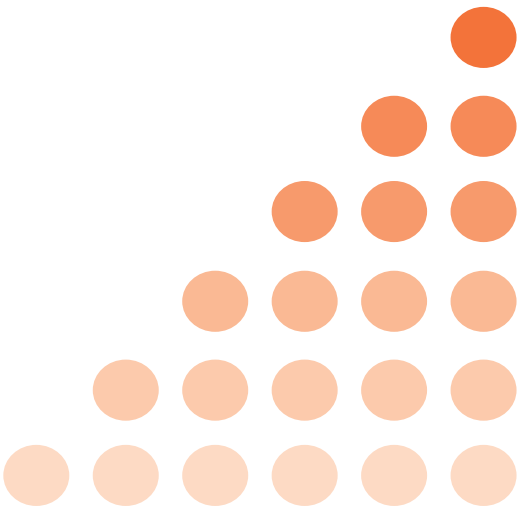
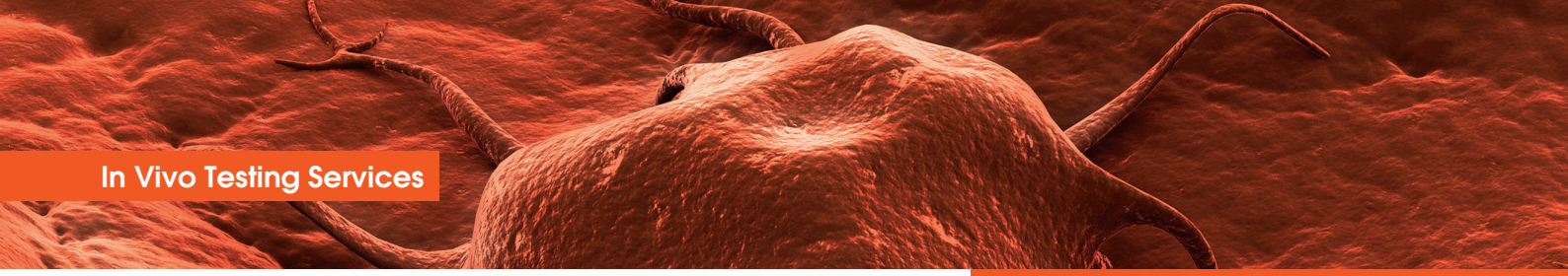
In Vivo Pharmacology

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 Testing Services

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")

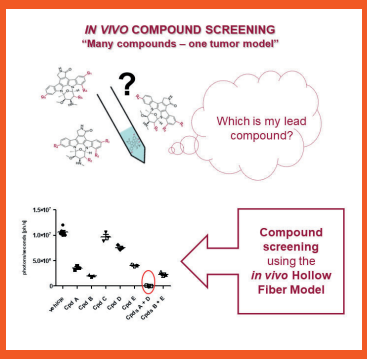
The method allows simultaneous evaluation of test items against up to three different tumor cell lines in two different compartments (s.c. & i.p. implantation) within the same mouse. Due to the low variability of the assay, studies can be performed with three mice per group. These characteristics predestine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

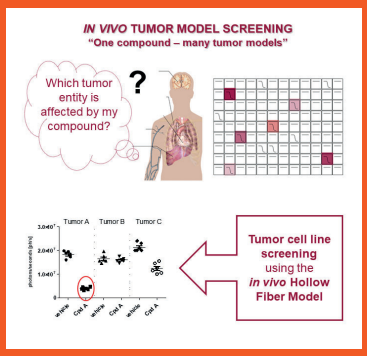
Our Service

➢ 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





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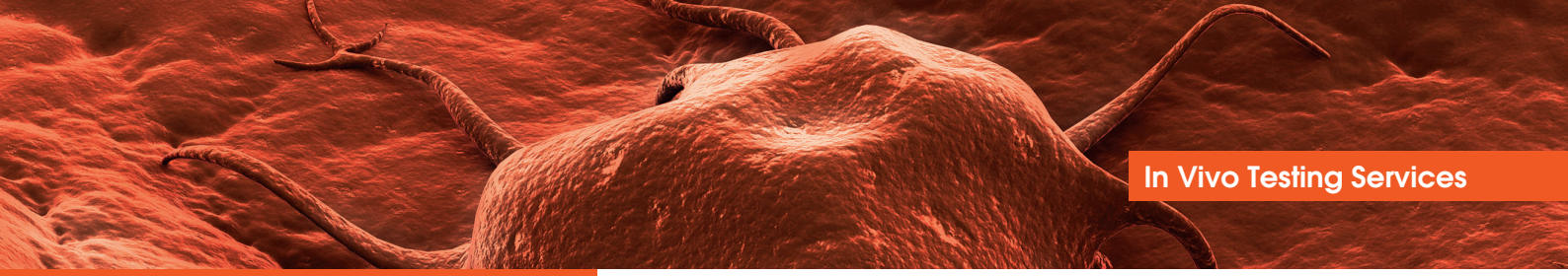


In Vivo Testing Services

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:

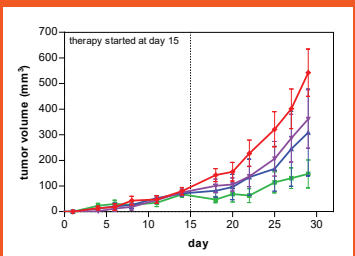
- Implantation results in larger tumors and longer treatment windows
- Homogeneous and reliable growth yields study outcomes with outstanding statistical value
- Tumors are measured via caliper, making the handling as easy and inexpensive as for subcutaneous models

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

Study Example

Effect of PQ-013 on subcutaneous tumor growth of MV4-11 cells.



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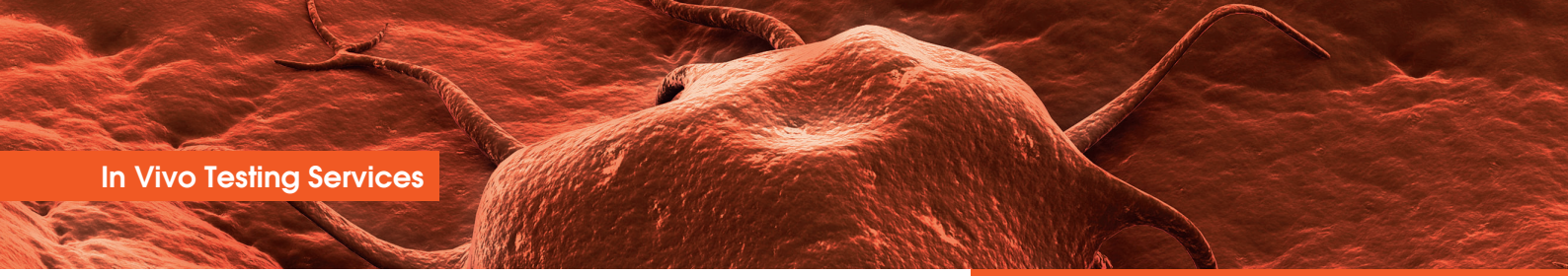

In Vivo Testing Services

Our Service

Human

#	Tumor Cell Line	Tissue Origin	Route of Application
1	Ramos	Ascites	orthotopic
2	Daudi	Blood	subcutaneous; orthotopic
3	HG-3		subcutaneous
4	HL-60		subcutaneous
5	KARPAS 299		subcutaneous
6	LP-1		subcutaneous; orthotopic
7	MOLM-13		subcutaneous; metastasis (i.v.)
8	MOLT-4		subcutaneous
9	MV4-11		subcutaneous; metastasis (i.v.)
10	RPMI-8226		subcutaneous; orthotopic
11	SU-DHL-6		subcutaneous
12	THP-1		subcutaneous
13	SJSA-1	Bone	subcutaneous
14	K-562	Bone Marrow	subcutaneous
15	NCI-H929		subcutaneous; orthotopic
16	Raji		subcutaneous; orthotopic
17	LN-229	Brain	subcutaneous; orthotopic
18	U-118 MG		subcutaneous
19	U-87 MG		subcutaneous; orthotopic
20	BT-474	Breast	subcutaneous; orthotopic
21	HCC1187		subcutaneous
22	HCC1569		orthotopic
23	HCC1954		subcutaneous; orthotopic
24	Hs 578T		subcutaneous
25	JIMT1		orthotopic; metastasis (i.c.)
26	MCF7		subcutaneous; orthotopic
27	MDA-MB-231		subcutaneous; orthotopic; metastasis (i.c.); metastasis (i.v.)
28	MDA-MB-361		subcutaneous
29	MDA-MB-453		subcutaneous
30	MDA-MB-468		subcutaneous; orthotopic
31	COLO 201	Colon	subcutaneous
32	COLO 205		subcutaneous
33	COLO 320DM		subcutaneous
34	DLD-1		subcutaneous
35	HCT 116		subcutaneous; orthotopic
36	HCT 15		subcutaneous
37	HT29		subcutaneous
38	LoVo		subcutaneous
39	LS 174T		subcutaneous
40	SW480		subcutaneous
41	SW620		subcutaneous; orthotopic
42	WiDr		subcutaneous
43	HT1080	Connective Tissue	subcutaneous; subQperior
44	HuTu80	Duodenum	subcutaneous
45	786-O	Kidney	subcutaneous
46	ACHN		subcutaneous

#	Tumor Cell Line	Tissue Origin	Route of Application
47	Hep3B2.1-7	Liver	subcutaneous; orthotopic
48	HepG2		orthotopic
49	Huh-7		subcutaneous; orthotopic
50	PLC/PRF/5		subcutaneous; orthotopic
51	SK-HEP-1		subcutaneous; orthotopic
52	A549	Lung	subcutaneous
53	Calu-3		subcutaneous
54	Calu-6		subcutaneous
55	EBC1		subcutaneous
56	LK-2		subcutaneous
57	NCI-H1437		subcutaneous
58	NCI-H146		subcutaneous
59	NCI-H226		subcutaneous
60	NCI-H292		subcutaneous
61	NCI-H441		subcutaneous
62	NCI-H460		subcutaneous; orthotopic
63	NCI-H520		subcutaneous
64	NCI-H596		subcutaneous
65	NCI-H69		subcutaneous; orthotopic
66	NCI-H82		subcutaneous
67	PC-9		subcutaneous
68	A2780	Ovary	subcutaneous; orthotopic
69	OVCAR-3		subcutaneous
70	SK-OV-3		subcutaneous
71	AsPC-1	Pancreas	subcutaneous; orthotopic
72	BxPC-3		subcutaneous; orthotopic
73	L3.6pL		subcutaneous; orthotopic
74	MiaPaCa2		subcutaneous; orthotopic
75	PANC-1		subcutaneous; orthotopic
76	DU 145	Prostate	subcutaneous
77	LNCaP		subcutaneous
78	PC-3		subcutaneous; orthotopic
79	PC-3-M		subcutaneous; orthotopic
80	A2058	Skin	subcutaneous
81	A375		subcutaneous
82	A431		subcutaneous
83	C32		subcutaneous
84	C8161		subcutaneous; orthotopic
85	CHL-1		subcutaneous
86	G-361		subcutaneous; orthotopic
87	HT 144		subcutaneous
88	Hs 746T	Stomach	subcutaneous
89	MKN-1		subcutaneous
90	MKN-45		subcutaneous
91	HeLa	Uterus, Cervix	subcutaneous

Mouse

#	Tumor Cell Line	Tissue Origin	Route of Application
1	MB-49	Bladder	subcutaneous; orthotopic
2	C1498	Blood	subcutaneous
3	E.G7-OVA		subcutaneous
4	GL261	Brain	subcutaneous; orthotopic
5	4T1	Breast	subcutaneous; orthotopic; subQperior_orthotopic; metastasis (orthotopic); metastasis (i.v.)
6	EMT6		subcutaneous; orthotopic; subQperior_orthotopic
7	HC11-NeuT		subcutaneous
8	CT26wt	Colon	subcutaneous; subQperior; orthotopic
9	MC38		subcutaneous
10	MC38-CEA#		subcutaneous; subQperior; orthotopic
11	RENCA	Kidney	subcutaneous; subQperior; orthotopic
12	Hepa1-6	Liver	subcutaneous; subQperior; orthotopic
13	AB12	Lung	subcutaneous; subQperior
14	LL/2 (LLC1)		subcutaneous, subQperior; orthotopic
15	Pan02	Pancreas	subcutaneous, subQperior; orthotopic
16	B16-F0	Skin	subcutaneous; orthotopic
17	B16-F10		subcutaneous; subQperior; orthotopic; metastasis (i.v.)
18	Clone M3/ Cloudman S91		subcutaneous, subQperior

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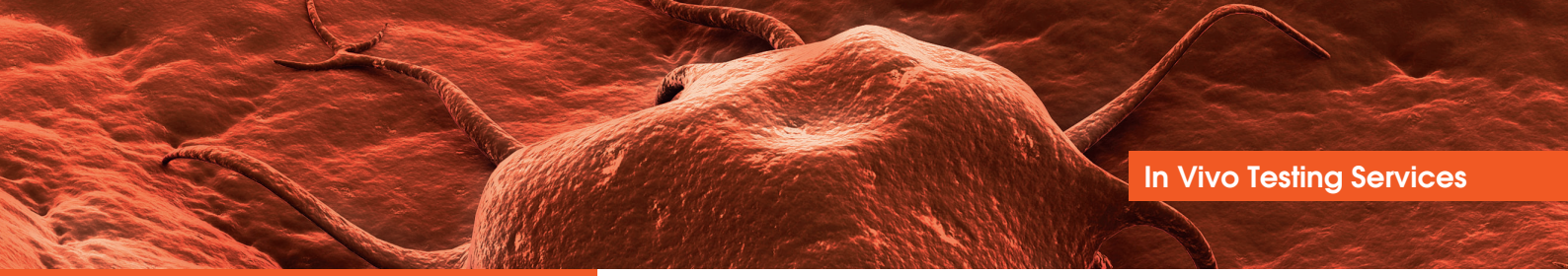


In Vivo Testing Services

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

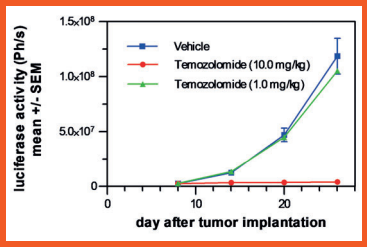
#	Tumor Cell Line	Tissue Origin	Species
1	LN-229	Brain	Human
2	U-87 MG		Human
3	GL261		Mouse
4	4T1	Breast	Mouse
5	HCC-1569		Human
6	JIMT-1		Human
7	MDA-MB 231 (Z1)*		Human
8	EMT6		Mouse
9	CT26 wt	Colon	Mouse
10	MC38-CEA		Mouse
11	HCT-116		Human
12	SW620		Human
13	RENCA	Kidney	Mouse
14	Hepa1-6	Liver	Mouse
15	LL/2	Lung	Mouse
16	AsPC1	Pancreas	Human
17	BxPC-3		Human
18	MiA-PaCa2		Human
19	Pan02		Mouse
20	PC-3	Prostate	Human
21	B16-F10	Skin	Mouse

* 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



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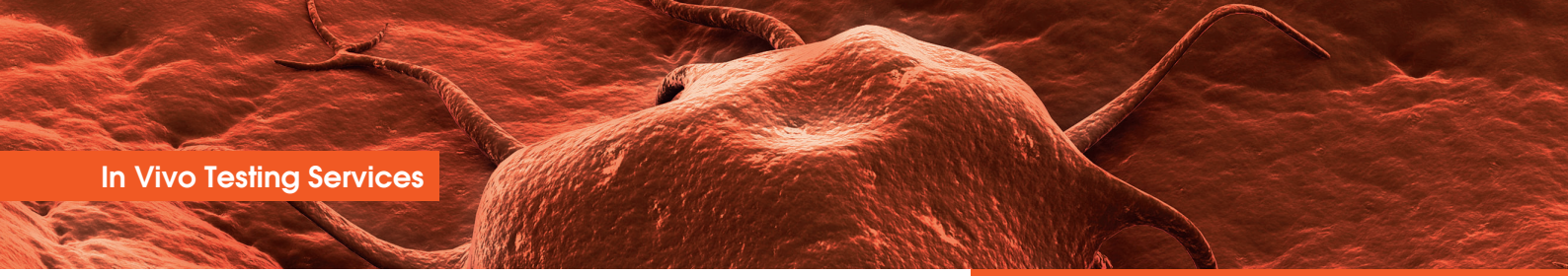


In Vivo Testing Services

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

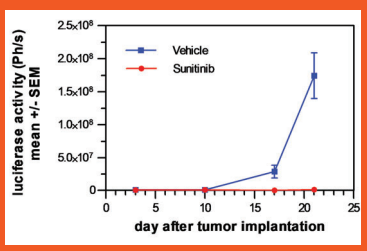
#	Tumor Cell Line	Tissue Origin	Route of application	Species
1	MOLM-13	Blood / Leukemia	i.v.	Human
2	MV4-11		i.v.	Human
3	4T1 (M3)*	Breast	orthotopic	Mouse
4	4T1		i.v.	Mouse
5	JIMT-1		i.c.	Human
6	MDA-MB-231 (Z1)*		i.v.	Human
7	MDA-MB-231 (Z1)*		i.c.	Human
8	B16-F10	Skin	i.v.	Mouse

* 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

Study Example

Effect of Sunitinib (red) on metastatic tumor growth of MOLM-13 cells (blue, vehicle control) determined by in vivo bioluminescence imaging.



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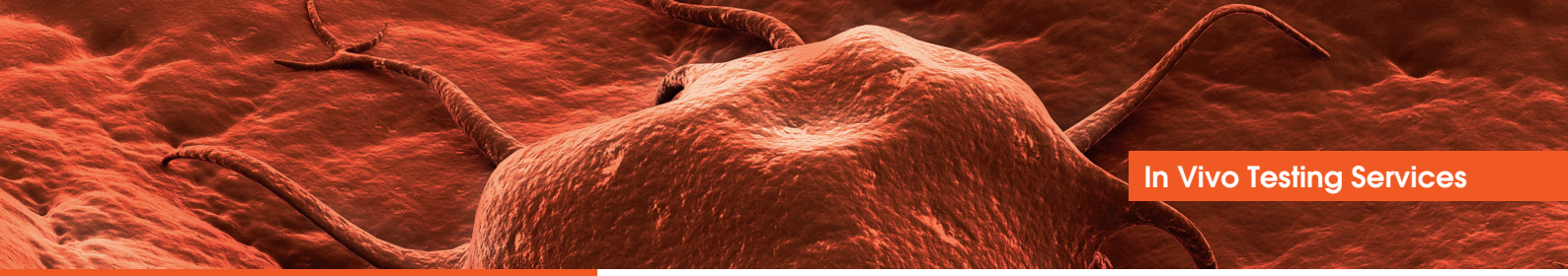


In Vivo Testing Services

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

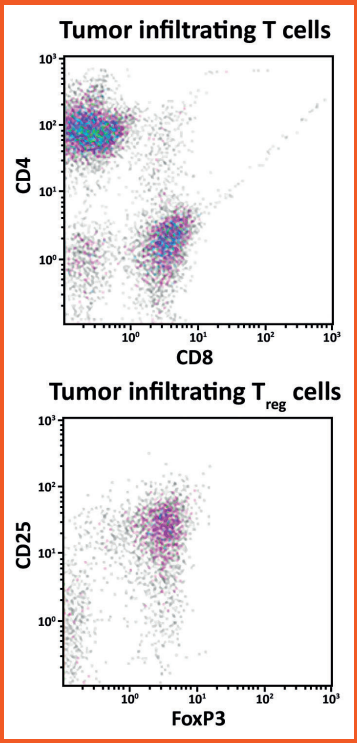
Anti-mPD-L1, anti-mPD-1 and anti-mCTLA4 antibodies were evaluated in all subcutaneous, subQperior™ and some orthotopic models

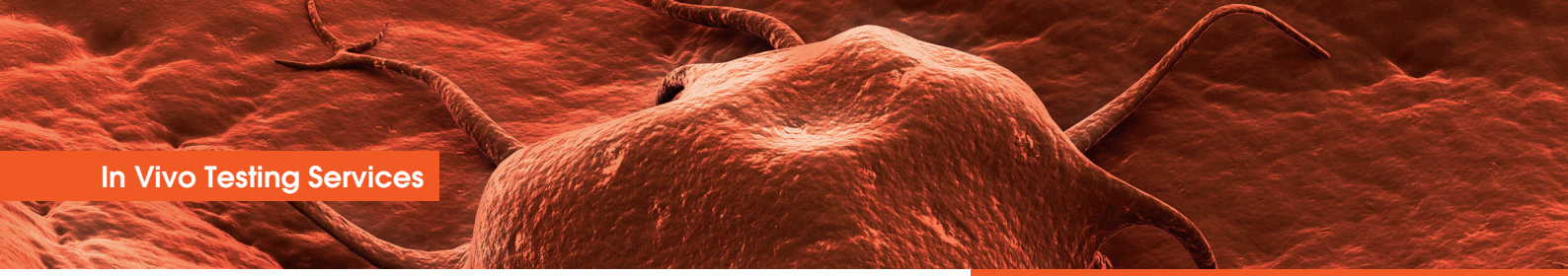
➢ Panel Screening

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.

Study Example

Flow cytometry analysis of CT26 wt tumor infiltrating leucocytes.





In Vivo Testing Services

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	RNA-seq	anti-PD1	anti-CTLA-4	Combi-nation	thera-peutic 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

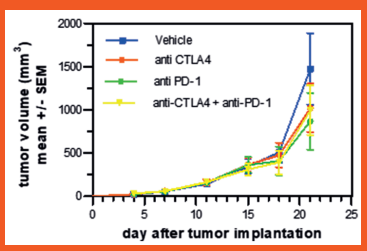
Comprises among other things:
(i) Subcutaneous implantation of tumor pieces
(ii) Measurement of animal weight (3x / week);
(iii) Determination of tumor size

➢ 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

Study Example

Effect of anti CTLA4 and anti PD-1 alone and in combination on subcutaneous tumor growth of JA-0009



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In Vivo Testing Services

In Vivo Angiogenesis Model

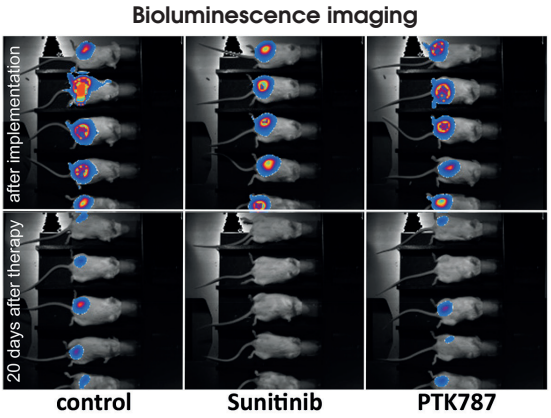
Field of Application

The spheroid-based in vivo angiogenesis model allows to study the effect of pro- and anti-angiogenic compounds in a living organism. For this model, spheroids from human umbilical vein endothelial cells (HUVECs) are implanted subcutaneously in mice in an extracellular matrix containing angiogenic growth factors. Subsequently, the formation of a human vasculature and the effect of treatment can be monitored in vivo and ex vivo. The in vivo angiogenesis model is suitable for the testing of the pro- or anti-angiogenic in vivo efficacy of antibodies or other biologicals and compounds.

Our Service

➢ Base Package

Implantation of spheroids from luciferase-transduced HUVECs; (i) administration of test compound (21 days); (ii) bioluminescence imaging (1x / week); (iii) measurement of ex vivo luciferase-activity at the end of the study; (iv) protocol & report



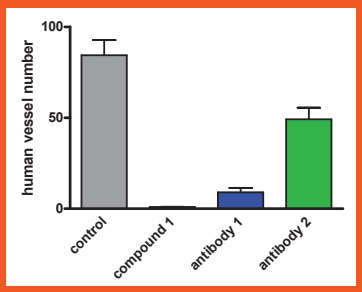
Our Service

➢ Reference Cpd

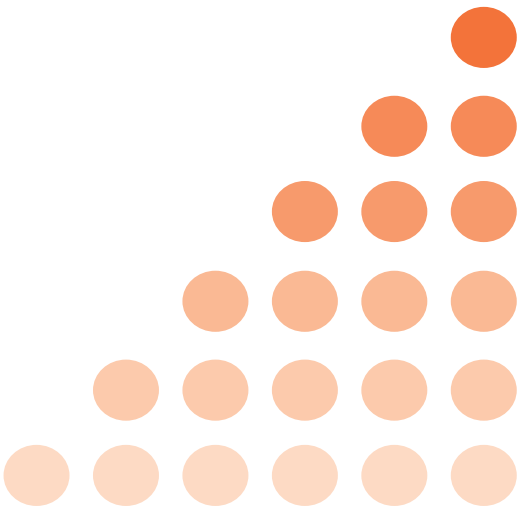
Testing of Sunitinib included in studies with anti-angiogenic agents

Study Example

Effect of a small molecule and two antibodies on human vessel number in the spheroid-based in vivo angiogenesis model.



Let’s discover together.



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 & Adme-Tox

- 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

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