell Activation Marker Panel, DC Panel, and the All-in-One Flow Panel covering T cells, NK cells, B cells, MDSCs, DCs, macrophages, and neutrophils.
2. Multiplexing immune markers including cytokines and chemokines can be performed by the highly sensitive mesoscale discovery (MSD) platform.
3. Tissue cores of tumors of all our syngeneic tumor models are available in a tumor tissue microarray (TMA). This enables thorough up-front analysis of the presence of target immune cells in the tumors.
4. Tumor tissue can be isolated and snap-frozen or embedded for histology or immunohistochemical investigation with a large variety of antibodies.

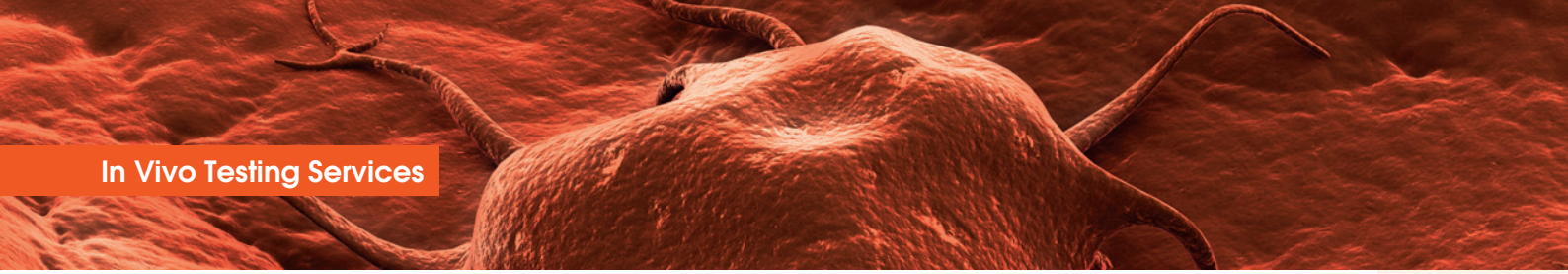


Tested Checkpoint Inhibitors

Anti-mPD-L1, anti-mPD-1 and anti-mCTLA4 antibodies were evaluated in all subcutaneous, subQperior™ and some orthotopic models

Panel Screening

On a regular basis, Reaction Biology performs testing on a panel of 6 SubQperior™ tumor models. Customers can choose from a selection of 8 tumor models. They pay for their treatment arms and receive data of vehicle control as well as anti-mPD1 antibody control in addition.



Mouse-derived Isograft Tumor Models (MDI)

Field of Application

Today, cancer research tends to focus on the development of novel cancer immunotherapies using syngeneic mouse models for the development of such compounds. While classical syngeneic mouse models are based on the implantation of cultured tumor cell lines, for Reaction Biology's mouse-derived isograft (MDI) tumor models are propagated subcutaneously in mice only. Implanted tumor pieces of low in vivo passage are originally derived from spontaneous- or carcinogen-induced mouse tumors. Hence, the major advantage of these novel and unique MDI tumor models is the preservation of primary tumor phenotype and intratumoral immune cell populations.

Our Service

MDI tumor models:

#	Model	Origin	Creation	RNAseq	anti-PD1	anti-CTLA-4	Combination	Therapeutic window	Relevant immune cells
1	JA-0009	adeno-carcinoma	spontaneous	yes	low	low	low	2 weeks	M2 macrophages
2	JA-0017		spontaneous	ongoing	nd	nd	nd	>6 weeks	nd
3	JA-0032		spontaneous	ongoing	nd	nd	nd	4 weeks	nd
4	JA-2011	sarcoma	carcinogen	yes	low	low	nd	2 weeks	neutrophils
5	JA-2019		carcinogen	ongoing	high	high	nd	2 weeks	MDSCs/Tcells
6	JA-2041		carcinogen	ongoing	moderate	moderate	nd	3 weeks	MDSCs/Tcells
7	JA-2042		carcinogen	yes	moderate	moderate	high	2 weeks	Treg cells

nd = not determined

Characterization

- All models were histologically characterized
- Growth curves were established for all models
- All models were tested with reference compounds
- RNA sequence data available for selected models

Flow Cytometric Analysis

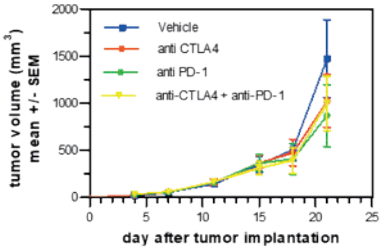
- Analysis of tumor infiltrating leukocytes and cells isolated from spleen and/or lymph nodes
- Multicolor 17 marker panel: T cells, MDSCs, Macrophages, NK cells, B cells, DCs
- Customized staining procedures are possible

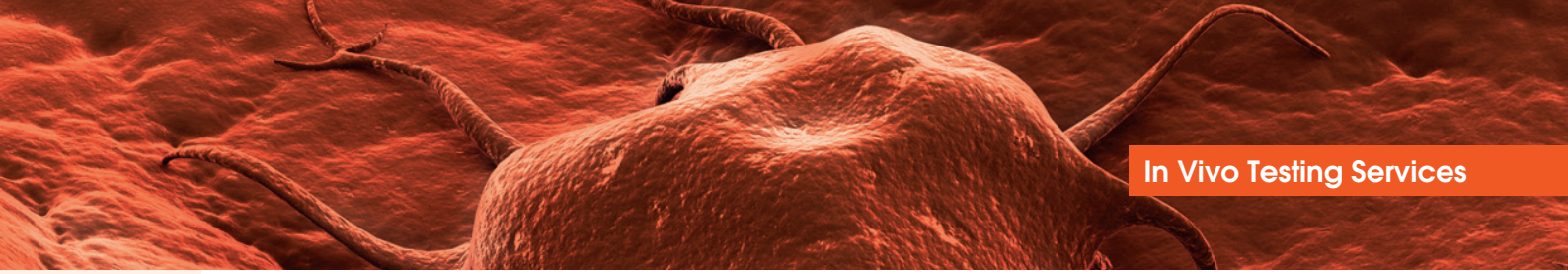
Standard Study

Comprises among other things:
(i) Subcutaneous implantation of tumor pieces
(ii) Measurement of animal weight (3x/week)
(iii) Determination of tumor size

Optional Services

(i) Blood sampling (ii) Characterization of abundance and relative distribution of different immune cell subsets in the tumor and lymphatic tissues by flow cytometry (iii) Paraffin embedding of tumor tissue (iv) Histological & pathological analysis (v) Cytokine determination (vi) Provision of tumor tissue for target validation (xii) MRT





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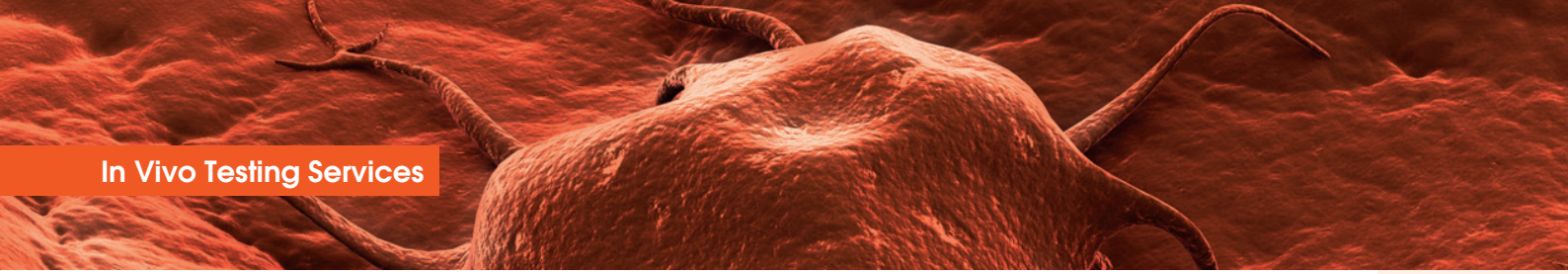

Notes

LET'S DISCOVER TOGETHER.

Recombinant Proteins

- Kinase proteins
- Epigenetic proteins
- Substrates
- Custom-tailored protein production

Target-Specific Assays

- Biochemical and cell-based assays
- Enzymatic activity testing
- Protein: Protein Interaction assays
- Receptor Biology

Cellular Oncology

- 2D and 3D proliferation assays
- Drug combination screening
- Invasion and migration assays
- Angiogenesis assay

Biophysical Assays

- Surface Plasmon Resonance
- Thermal Shift Assay
- Isothermal Titration Calorimetry
- Microscale Thermophoresis

In Vivo Pharmacology

- In Vivo Hollow Fiber Model
- Xenograft models
- Orthotopic models
- Metastasis models

Safety & Toxicology

- Cardiac Safety Panel
- CYP inhibition
- PK/PD studies
- In Vitro Safety Panel

Integrated Drug Discovery

- Target research
- Hit identification
- Hit-to-Lead
- Lead optimization

Biomarker Discovery

- Genomic biomarkers
- Protein biomarkers
- Immunophenotyping

Immuno-Oncology

- In Vitro Killing Assays
- Syngeneic Mouse Models
- Propriatary Tumor Models
- Immunophenotyping

REACTION BIOLOGY

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