Angiotargeting

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MIC
Molecular Imaging Center

MR Imaging of Malignant Brain Tumor Models in Rodents

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Overview

1. Introduction to malignant brain tumors.
2. Rat model: Intracranial implantations of human brain metastasis.
3. Mouse model: Intracardial injections of human melanoma cells.
4. Mouse model: Intradermal injections of human melanoma cells.
5. Can single tumor cells be tracked in vivo?

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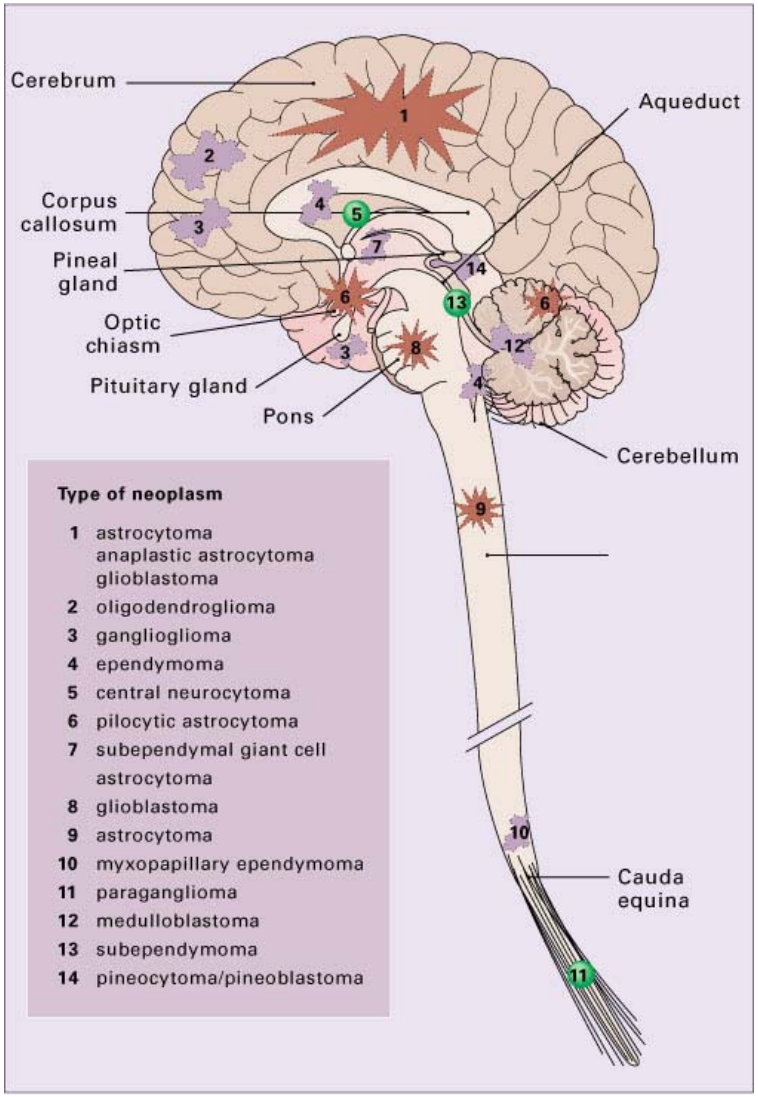
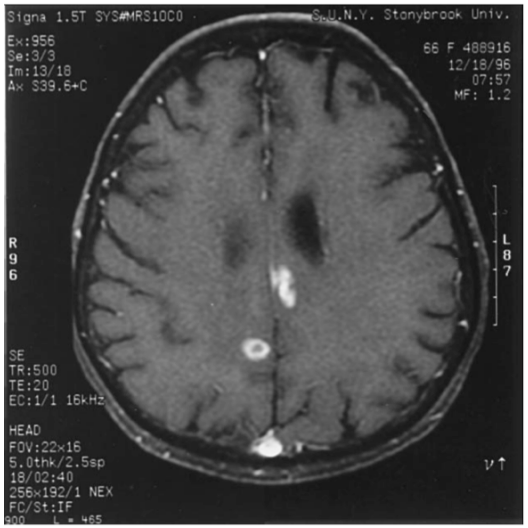
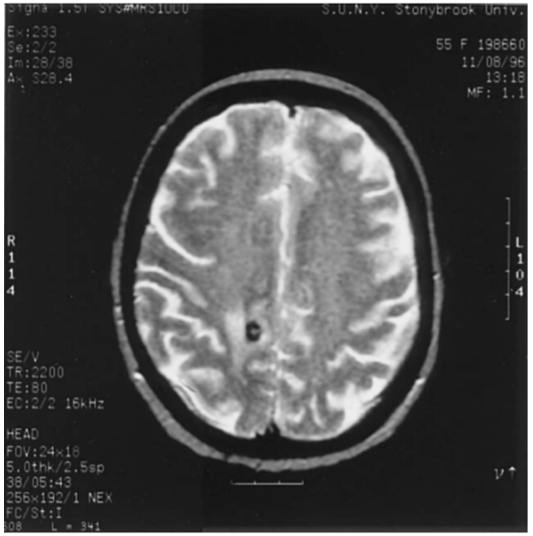
Primary and secondary brain tumors



Ellison & Love: Neuropathology 2e © 2004 Elsevier Ltd.

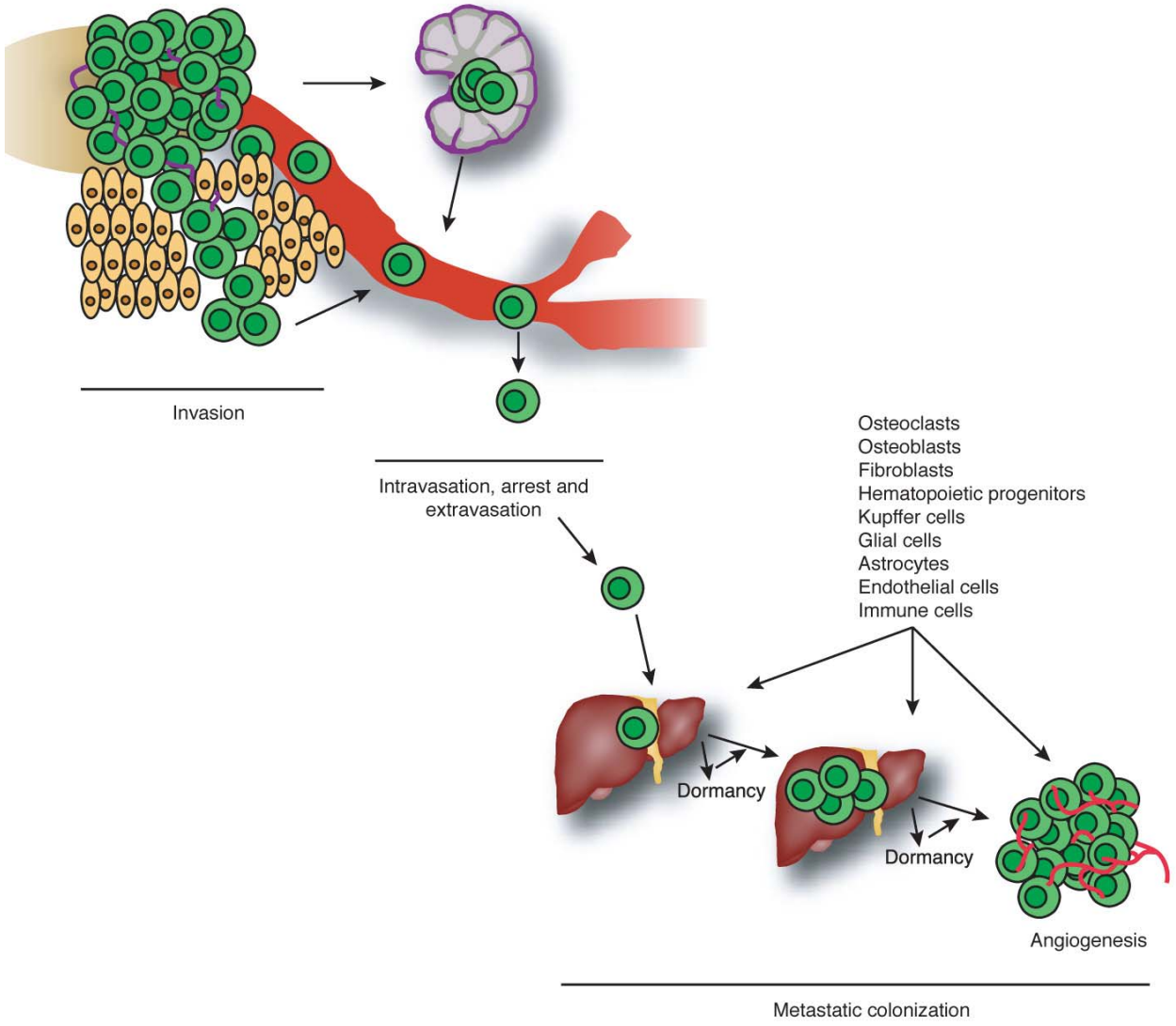


Brain met from lung cancer



Brain met from endometrial cancer

Cancer and the metastatic process



Steeg, Nature Med 2006

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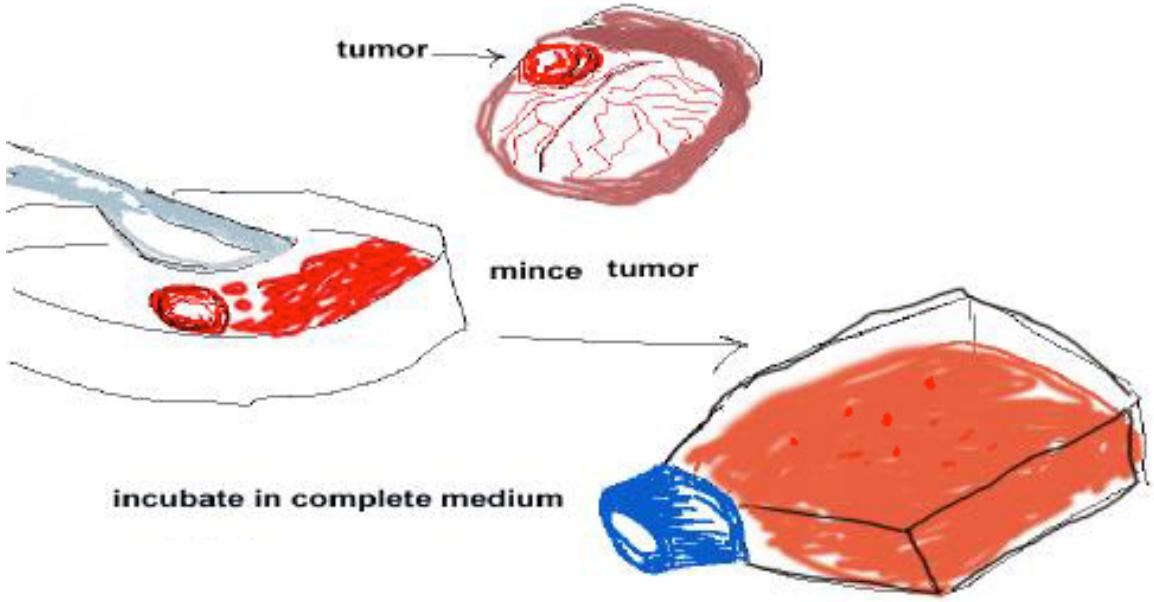
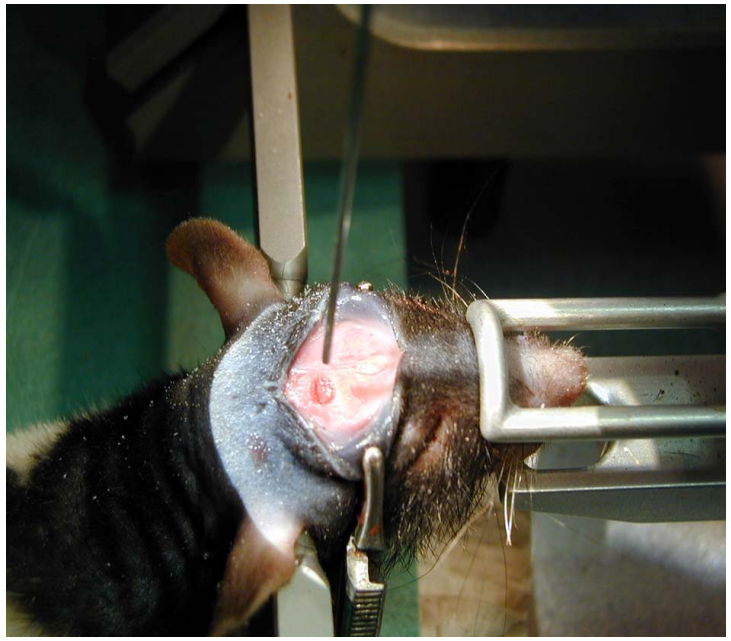
Collection of metastases

Brain metastases from patients are collected during operation, minced, and put in culture. After 2-4 weeks, tumor spheroids are implanted stereotactically into brains of immunodeficient animals.





Intracranial tumor take

Patient	Diagnosis of primary disease	i.c. tumour take [# of tumor take/ # of animals]	Animal survival [mean # of days ± SD]	s.c. tumor take [# of tumor take/ # of implanted animals]	Number of passages in animals [# of tumor take/ # of animals last passage]	Animal survival at last passage [mean # of days ± SD]
1	Serous papillary adenocarcinoma from ovary	Yes [1/2]	212	n.d.	2 [1/1]	156
2	Serous papillary adenocarcinoma from ovary	Yes [3/4]	102 ± 30	n.d.	n.d.	n.d.
3	Adenocarcinoma from colon	Yes [6/6]	55 ± 15	n.d.	4 [6/6]	41 ± 15
4	Large cell neuroendocrine carcinoma from colon	Yes [7/7]	55 ± 9	n.d.	3 [4/4]	30 ± 3
5	Malignant melanoma from skin	Yes [7/7]	27 ± 7	n.d.	2 [2/2]	120 ± 132
6	Malignant melanoma from skin	No [0/4]	-	n.d.	n.d.	n.d.
7	Malignant melanoma from skin	Yes [1/4]	94	No [0/3]	n.d.	n.d
8	Squamous carcinoma from lung	No [0/4]	-	No [0/2]	n.d.	n.d
9	Non-small cell adenocarcinoma from lung	Yes [3/4]	32 ± 9	No [0/2]	n.d.	n.d.

Abbreviations: i.c., intracranial; s.c., subcutaneous; n.d., not done; -, no data.

Primary Abs and Dilutions Used

Antibodies	Clone	Dilution	Source
CK7	OV-TL 12/30	1:100	DAKOCytomation (Glostrup, DK)
CK20	K_S 20.8	1:1000	DAKOCytomation
CA125	OC125	1:50	DAKOCytomation
CEA	II7	1:400	DAKOCytomation
ERα	1D5	1:50	DAKOCytomation
PR	PgR 636	1:150	DAKOCytomation
Melan-A	A103	1:50	DAKOCytomation
HMB45		1:100	DAKOCytomation
S100		1:3000	DAKOCytomation
Vimentin	V9	1:1000	DAKOCytomation
TTF1	8G7G3/1	1:100	DAKOCytomation
CD56	1B6	1:25	Novocastra Laboratories Ltd (Newcastle, UK)
NSE	BBS/NC/VI-H14	1:1000	DAKOCytomation
Syn		1:150	DAKOCytomation
CgA		1:3000	DAKOCytomation
p53	DO-7	1:1000	DAKOCytomation

CEA: Carcinoembryonic antigen; ERα: Estrogen receptor α; PR: progesterone receptor; TTF1: Thyroid transcriptional factor 1; NSE: Neuron specific enolase; Syn: Synaptophysin; CgA: Chromogranin A.

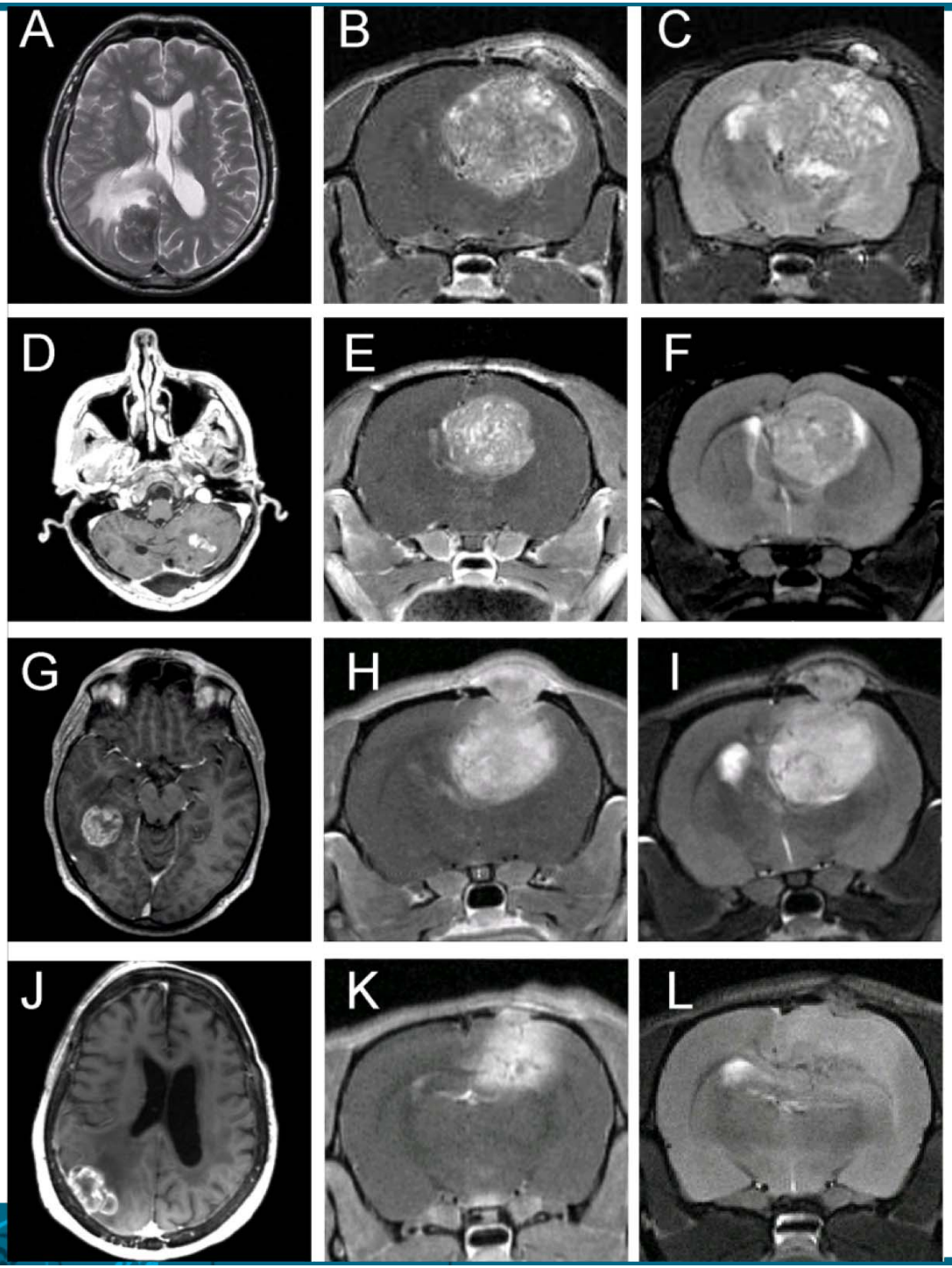
Tumor marker expression

Tumor Marker	Patient 1 Ovarian cancer			Patient 2 Ovarian cancer			Patient 3 Colon cancer			Patient 4 Colon cancer			Patient 5 Skin cancer			Patient 9 Lung cancer		
	PT	PM	RM	PT	PM	RM	PT	PM	RM	PT	PM	RM	PT	PM	RM	PT	PM	RM
CK7	+++	+++	nt	+++	+++	+++	-	-	-	+++	+++	+	nt	-	-	+++	+++	-
CK20	-	-	-	-	-	-	+++	nt	+++	+++	+	-	nt	-	-	-	-	-
CA125	+++	+++	nt	+++	+++	+++	-	-	-	-	-	-	nt	-	-	-	-	-
CEA	-	-	nt	-	-	-	+++	+++	+++	+++	+++	+++	nt	-	-	-	-	-
ER	++	++	nt	+	+	-	-	-	-	-	-	-	nt	-	-	-	-	-
PR	+	+	nt	+	+	+++	-	-	-	-	-	-	nt	-	-	-	-	-
Melan-A	-	-	-	-	-	-	-	nt	-	-	-	-	+++	-	+	-	-	-
HMB45	-	-	nt	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S100	+	-	nt	+	+	+	-	-	-	-	+	+	+++	+	+	-	++	-
Vimentin	-	-	nt	+	+	-	-	-	-	-	-	-	nt	+++	+++	-	+++	+++
TTF1	-	-	nt	-	-	-	-	nt	-	-	-	-	nt	-	-	-	++	-
CD56	-	+	nt	++	+	+	-	-	-	+++	+++	++	nt	-	-	++	+	-
NSE	-	-	nt	+	-	-	-	-	-	++	++	+	nt	++	-	+	+	-
Syn	+	-	nt	-	+	+	+	+	-	+++	+++	+++	nt	-	-	-	-	-
CgA	-	-	nt	-	-	-	-	-	-	++	+	-	nt	-	-	-	-	-
p53	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	nt	+	+	+++	+	+

Common markers used to discriminate colorectal carcinomas from ovarian adenocarcinomas.
Melanocytic tumor markers.
Commonly used mesenchymal tumor markers.
Lung specific tumor marker.
Neuroendocrine cell markers.

+++: 70%-100% positive tumor cells
++: 30%-70% positive tumor cells.
+: Less than 30% positive tumor cells.
-: No positive tumor cells.
nt: No tumor cells available.

Experimental tumors have similar radiological appearance as in the patients



Primary: Colon c.

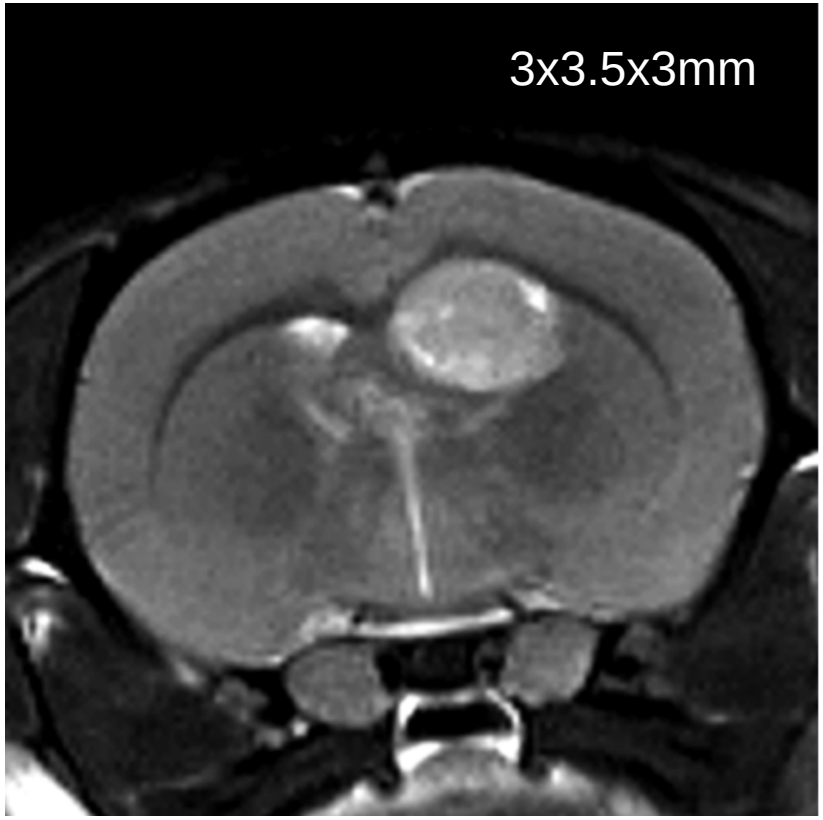
Primary: Ovarian c.

Primary: Melanoma

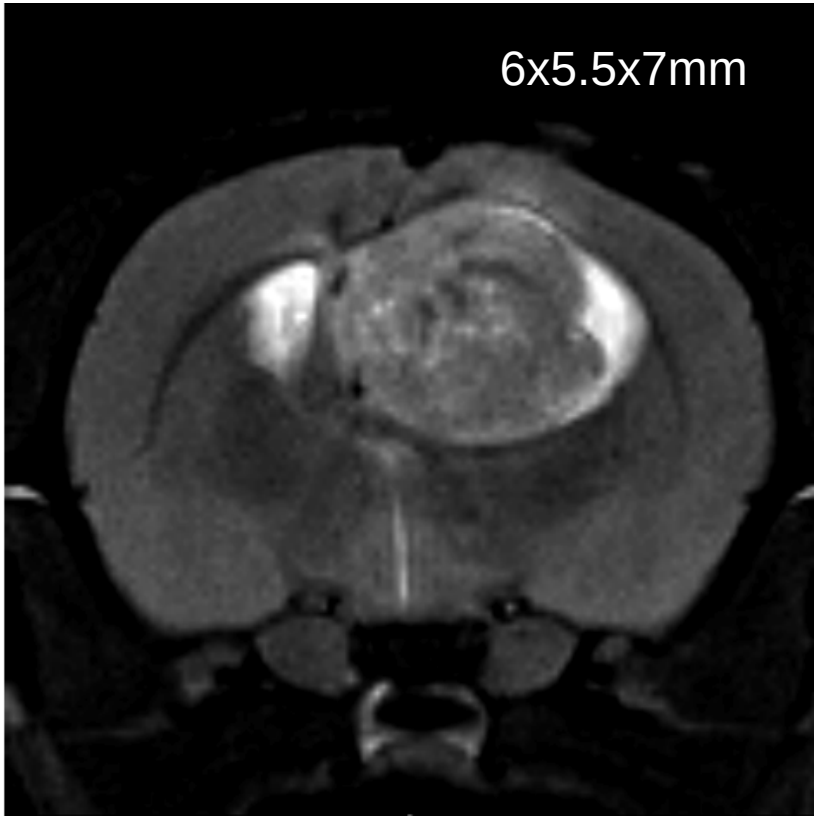
Primary: Lung c.

Brain metastases growth is monitored with MR

MR Scan 08.09.2006



MR Scan 28.09.2006

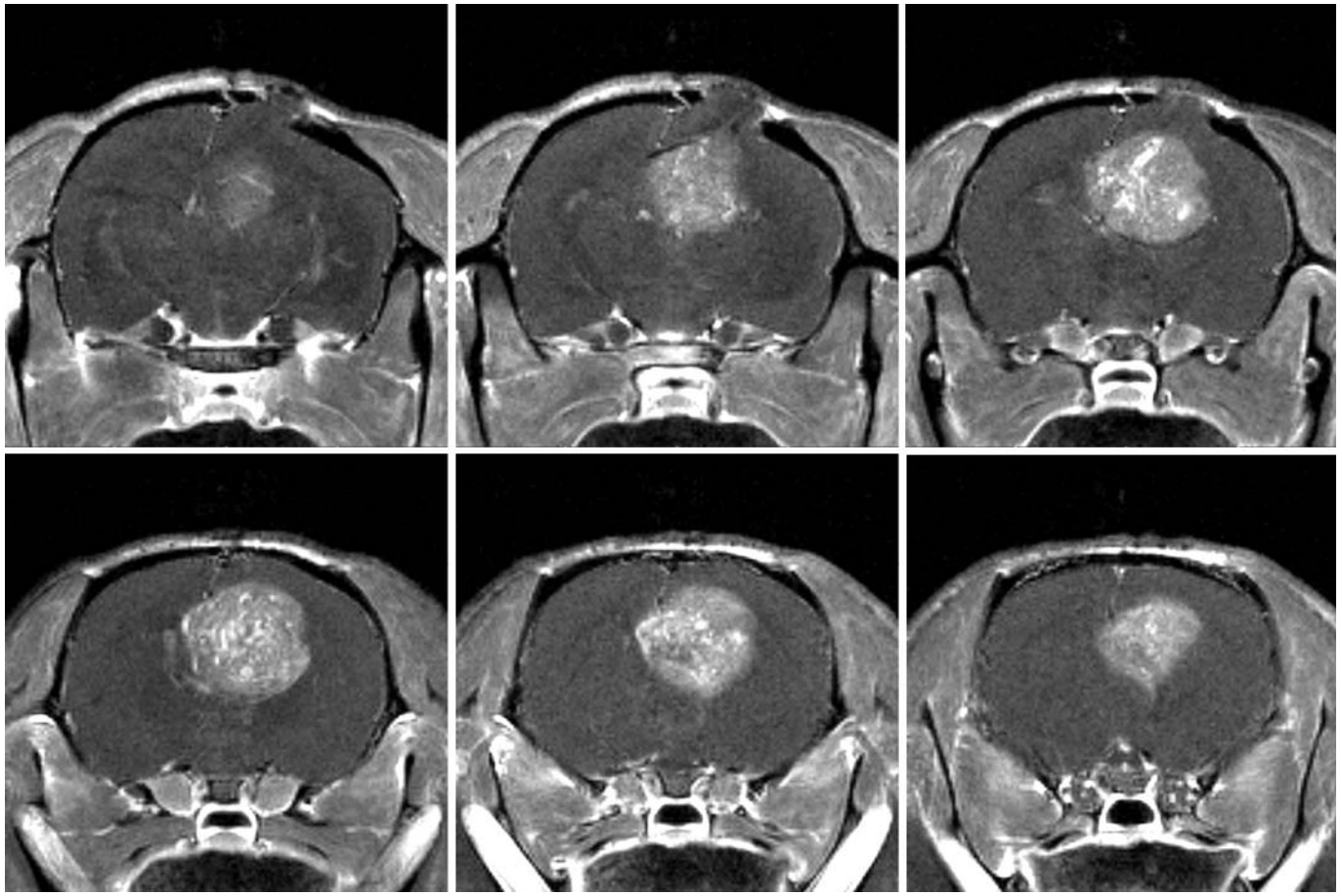


Primary cancer: Ovarian

RARE T2: TR4200ms, TE36ms, FA180, TA 4m28s800ms, Slice 1/1, NA2, 256X256, FOV3.5

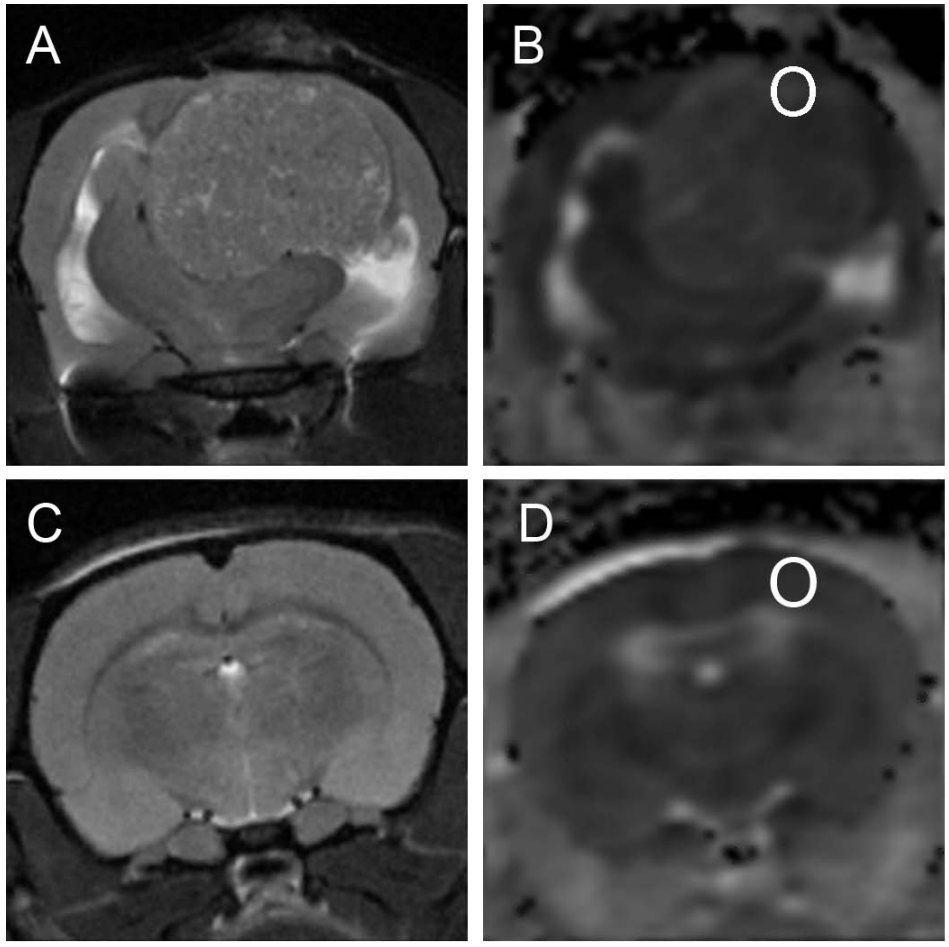
The metastases are highly angiogenic

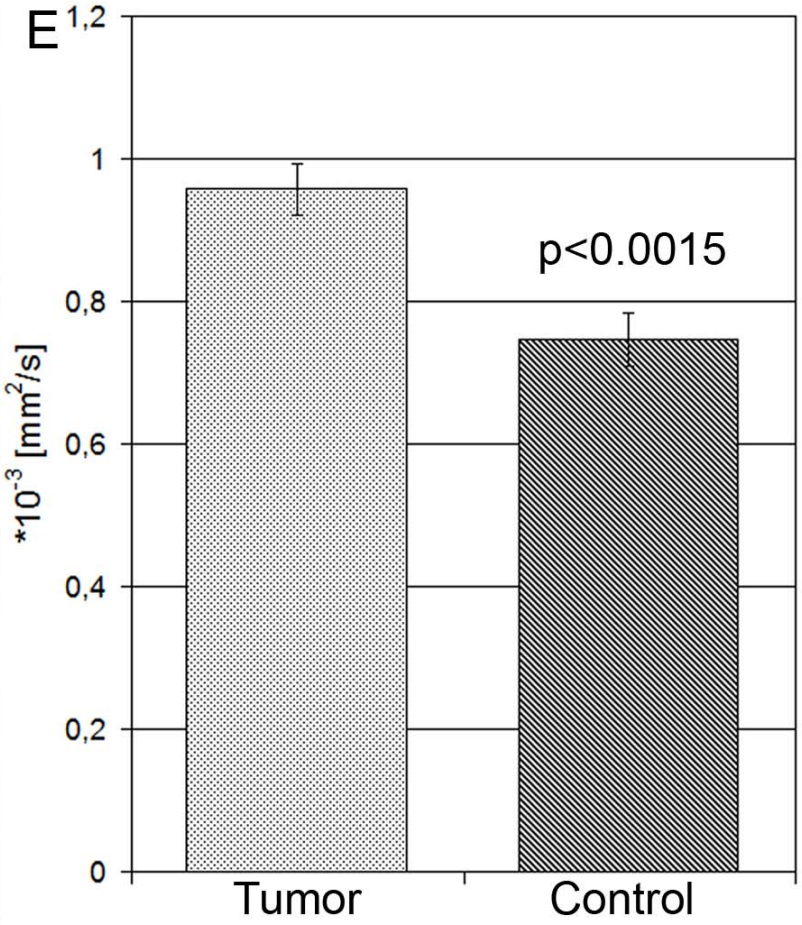
MR Scan 28.09.2006. T1 with Gadolinium Contrast



MSME: TR438.5ms, TE10.6ms, FA180, TA 5m36s786ms, Slice 1/1, TI0, NA3, 256X256, FOV3.5, Fat Sup. On, Mot. Sup. On

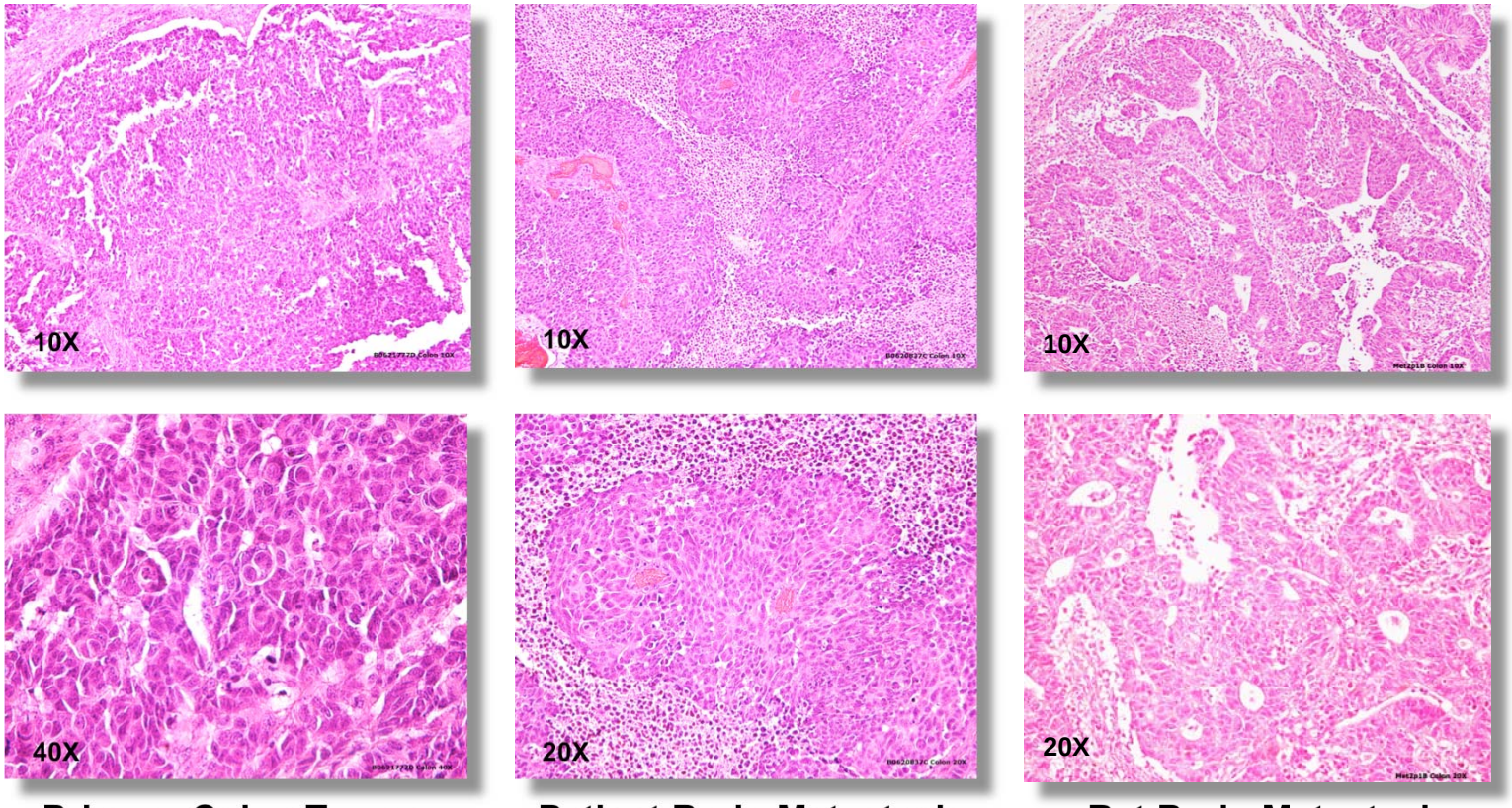
Intratumoral diffusion is increased





EPI:
TR3000ms, TE33.3ms, FA90, TA5m36s0ms, Slice 1/1, NEX4, 1 diffusion direction, 6 diff. exp. pr direction, 1 A0. B values 100, 200, 400, 600, 800, 1000 s/mm2.

Histology of patient and animal tumor is similar



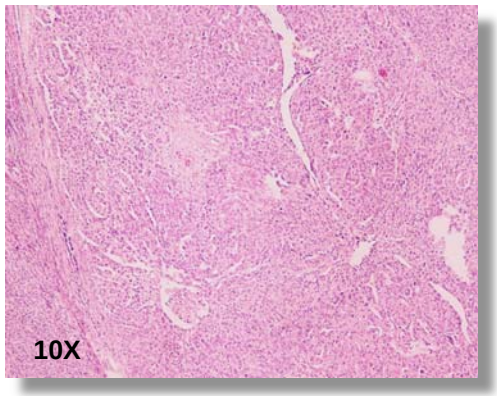
Primary Colon Tumor
B0117862B

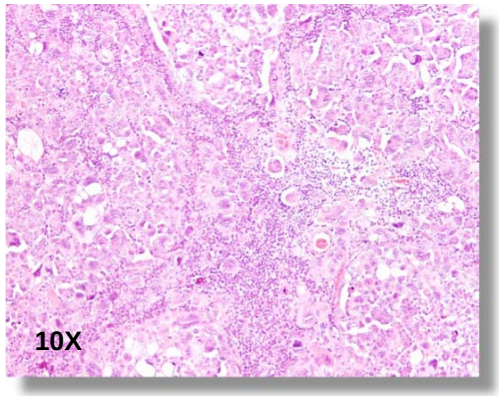
Patient Brain Metastasis
B0620837C

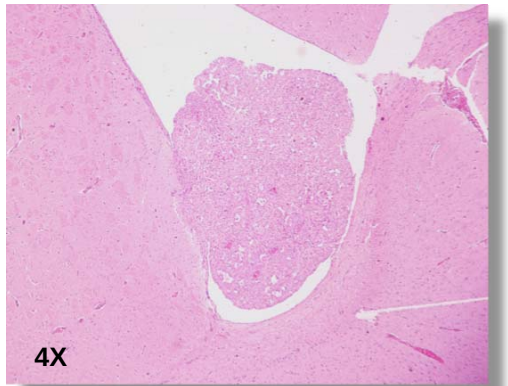
Rat Brain Metastasis
Met2p1B

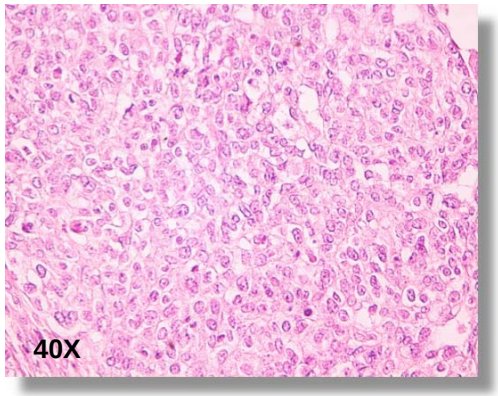
Morphology like adenocarcinomas of colorectal type. The tumor cells are polymorphic, eosinophilic, showing tubular growth pattern, vacuolated, tumor tissue sharply demarcated to the brain tissue, mitotic figures and necrotic areas are also present.

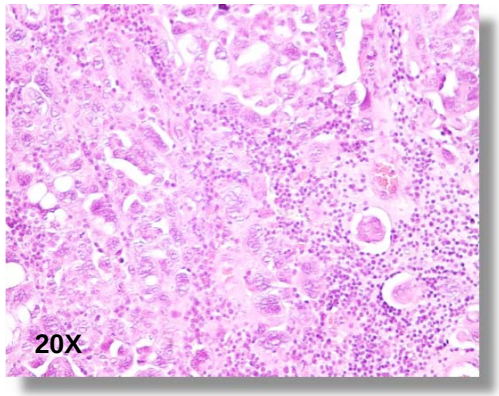
Histology of patient and animal tumor is similar

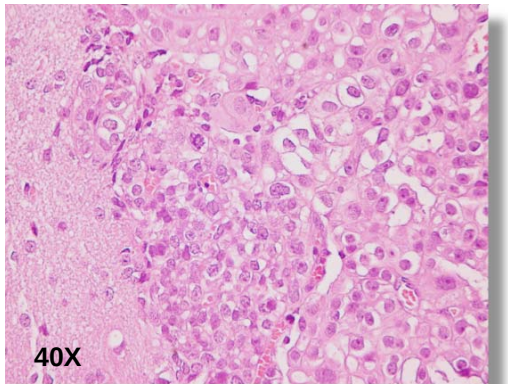












Primary Ovary Tumor

B04158871

Patient Brain Metastasis

B0615651A

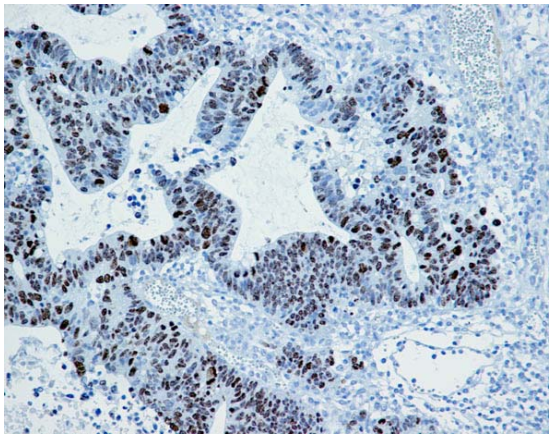
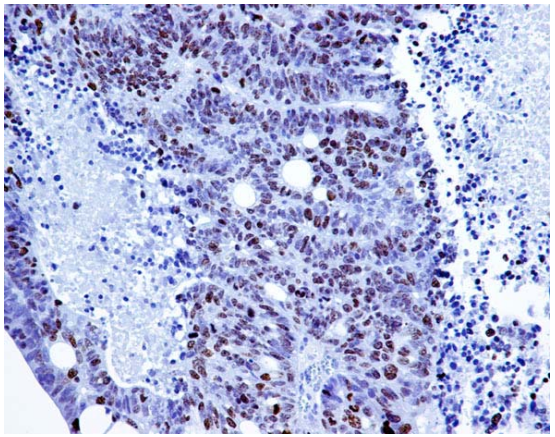
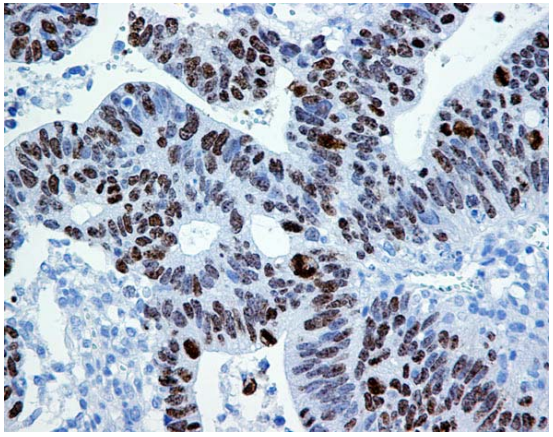
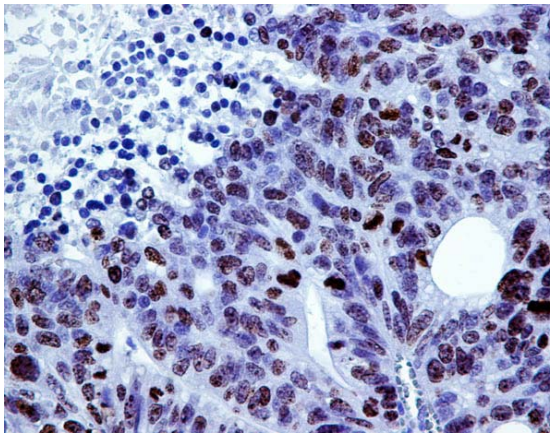
Rat Brain Metastasis

Met3p1A

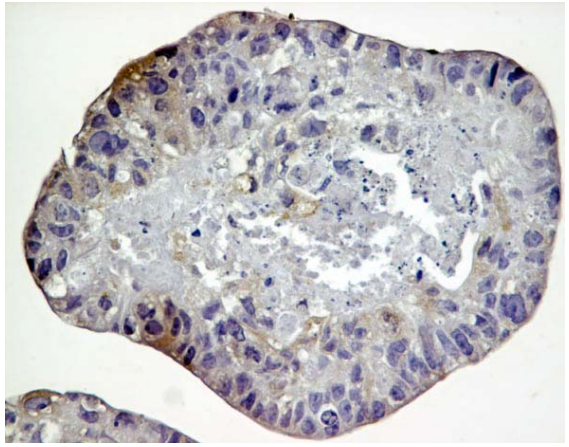
Low differentiated tumor tissue with solid arrangement of polygonal cells with enlarged roundish hyperchromatic nuclei, tight cell cohesion (low differentiated carcinoma). The tumor cells are epithelial, eosinophilic, sometimes clear, polymorphic, with diffuse growth pattern and mitotic figures.

The metastases show high proliferation rates also after serially passaging

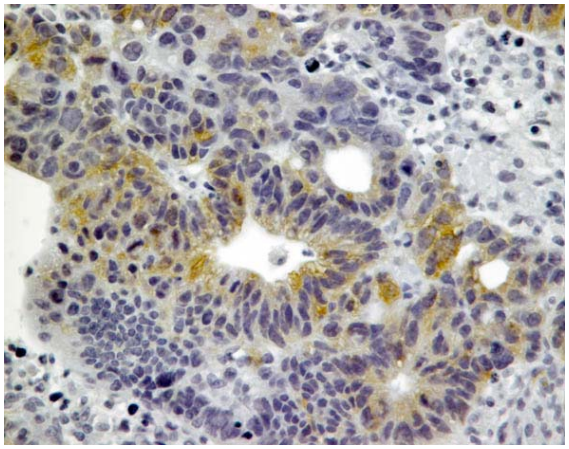
Colon met:

	1st Passage	3rd Passage	
20X			20X
40X			40X

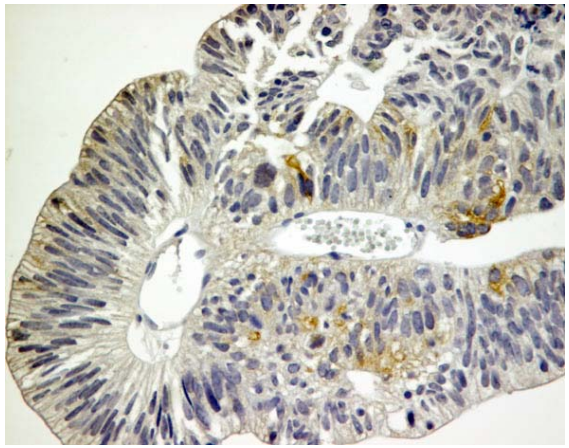
Colon mets express Nestin in primary biopsy as well as in serially passaged tumors



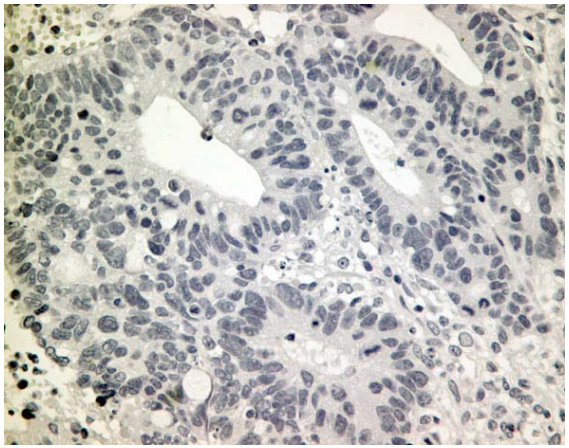
Patient biopsy



Passage 1



Passage 3

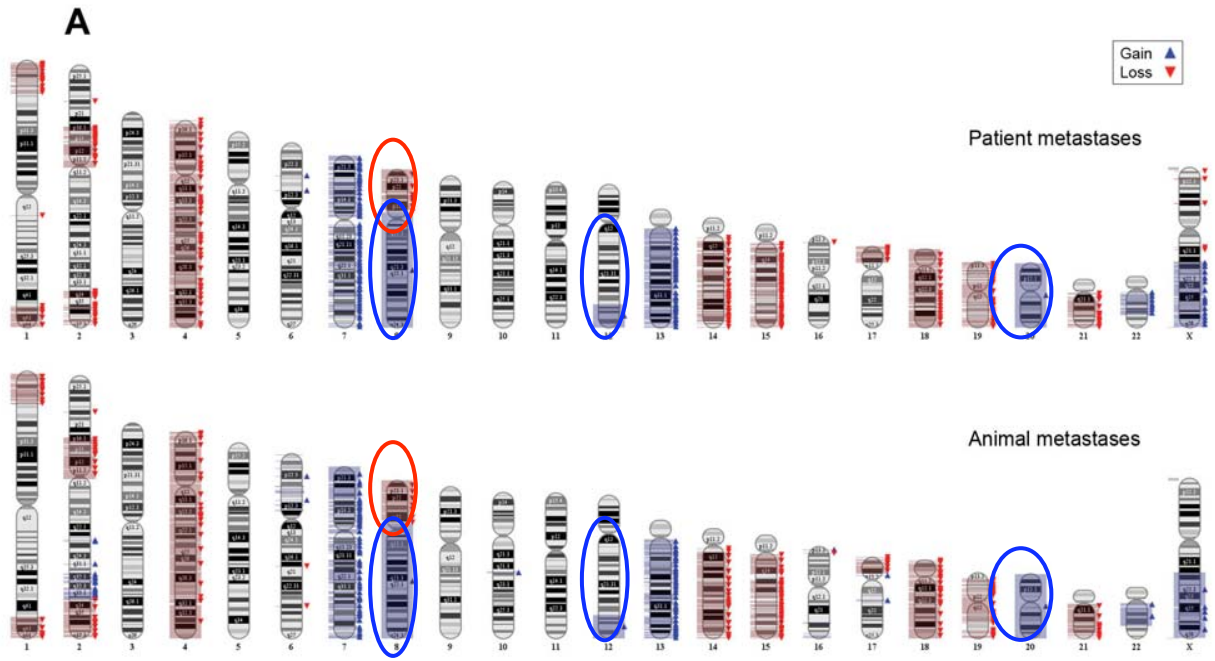


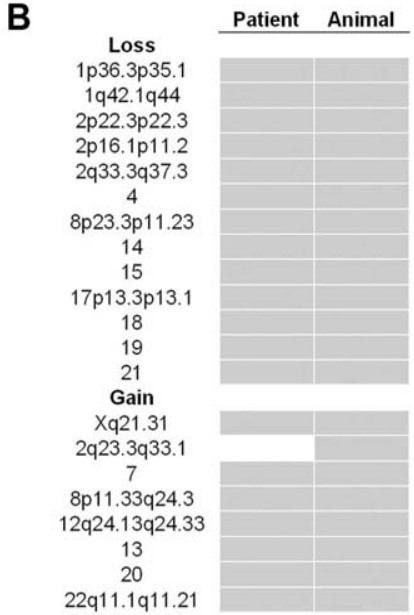
Negative control

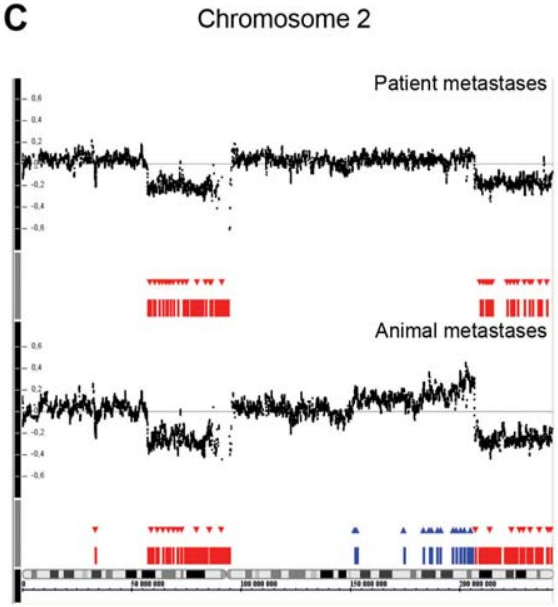
Nestin is a type IV intermediate filament protein.

Expressed (transient) by many cells during development, but does not persist into adulthood.

DNA Copy Analysis



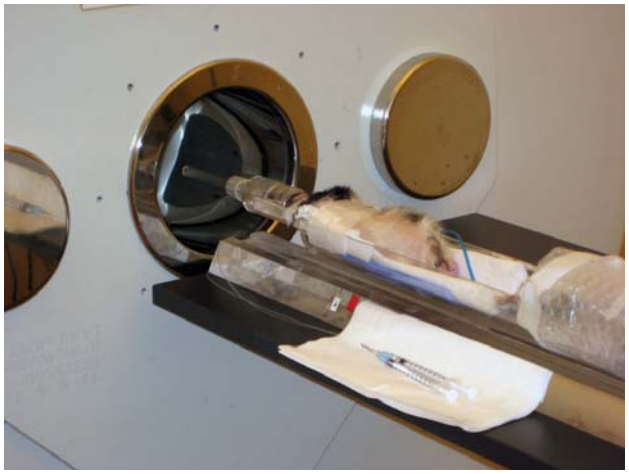


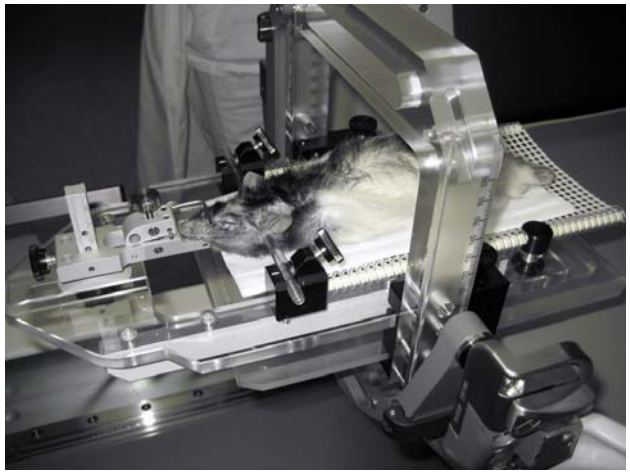


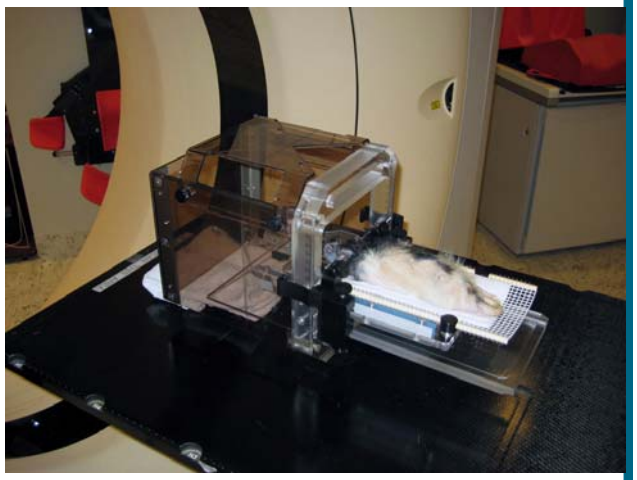
Only difference:

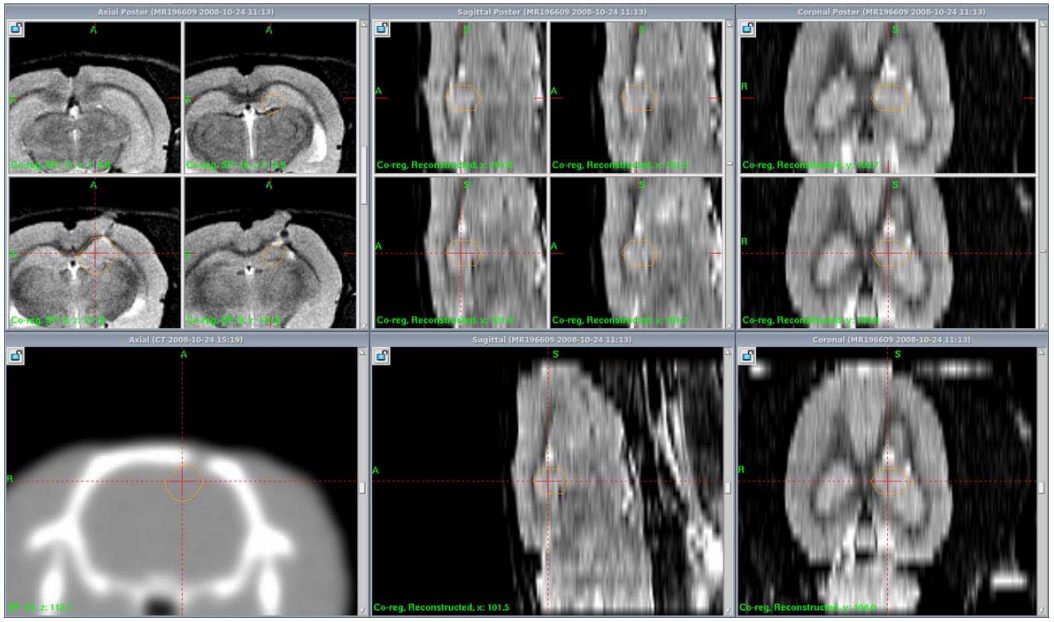
Gain in 2q23.3q33.1 seen in animal met, which was not present in the patient tumor.

Future work: Gamma Knife irradiation of metastases implanted intracranially in nude rats

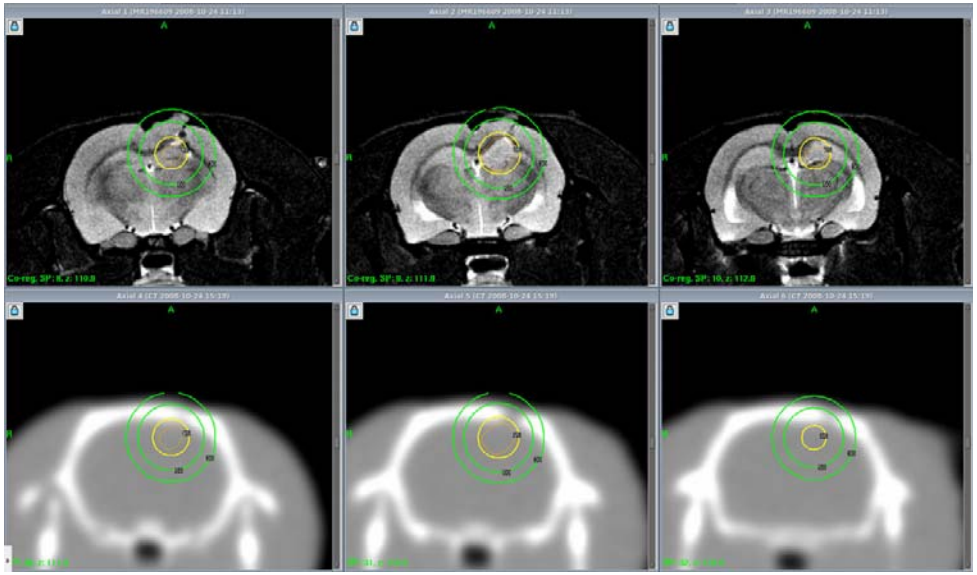


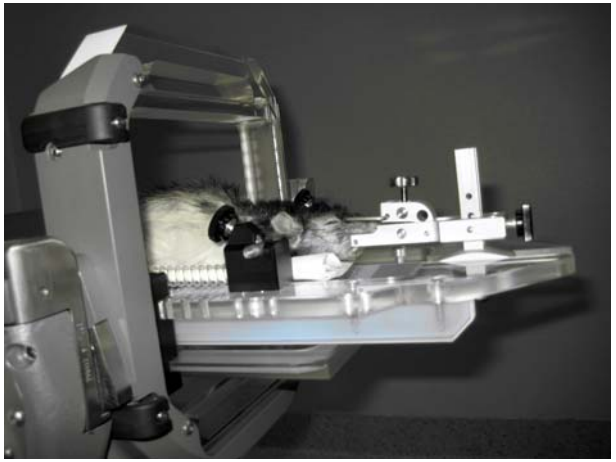






Gamma Knife pilot study





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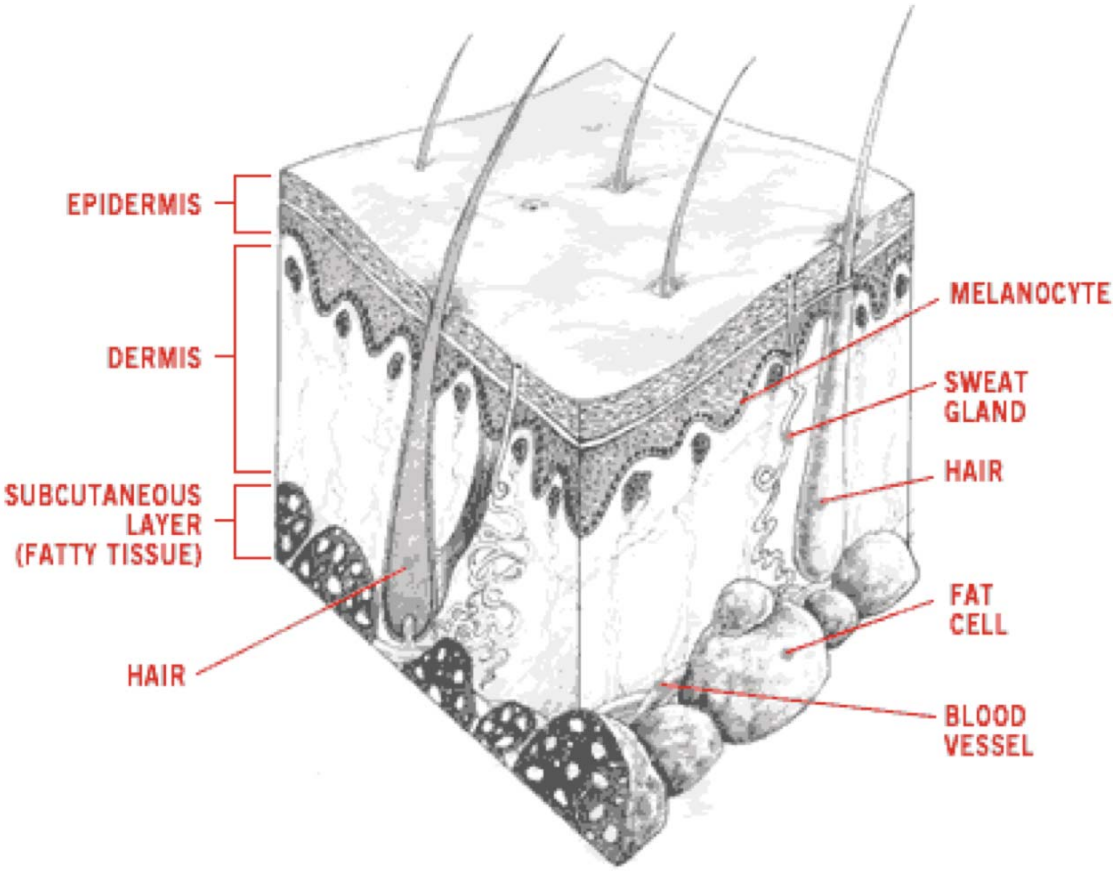
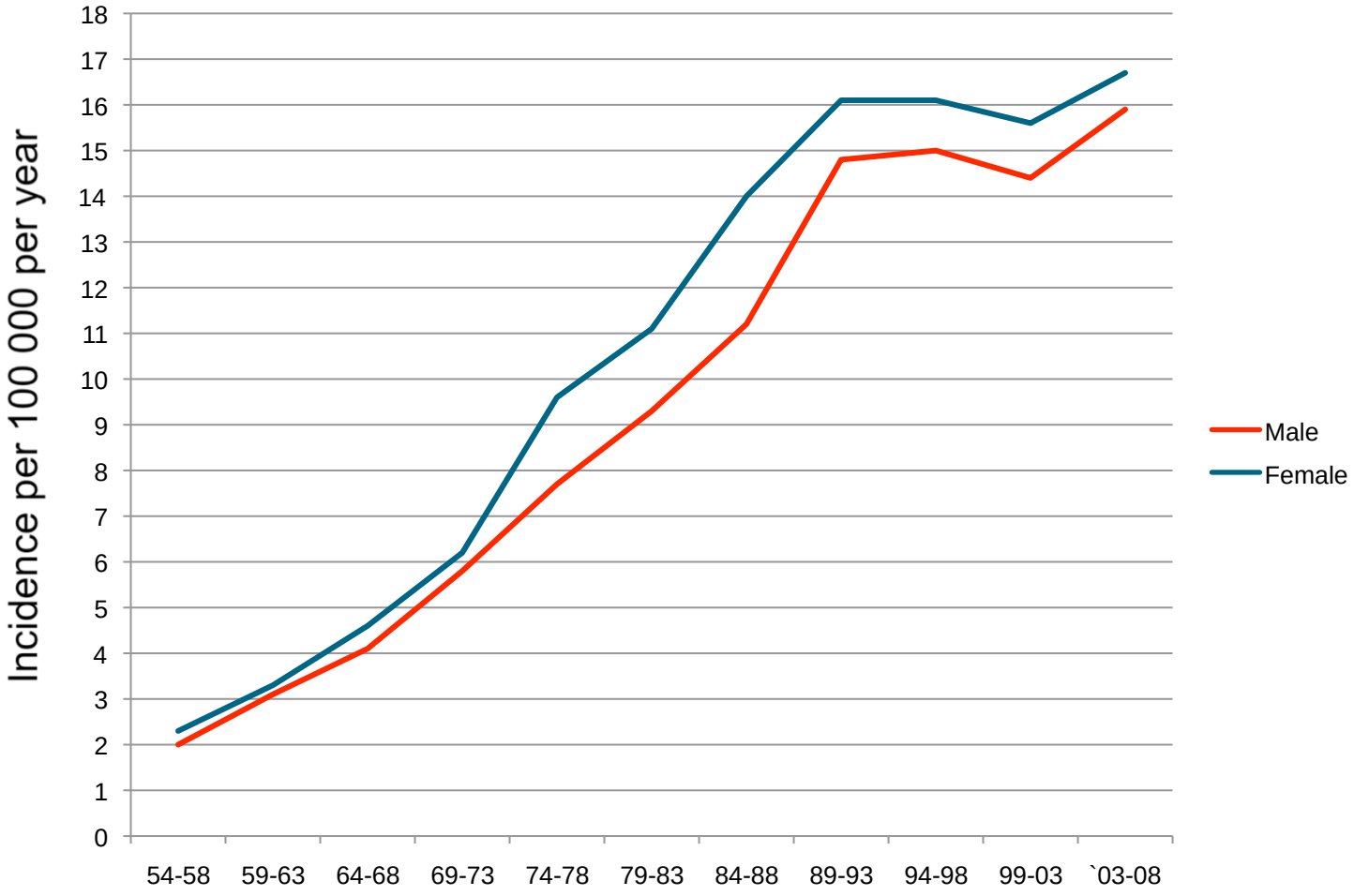
The skin



In epidermis:

Basal cells
Squamous cells
Melanocytes

Incidence of melanoma in Norway



5 year survival if distant metastasis: 21.9% (females), 6.5% (males).
In 2007, 177 men and 98 women melanoma deaths were registered.

Source: http://www.kreftregisteret.no/no/Registrene/Kreftstatistikk/

Some risk factors

Exposure to UV radiation.

Fair skin.

Lots of moles on the skin.

Previous melanomas.

History of many sunburns.

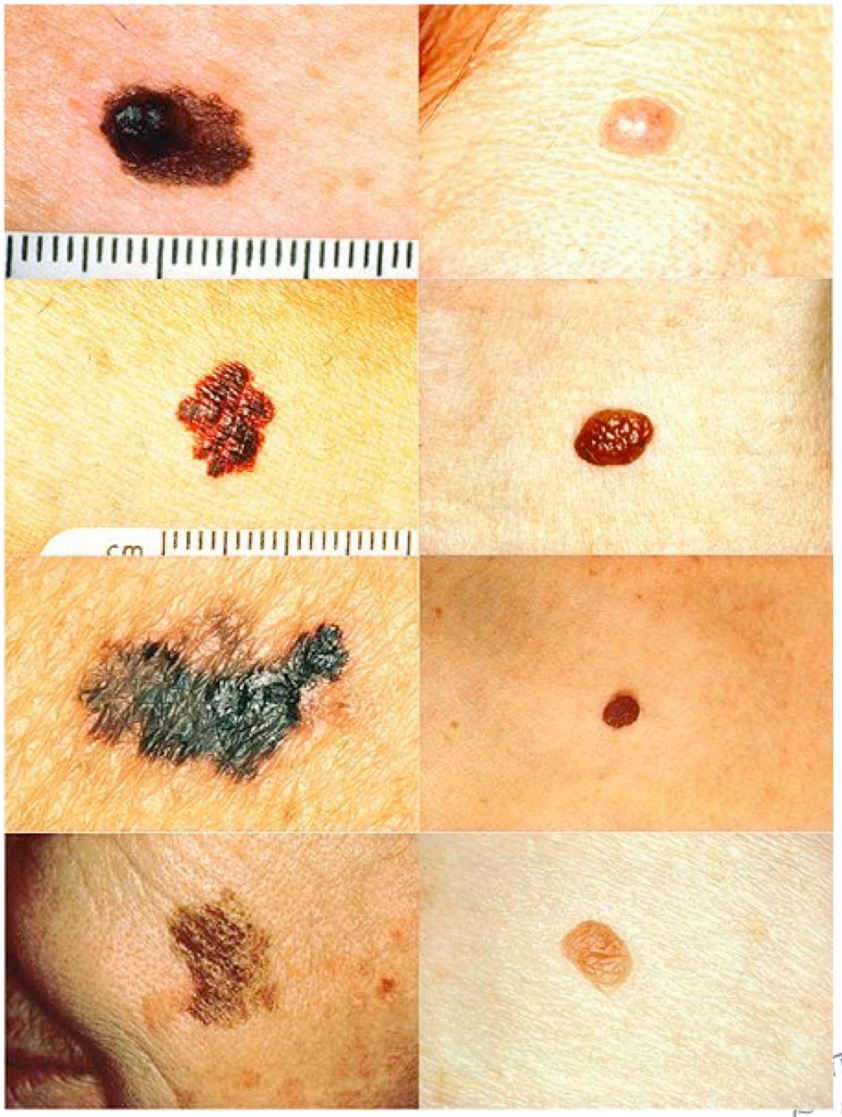
Family history.

Being older.

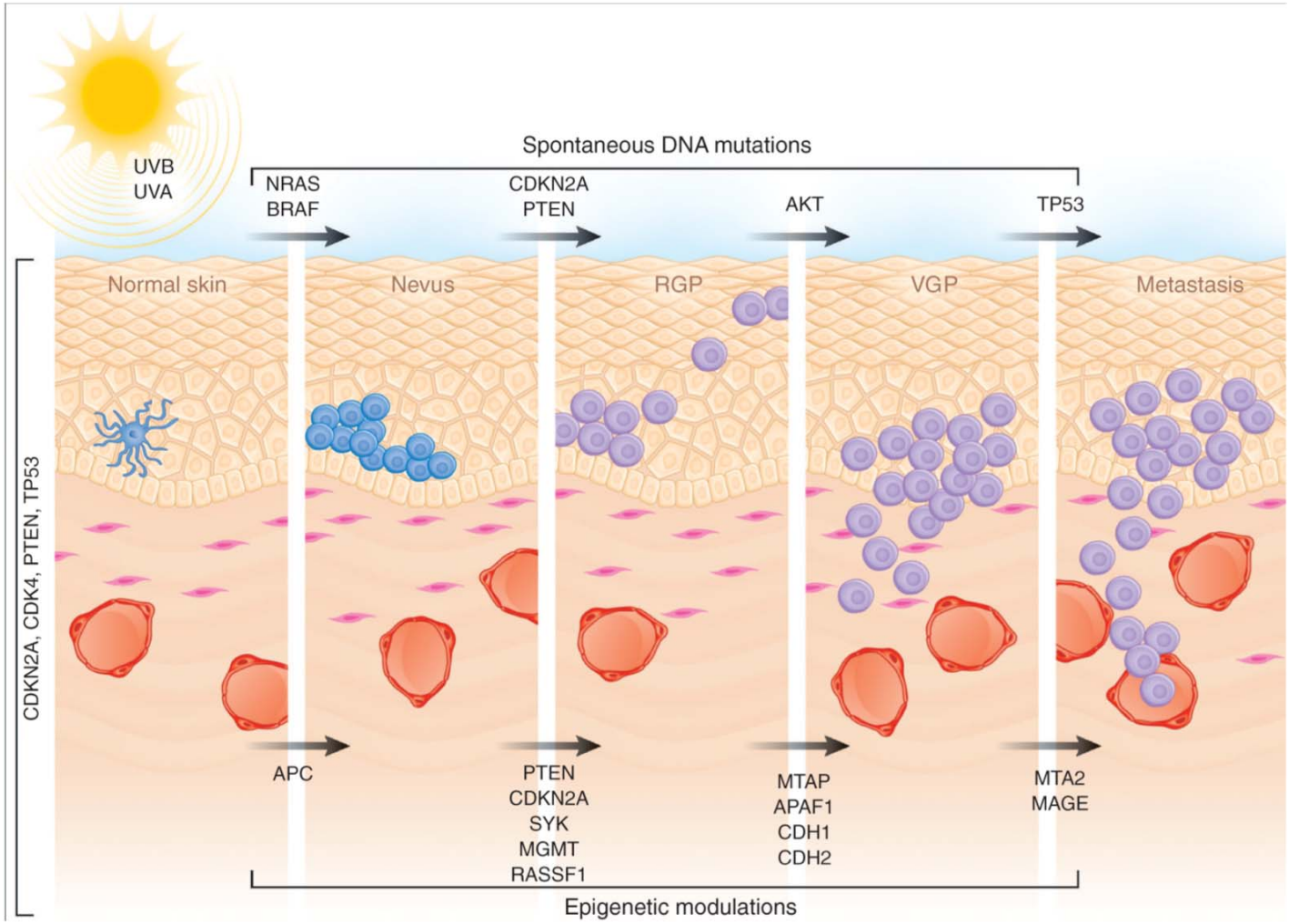
Genetic (5-10%):

CDKN2A (cell cycle regulator).

MC1R (controlling the type of melanin being produced).



Proposed molecular alterations



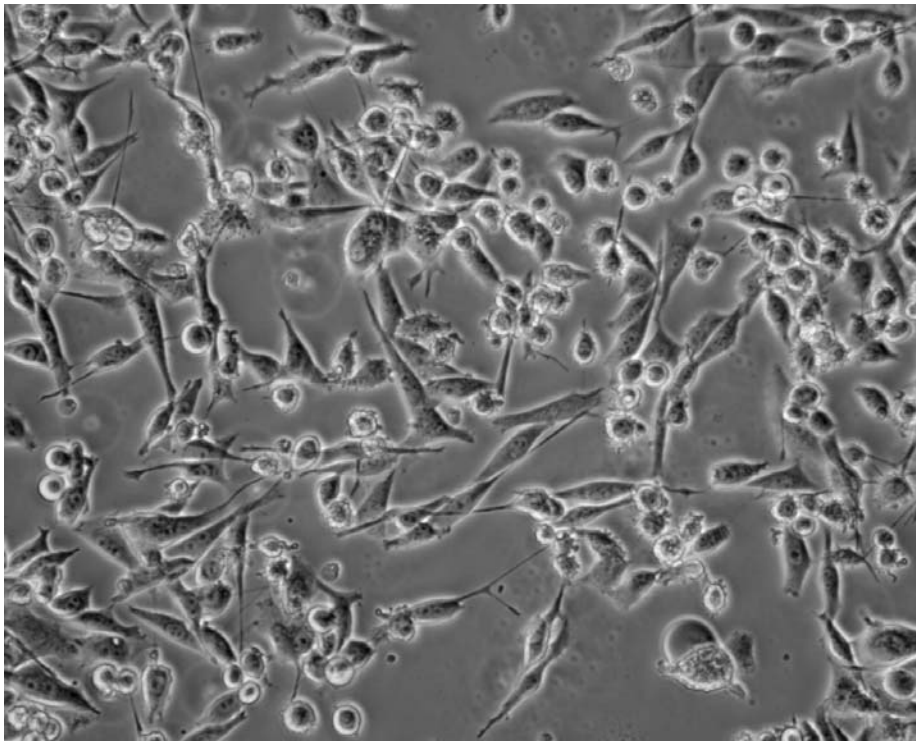
Zaidi et al, J Invest Dermatol 2008

Models of melanoma progression

40-60% of melanoma patients diagnosed with (often multiple) brain metastases.
Patient survival 4-6 months.

Available animal models:

Intracarotid injection of murine and human melanoma cell lines in syngeneic mice (Fidler el al, 1999).
Several murine and human cell lines have been established (see review; Cranmer et al, 2005).
Grafting human skin onto immunodeficient mice (Zaidi et al, 2008).

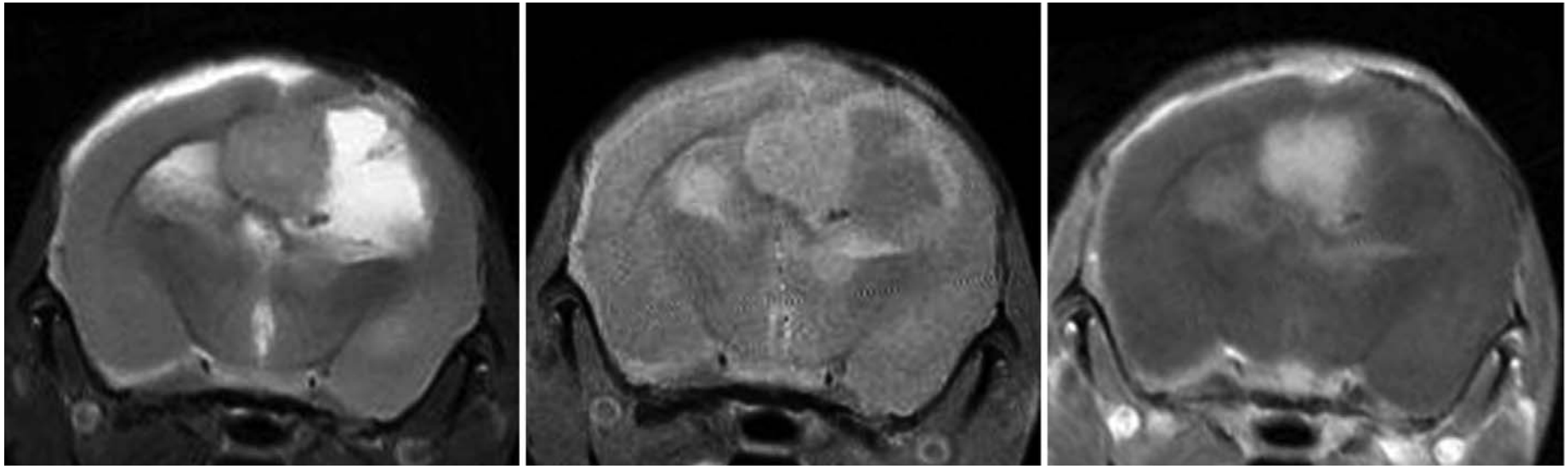


We obtained a brain metastases from a patient diagnosed with melanoma. Tumor pieces were put in cell culture. A cell line (H1) developed after 4 weeks.

The melanoma cells is tumorigenic in the mouse brain

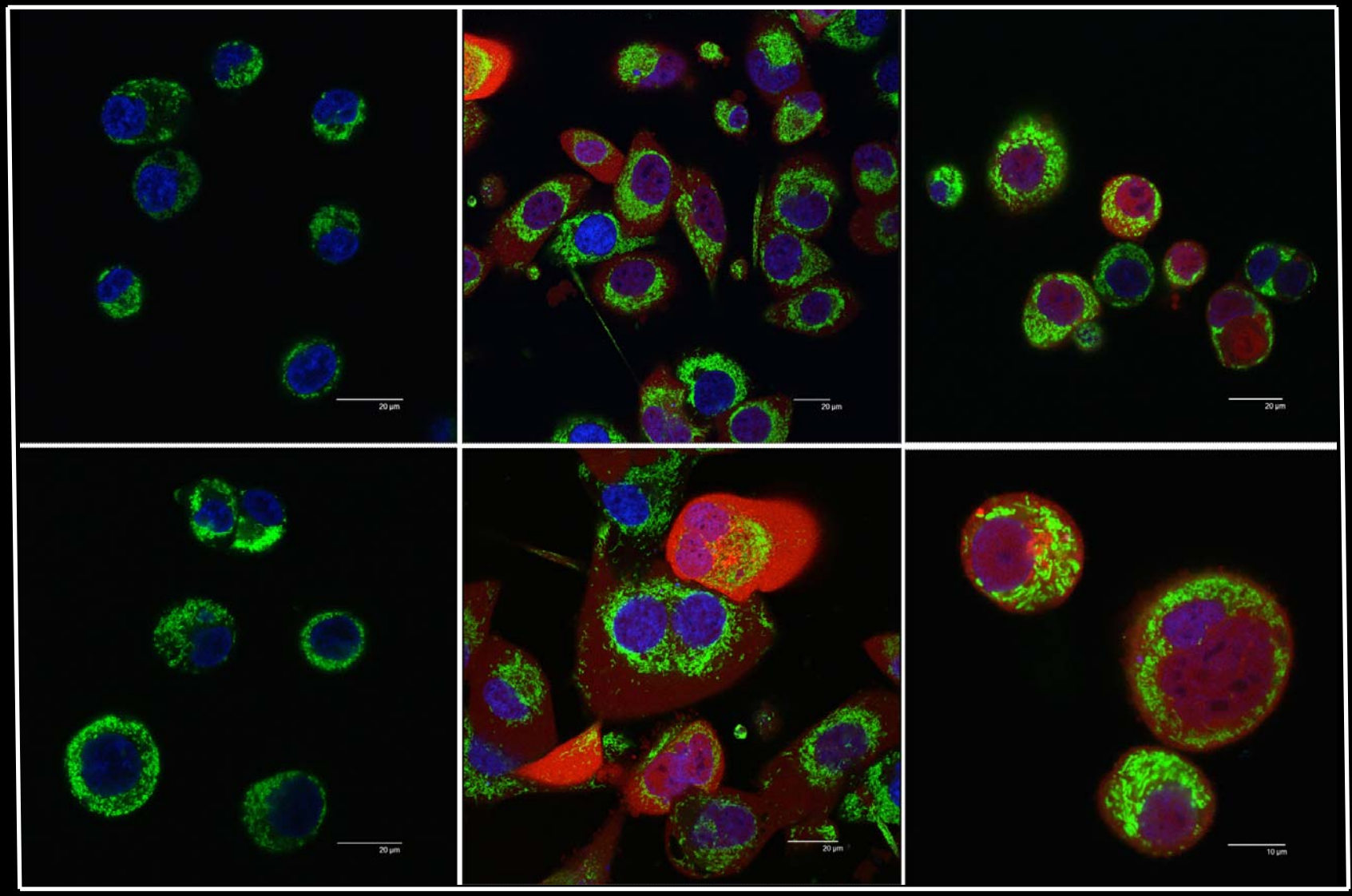
3 animals were stereotactically injected with 1 million H1 cells into the brain of nod/scid mice.

Tumors developed in 2/3 animals after 4 weeks.



T2 T1 T1+contrast

H1dsRed cell line is established from H1 cells

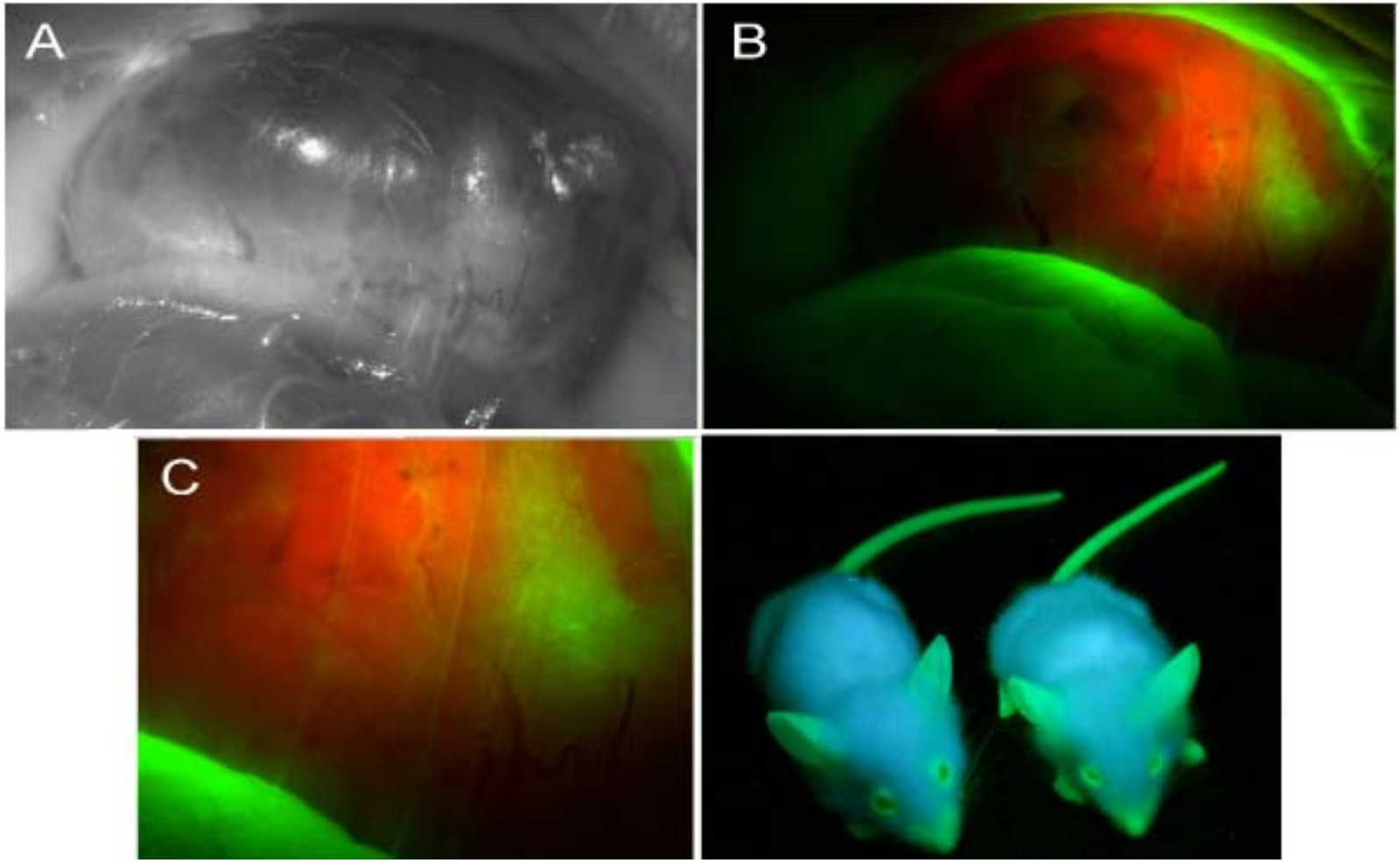


H1 (neg. Control)

H1_DsRed unsorted

H1_DsRed sorted

The H1dsRed cell line is tumorigenic sub. cut.

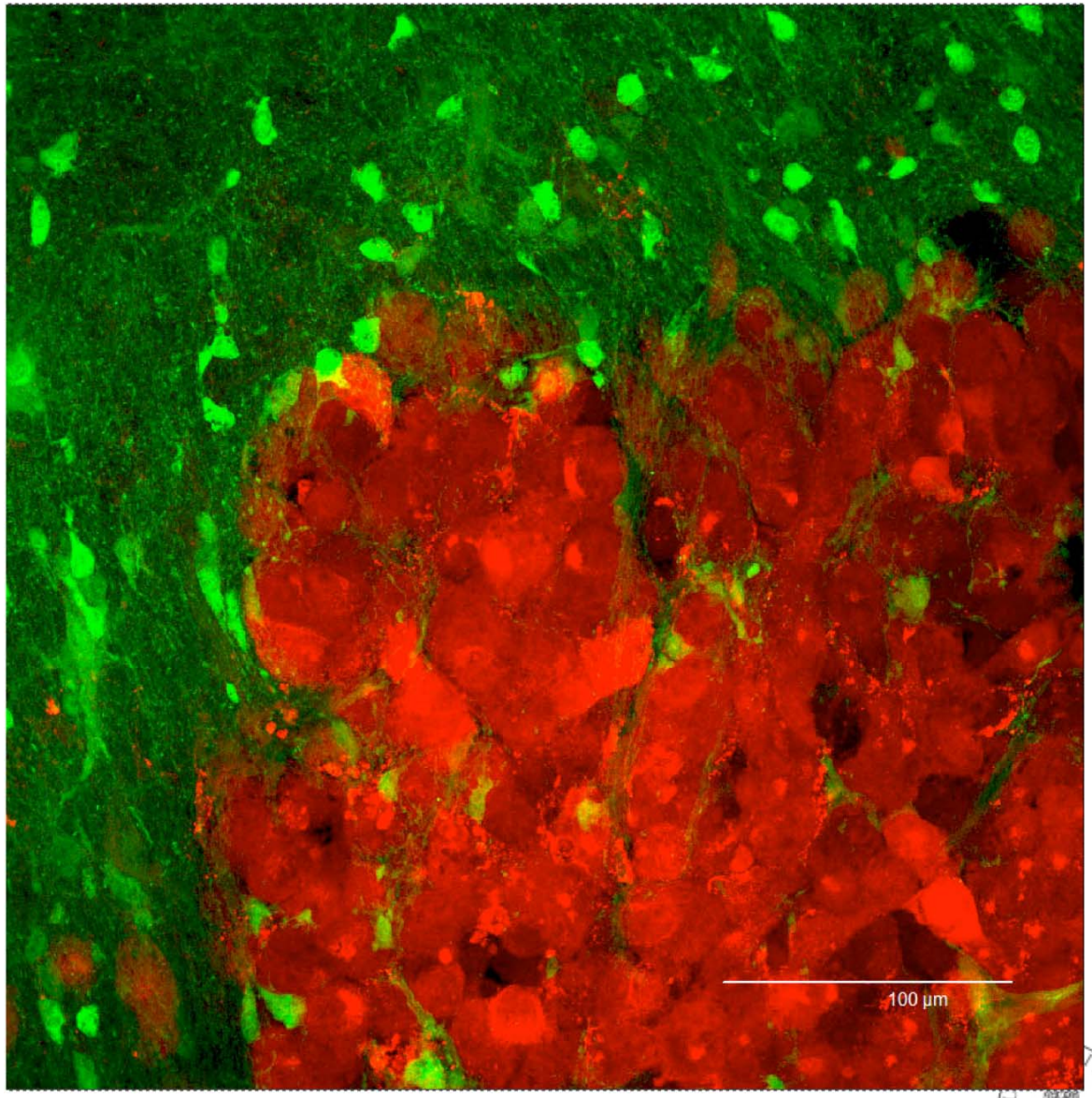


Tumor by H1dsRed 6 weeks after sub.cut injection in matrigel in eGFP nod/scid mice

The H1dsRed cell line is tumorigenic in the brain

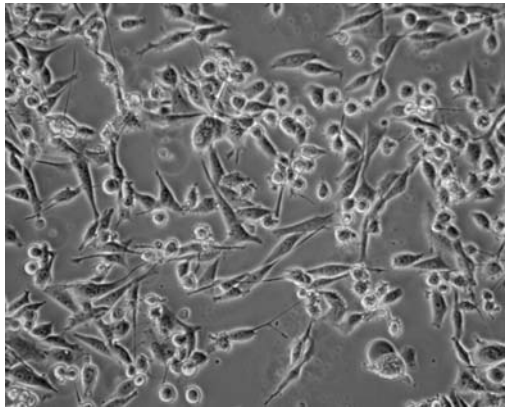
2 animals were stereotactically injected with 1 million melanoma cells into the brain of eGFP nod/scid mice.

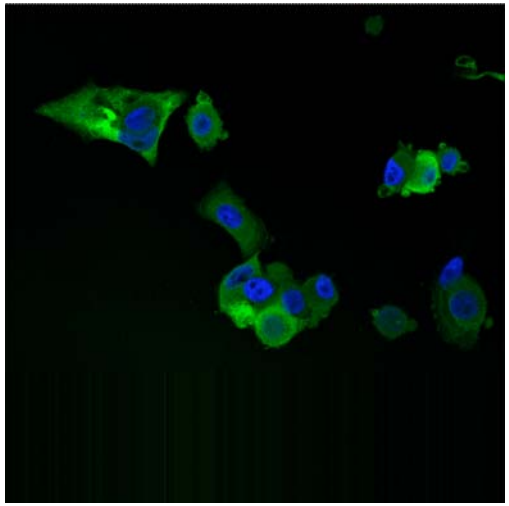
Tumors detected by confocal imaging after 4 weeks.

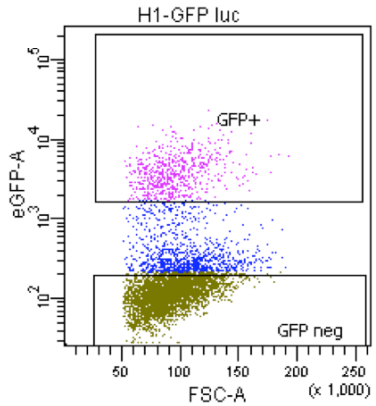


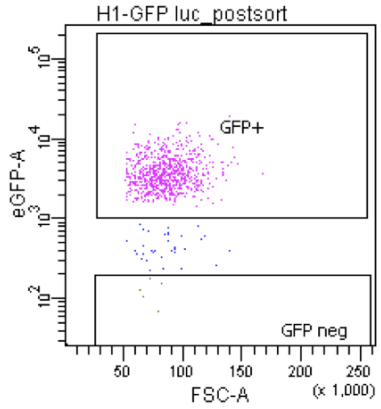
Development of H1_GFP_Luc cell line

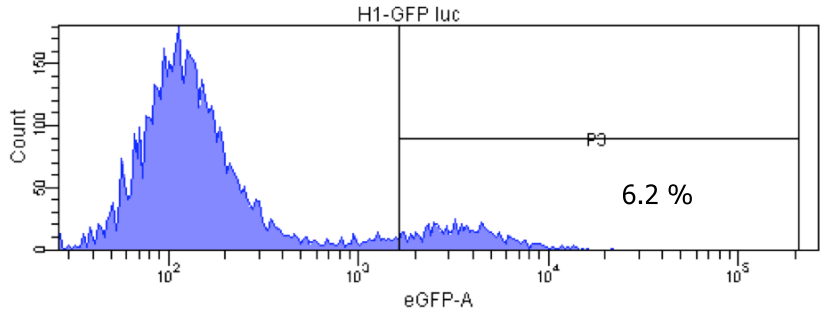
Lentiviral transfection of H1 cell line with genes for GFP and Luciferase.
The resulting H1_GFP_Luc cell line was then sorted by flow cytometry.



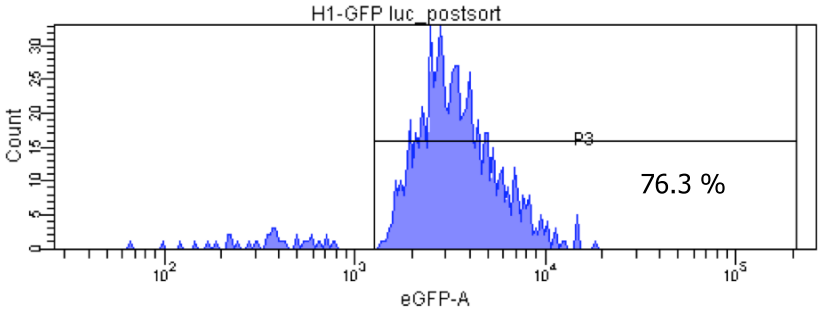




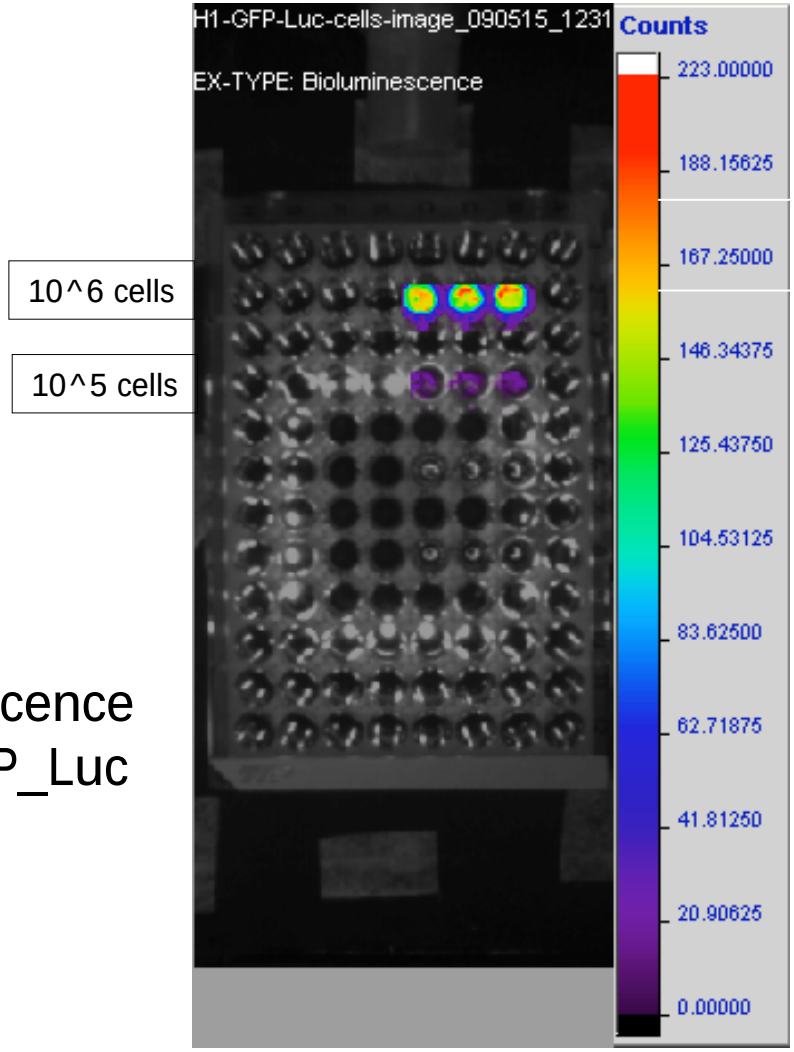




Double sorting



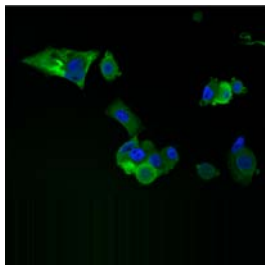
Determination of whether H1_GFP_Luc cells are suitable for in vivo bioluminescence imaging

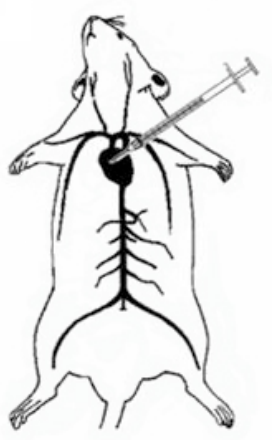


In vitro bioluminescence imaging of H1_GFP_Luc cell line

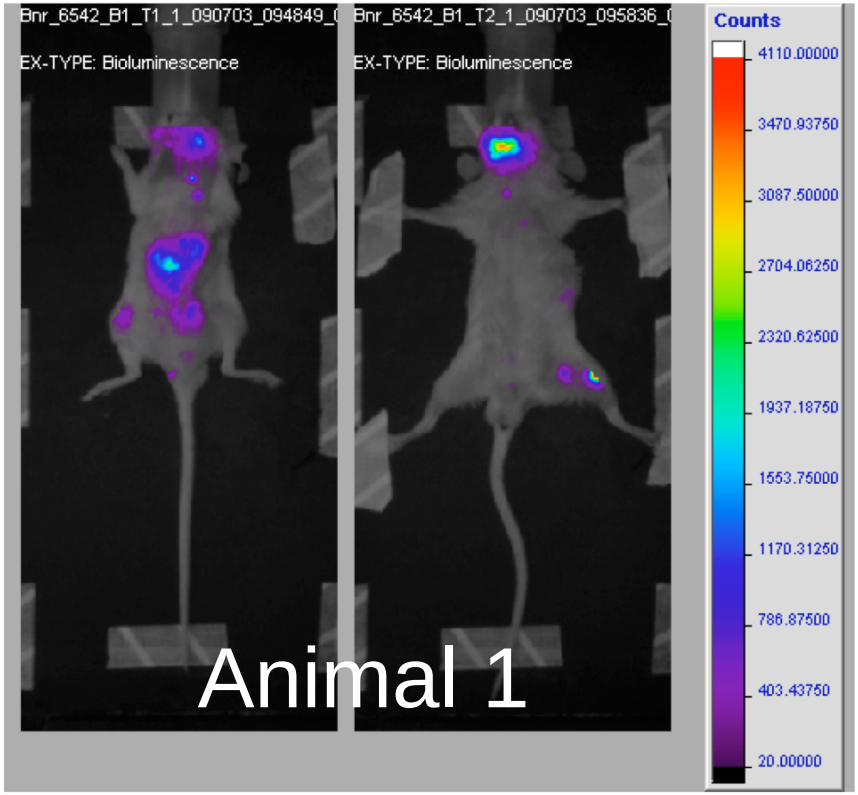
The H1_GFP_Luc cell line is tumorigenic in vivo

6 animals injected intracardially with 1 million H1_GFP_Luc cells.



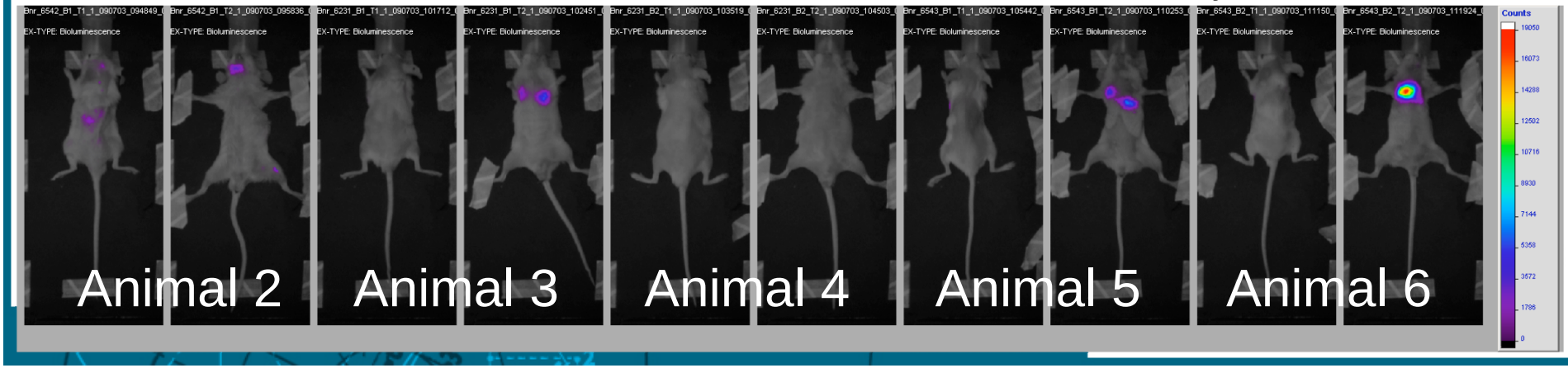


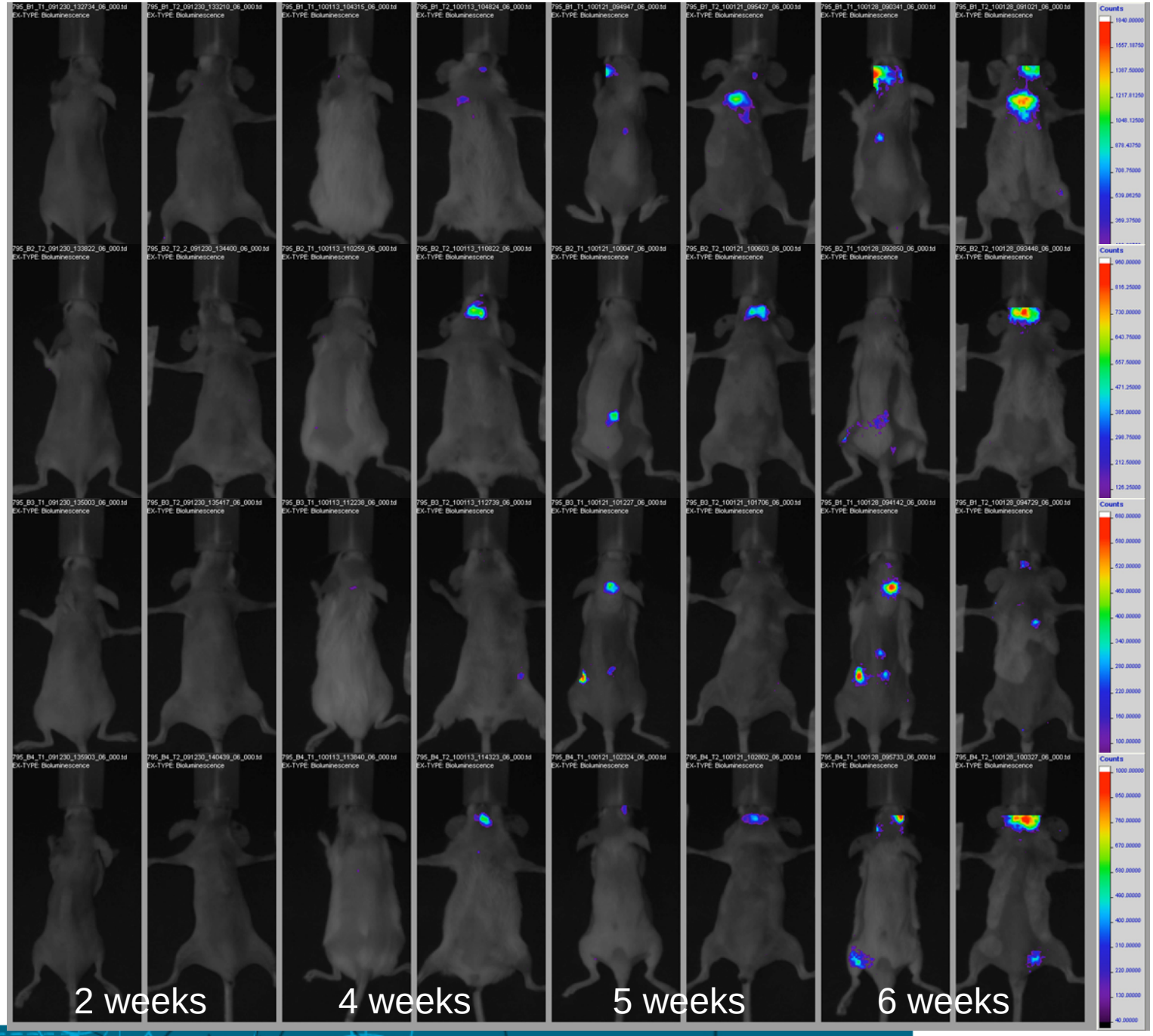
H1_GFP_Luc
Post sorted



Metastases in heart, lung and mandible after 3 weeks.
Metastases in heart, lung, mandible, brain and lymph nodes were seen after 5 weeks.

5 weeks after injections





Animal 1

Animal 2

Animal 3

Animal 4

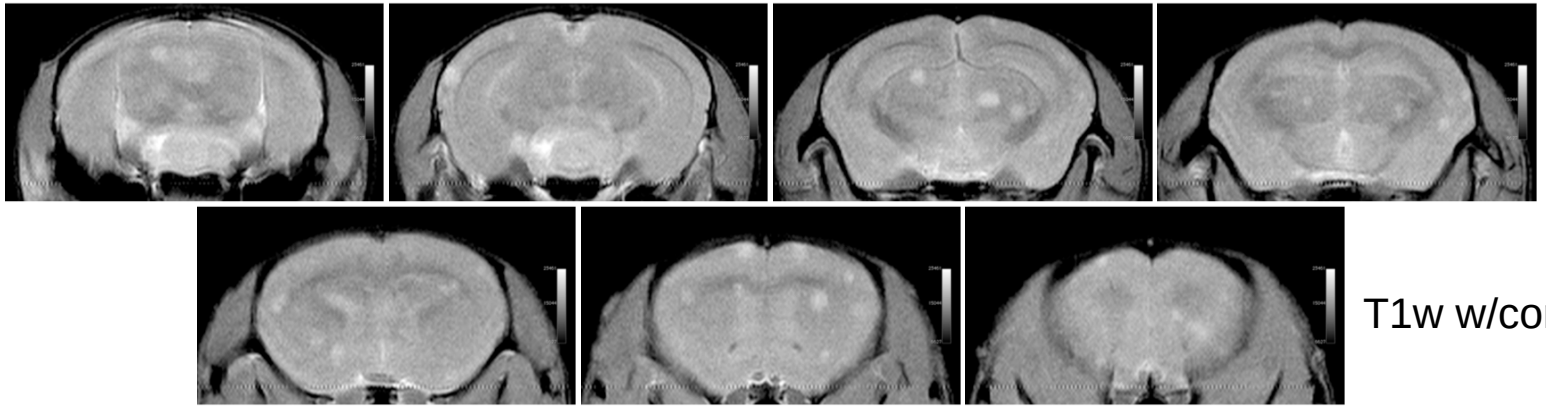
Progression of tumor metastases development

6542_B1	Week 3	Week 5
	Mandible, femur,lung	Mandible, femur, brain lung,spine,spleen/kidney

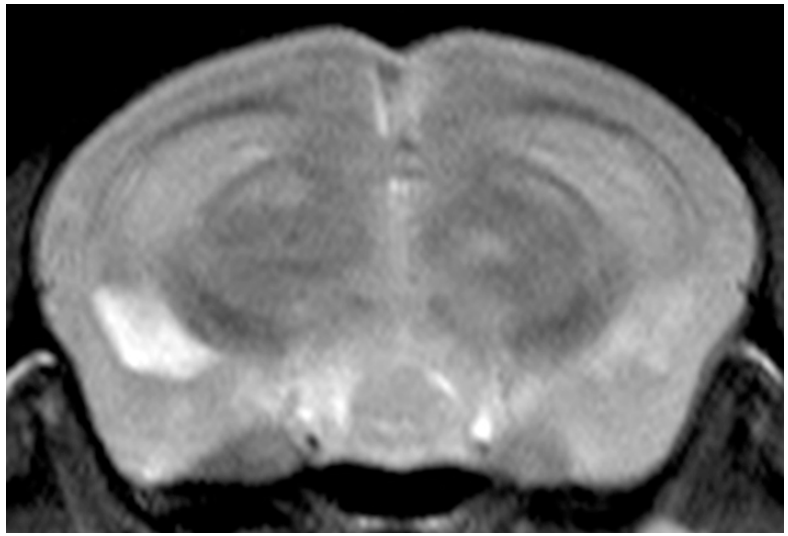
6543_B2	Week 3	Week4,5	Week6,7	Week 8
	Heart,lung/ lymph-node	Heart, lung	Heart, lung, mandible	Heart, lung, mandible, femur, spine, ovary, kidney,brain

Multiple brain metastases detected by MR

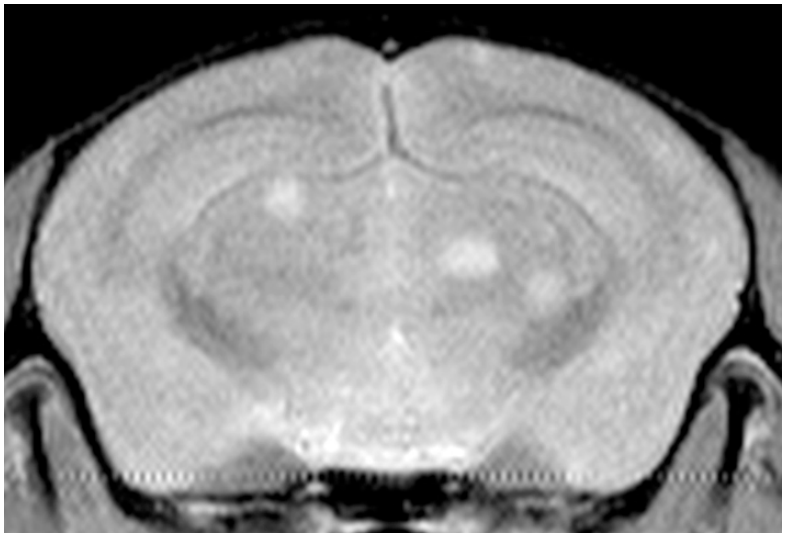
Multiple brain metastases were seen on MRI 5 weeks after injection of the H1_GFP_Luc cells.



T1w w/contrast

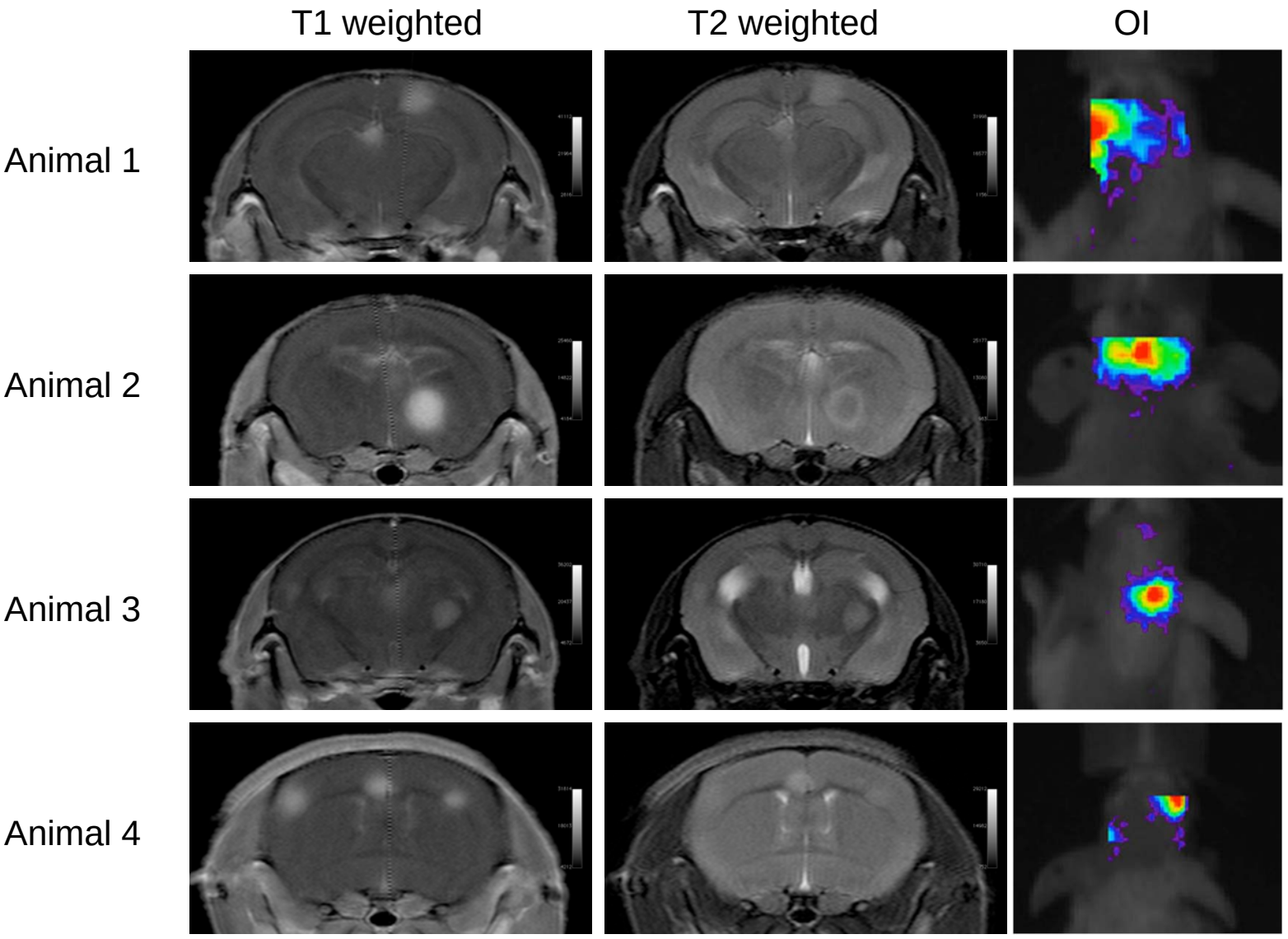


T2 weighted MR image

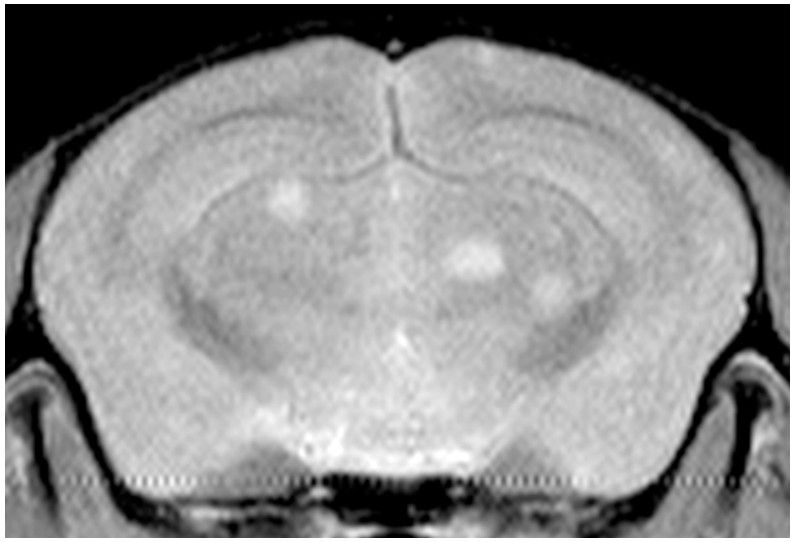


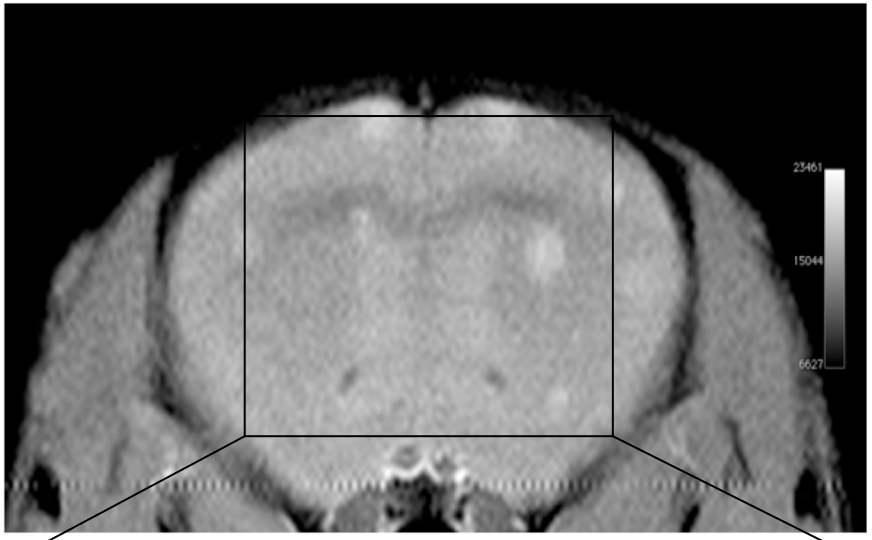
T1 weighted MR image with contrast

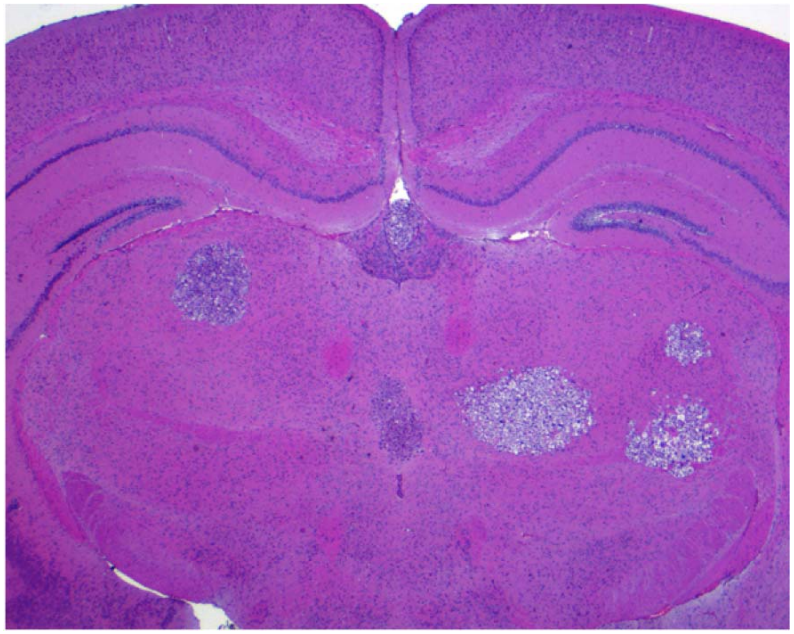
MRI 7 weeks after injections shows multiple brain mets

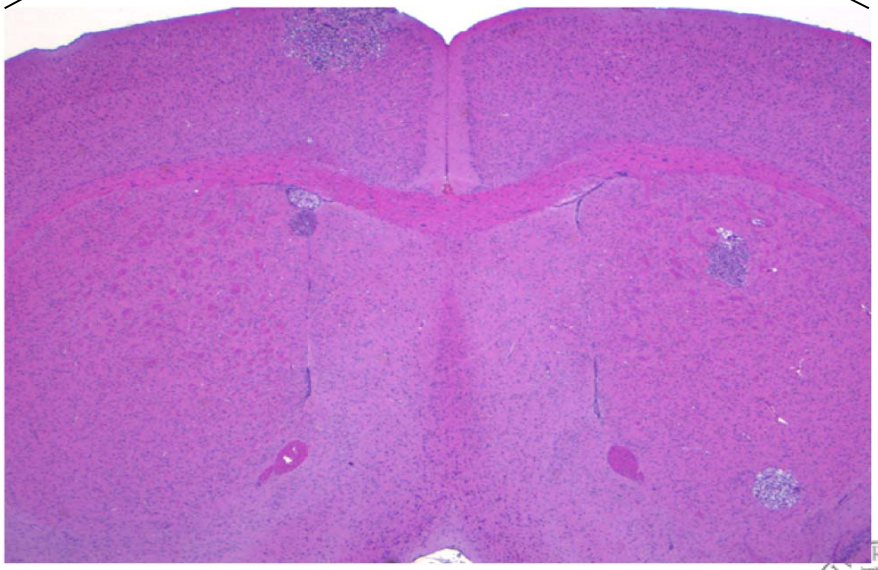


Multiple brain metastases confirmed by histology

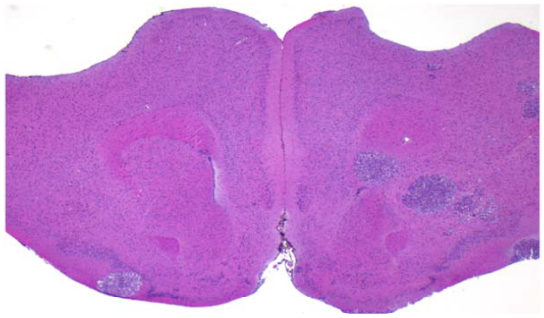




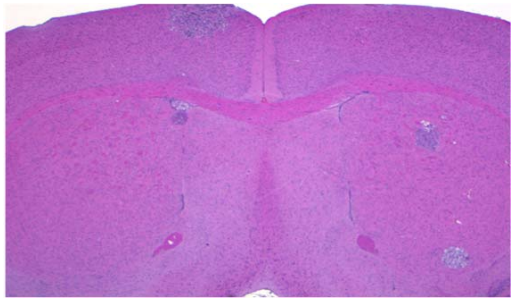




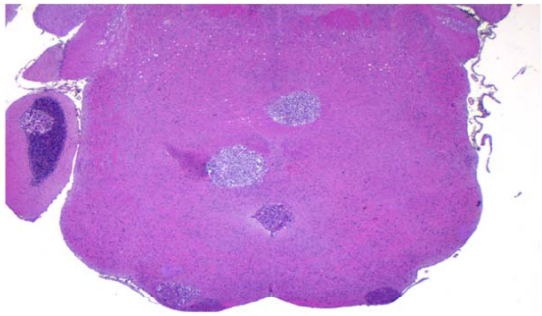
Multiple CNS metastases were seen by histology



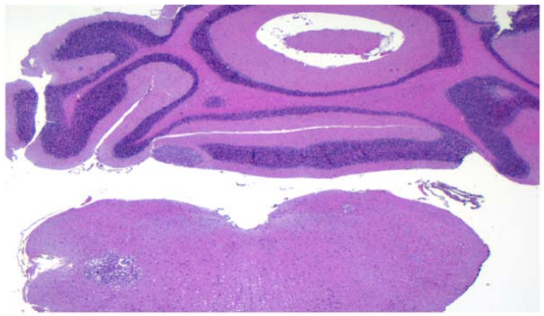
Cerebrum



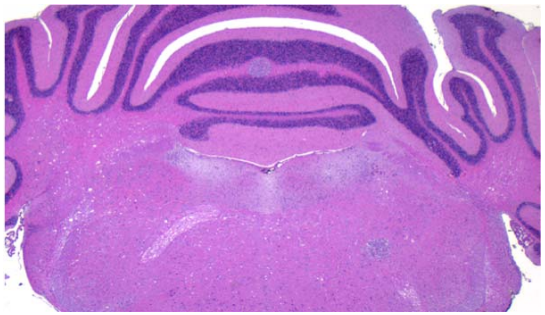
Cerebrum



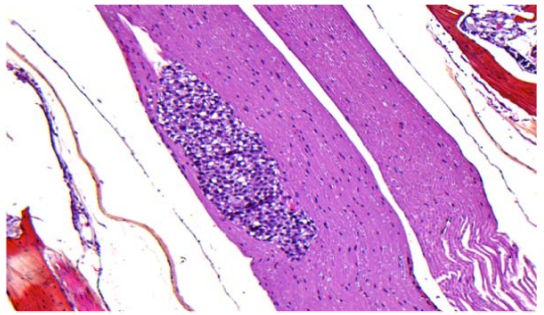
Pons



Medulla



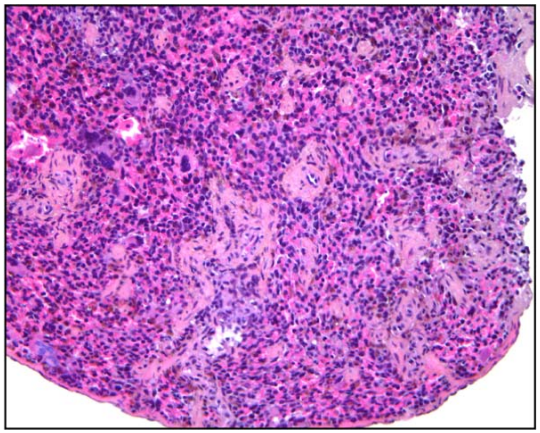
Cerebellum

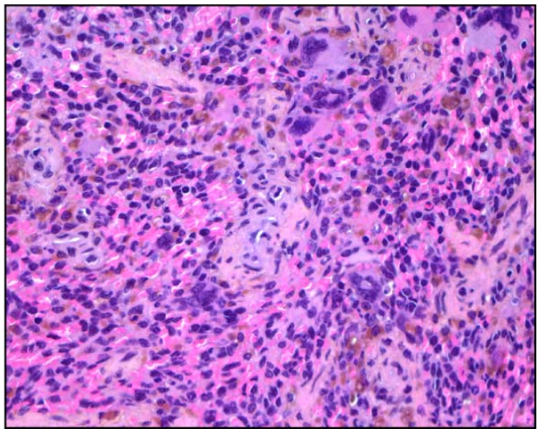


Spinal cord

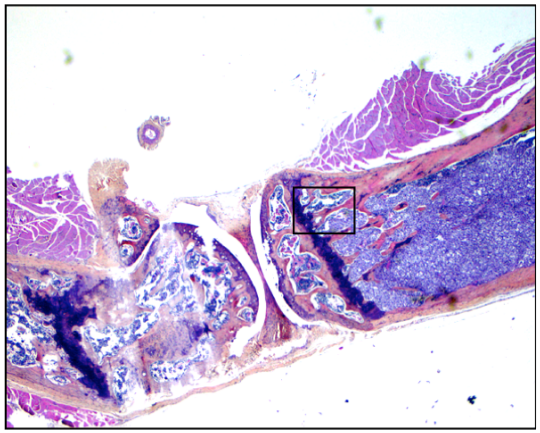
Multiple metastases were detected also in other organs

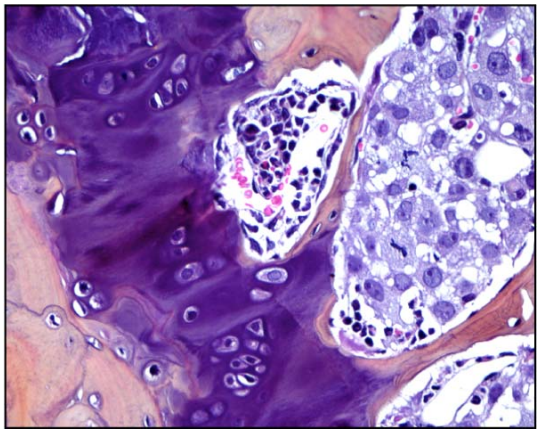
Spleen



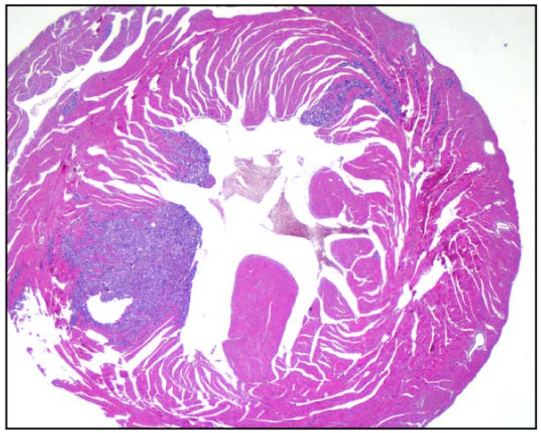


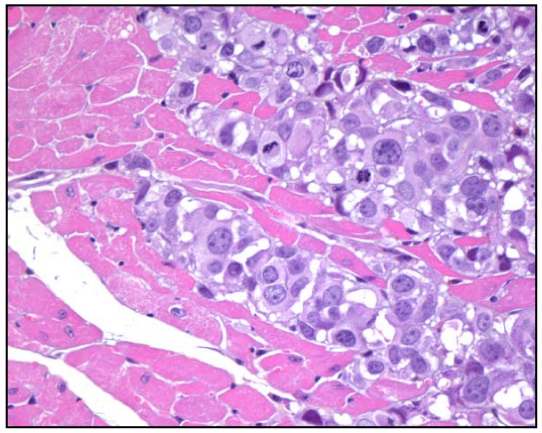
Os ilium





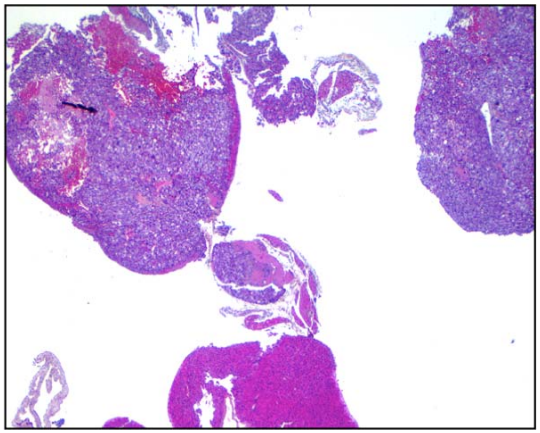
Heart

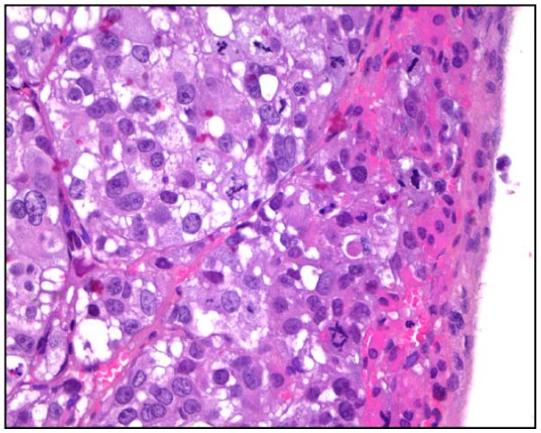




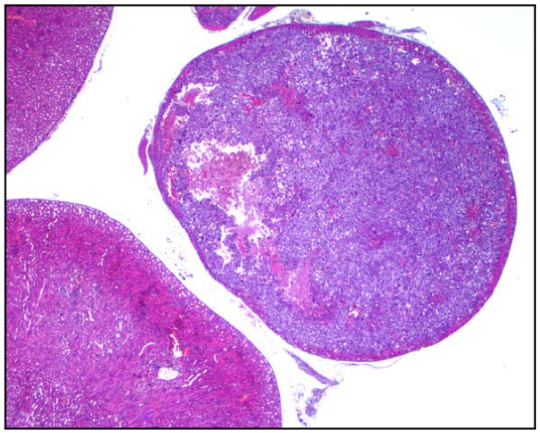
Multiple metastases were detected also in other organs

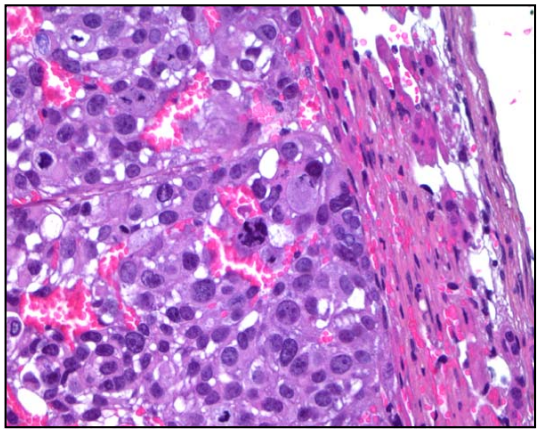
Liver hilus



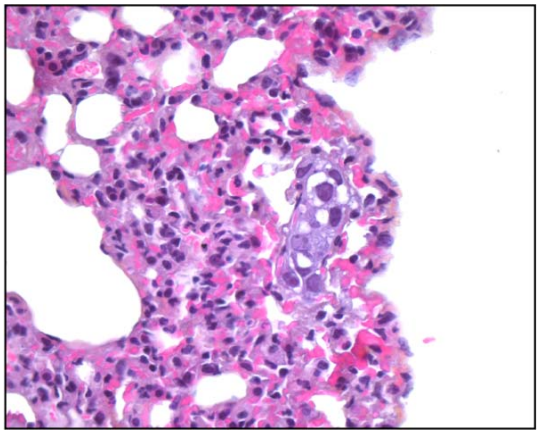


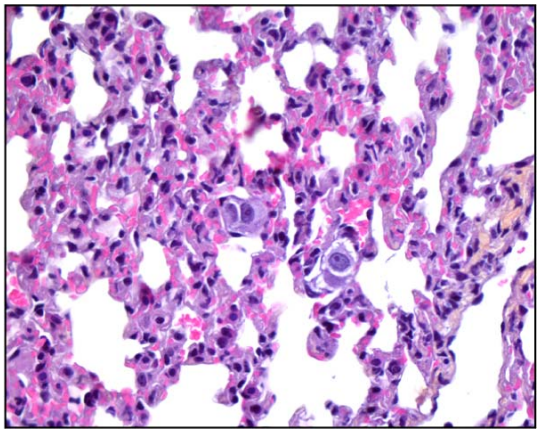
Adrenal



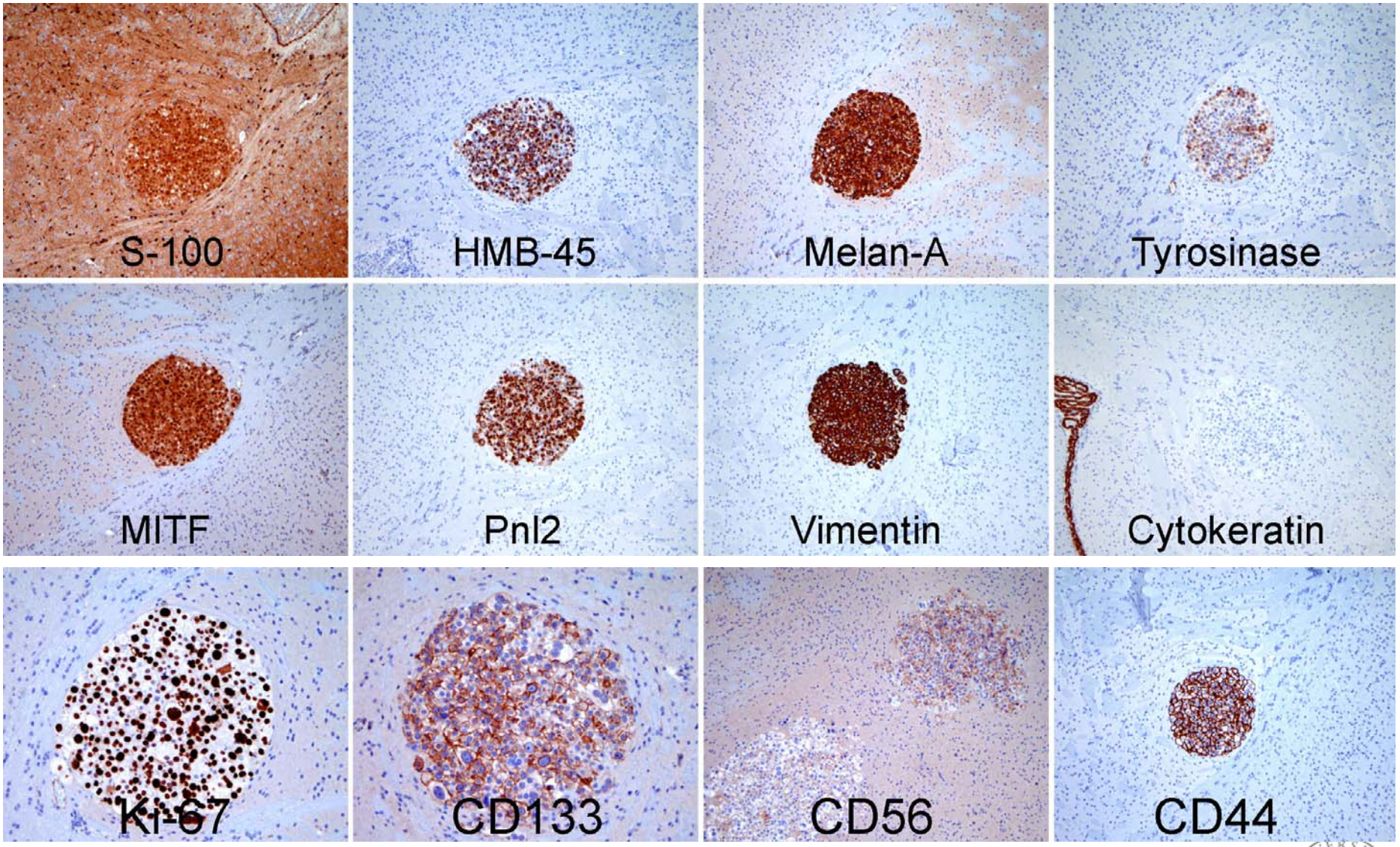


Lung

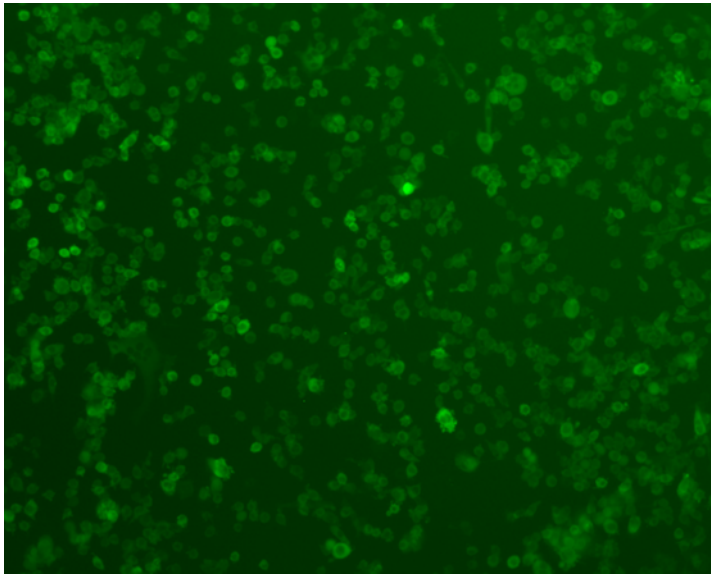




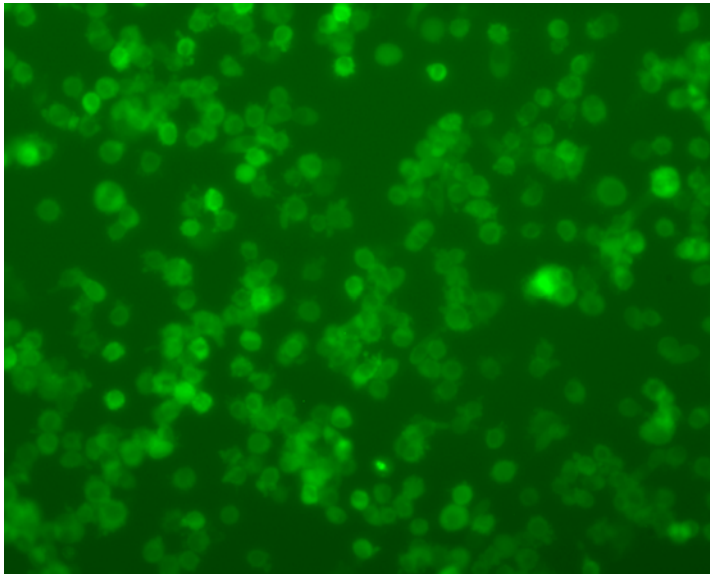
Expression of tumor markers in brain metastases



Ovary metastasis cells show strong GFP expression



6543_B2_ Right_Ovary_10X

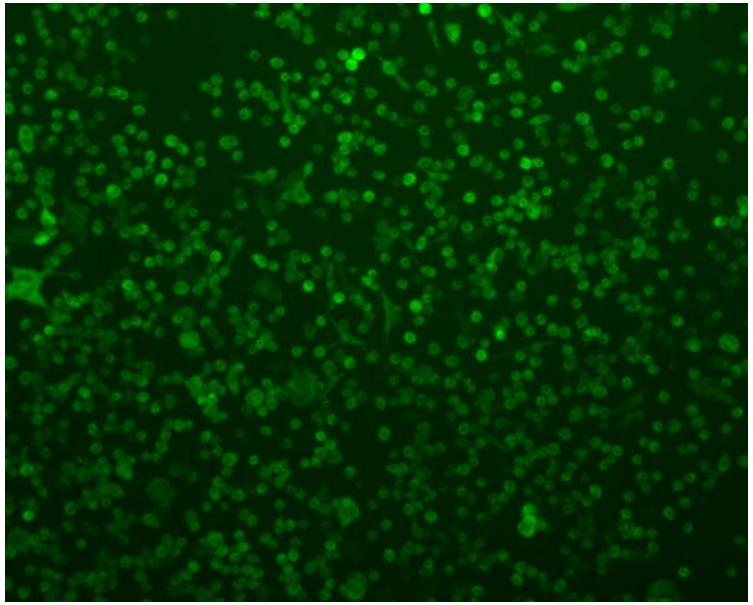


6543_B2_ Right_Ovary_20X

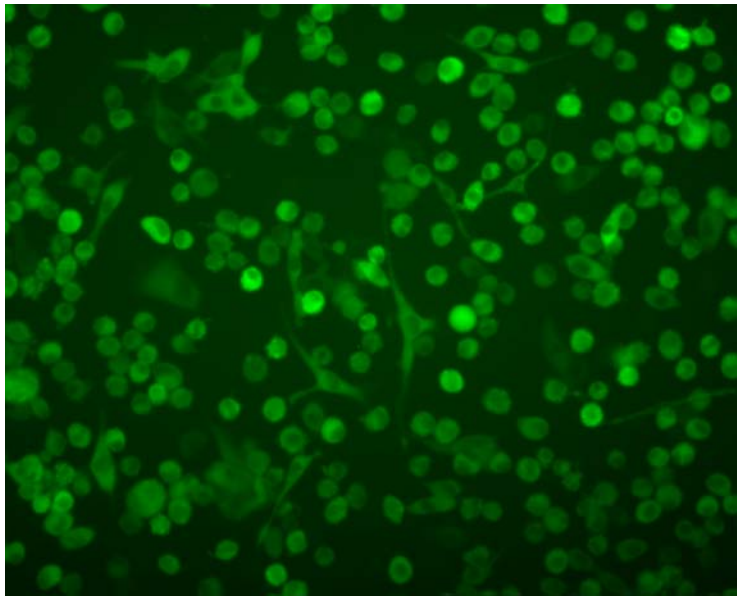
Ovary metastasis cell line shows strong GFP expression, similar to the parent H1_GFP_Luc cell line

Ovary cell line established after 2 weeks
Spinal cord and femur metastasis cell lines established after 4 weeks

Generation of cell line from mouse lung metastasis



6543_B2_ Right_Lung_10X



6543_B2_ Right_Lung_20X

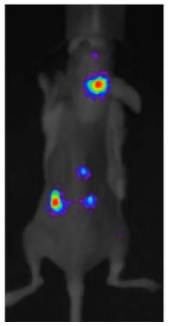
Lung metastasis cell line shows strong GFP expression, similar to the parent H1_GFP_Luc cell line

Lung metastasis cell line established after 1 week

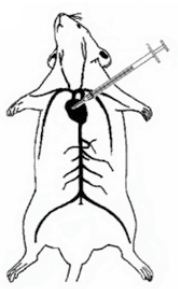
Genetic analysis of tumors developing in different organs

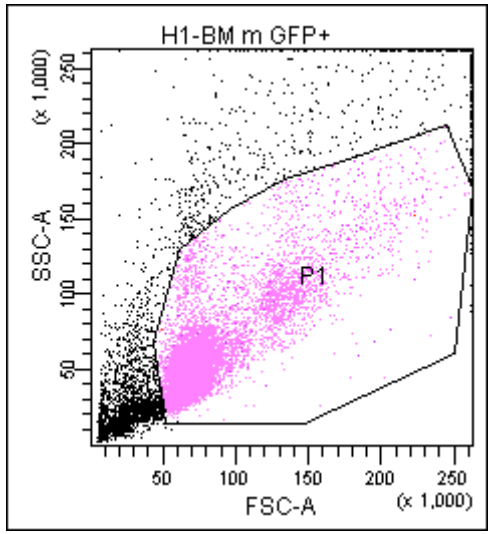
Do the melanoma brain metastatic cells also populate other organs than the brain?
If yes, is it possible to determine common/differentially expressed genes in the process?

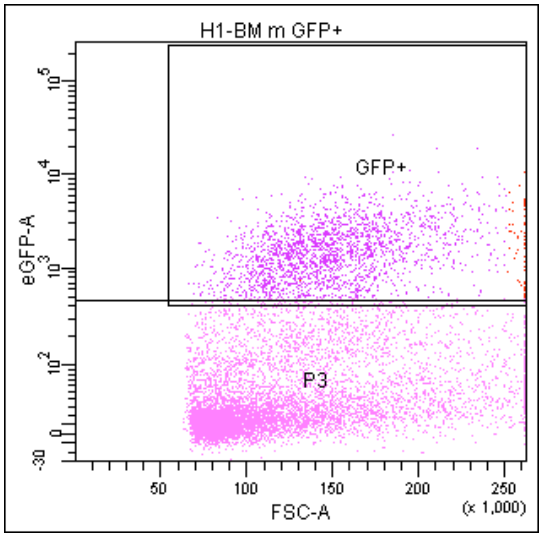
Strategy:

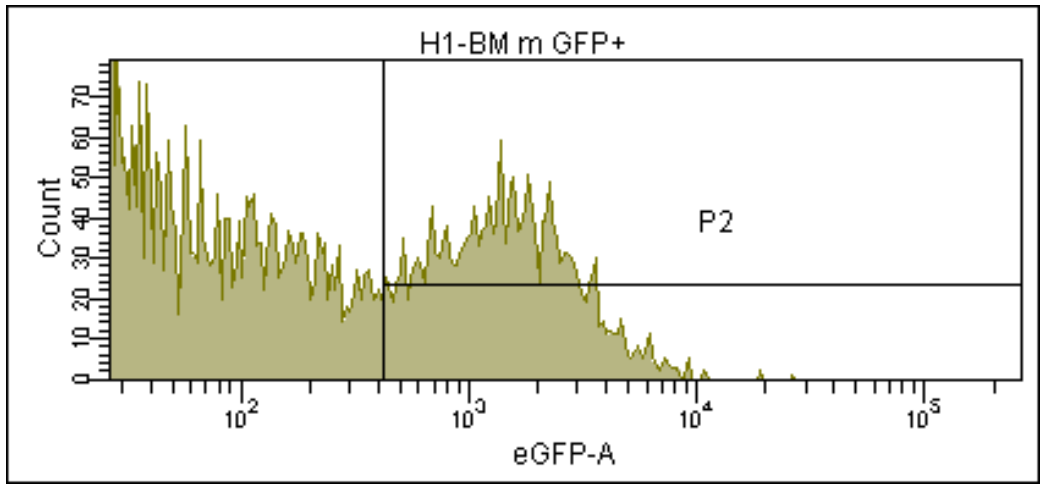


Brain
Bone marrow
Lung
Liver







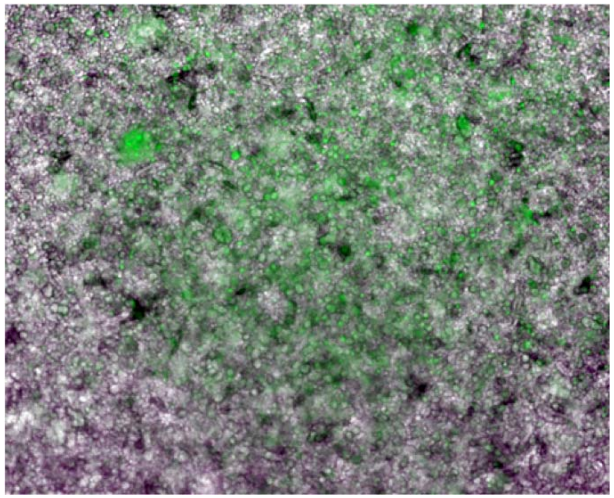


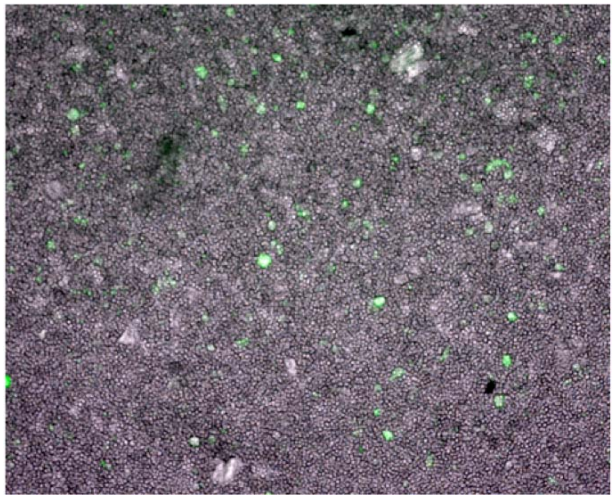
Organs are currently being dissociated and sorted by FACS

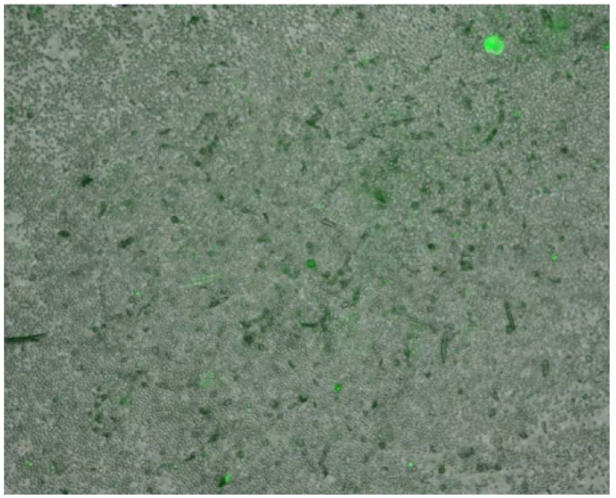
Liver cells presorted

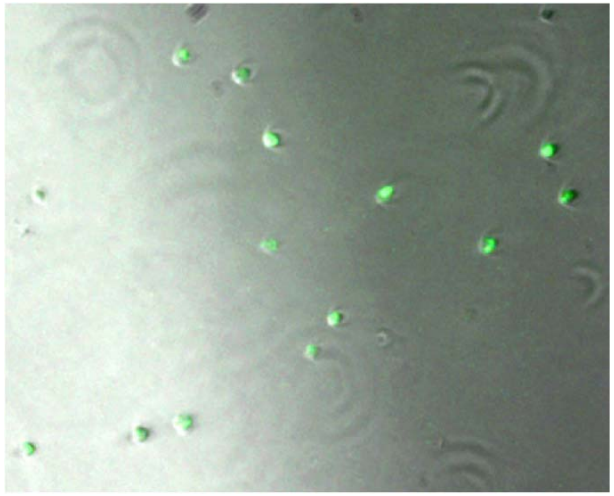
Bone marrow cells presorted

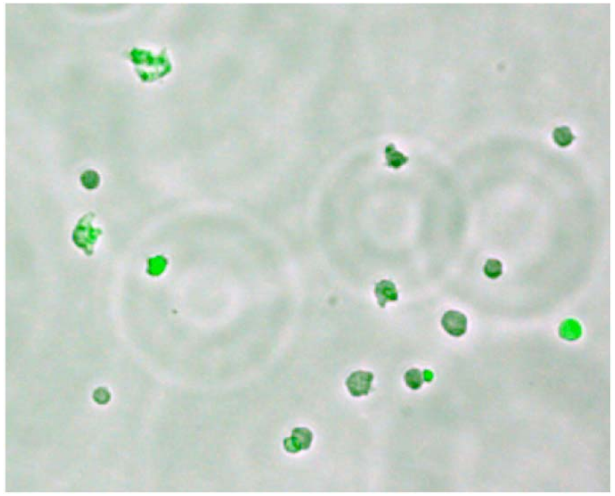
Lung cells presorted

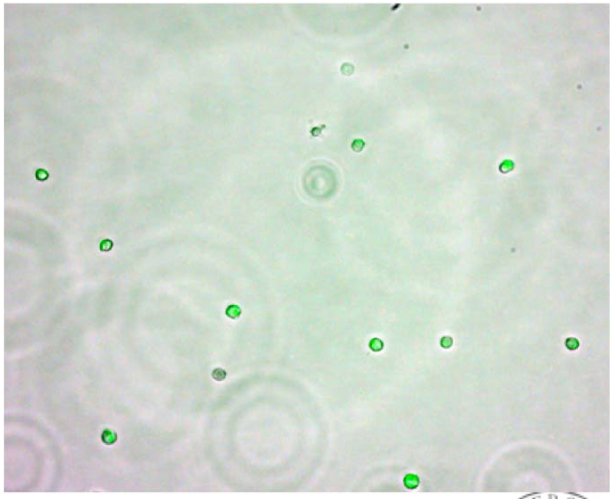












Liver cells postsorted

Bone marrow cells postsorted

Lung cells postsorted

1. Introduction to malignant brain tumors.
2. Rat model: Intracranial implantations of human brain metastasis.
3. Mouse model: Intracardial injections of human melanoma cells.
4. Mouse model: Intradermal injections of human melanoma cells.
5. Can single tumor cells be tracked in vivo?

Intradermal injections of H1_GFP_Luc cells

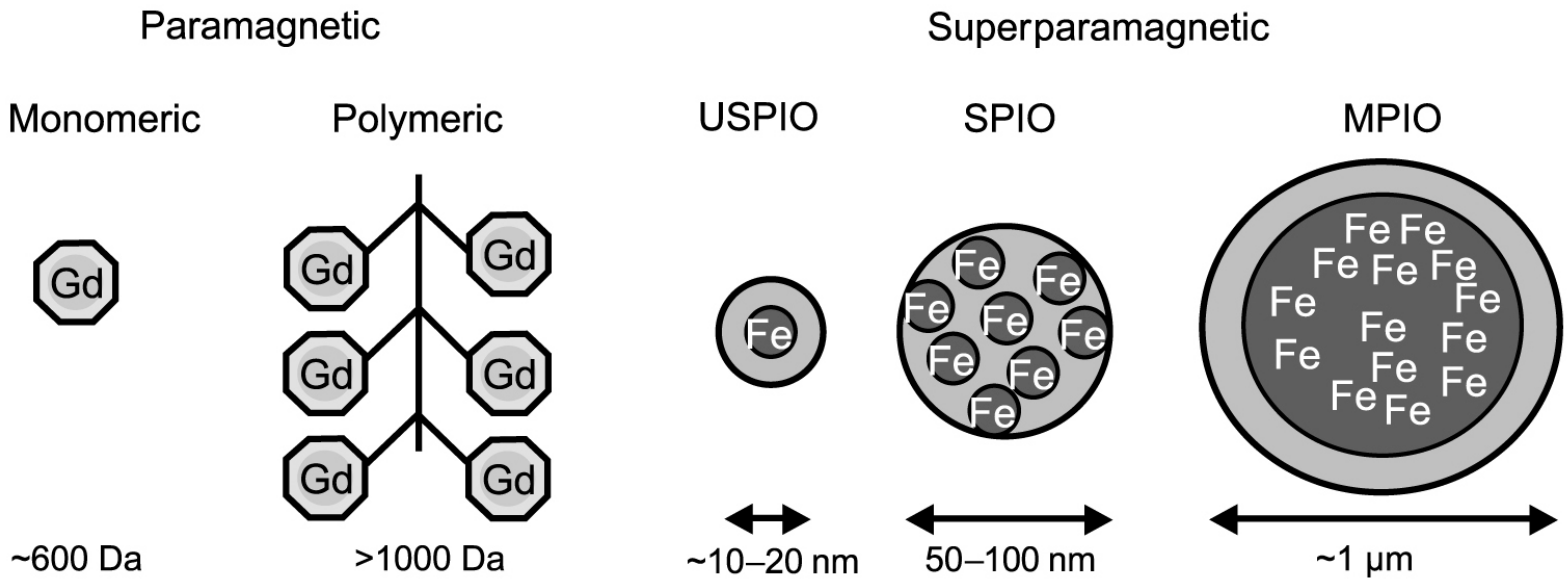
Injections Jan 4th 2010
0.5 million cells/100 µl
7 animals

→ Experiments ongoing

1. Introduction to malignant brain tumors.
2. Rat model: Intracranial implantations of human brain metastasis.
3. Mouse model: Intracardial injections of human melanoma cells.
4. Mouse model: Intradermal injections of human melanoma cells.
5. Can single tumor cells be tracked in vivo?

Cellular MR Imaging

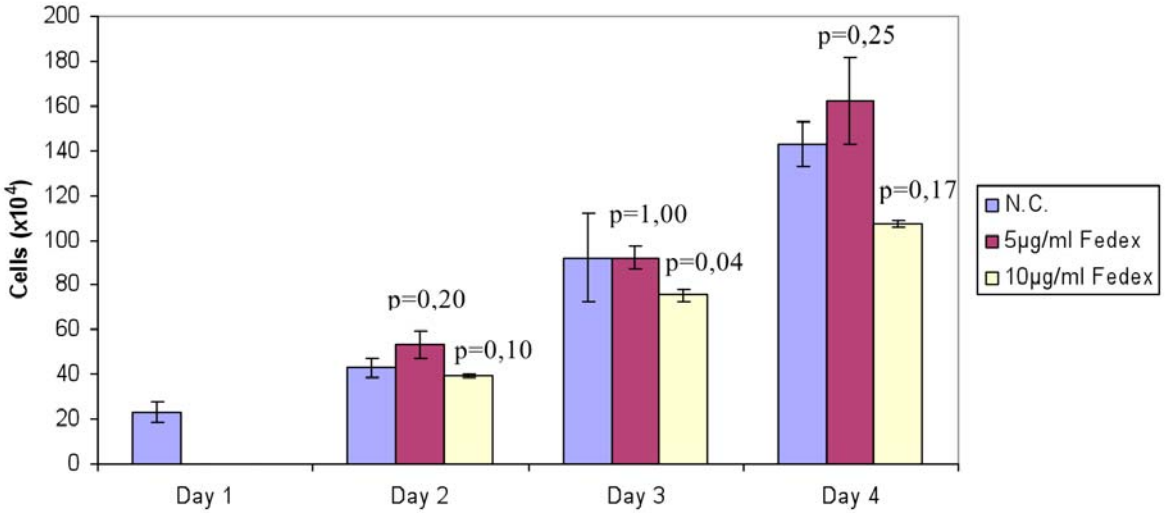
For cellular MR imaging, cells need to be labeled with MR contrast agents in order to make them stand out from surrounding tissue. The phagocytic activity of certain cell types allows the incorporation of MR contrast agents to visualize their traficking in vivo.

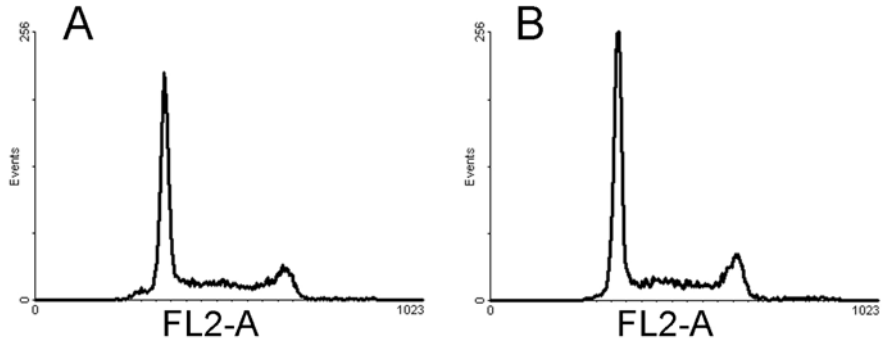


Modo M et al, Mol Imaging 2005

In vitro cell proliferation study

Performed on U251 and U373 glioma cells, and H1 metastatic melanoma cells.

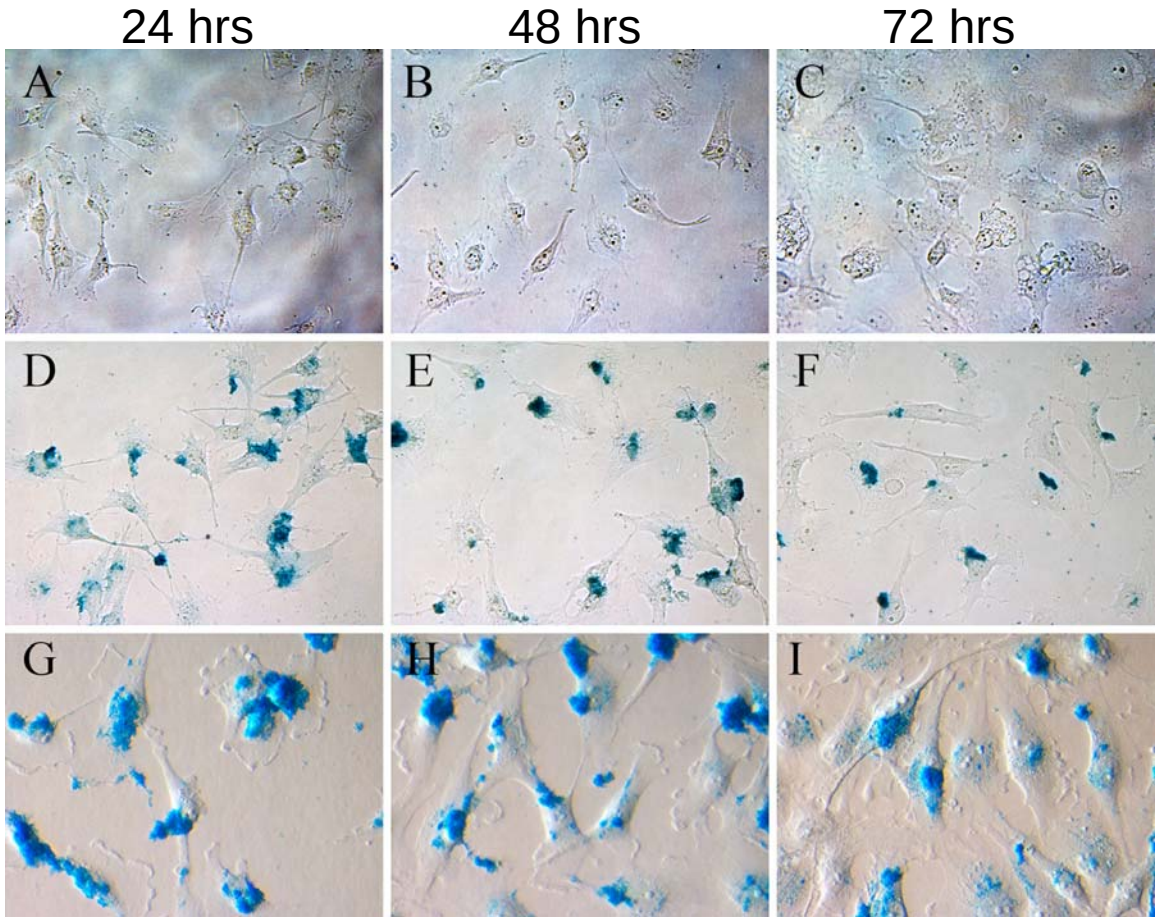




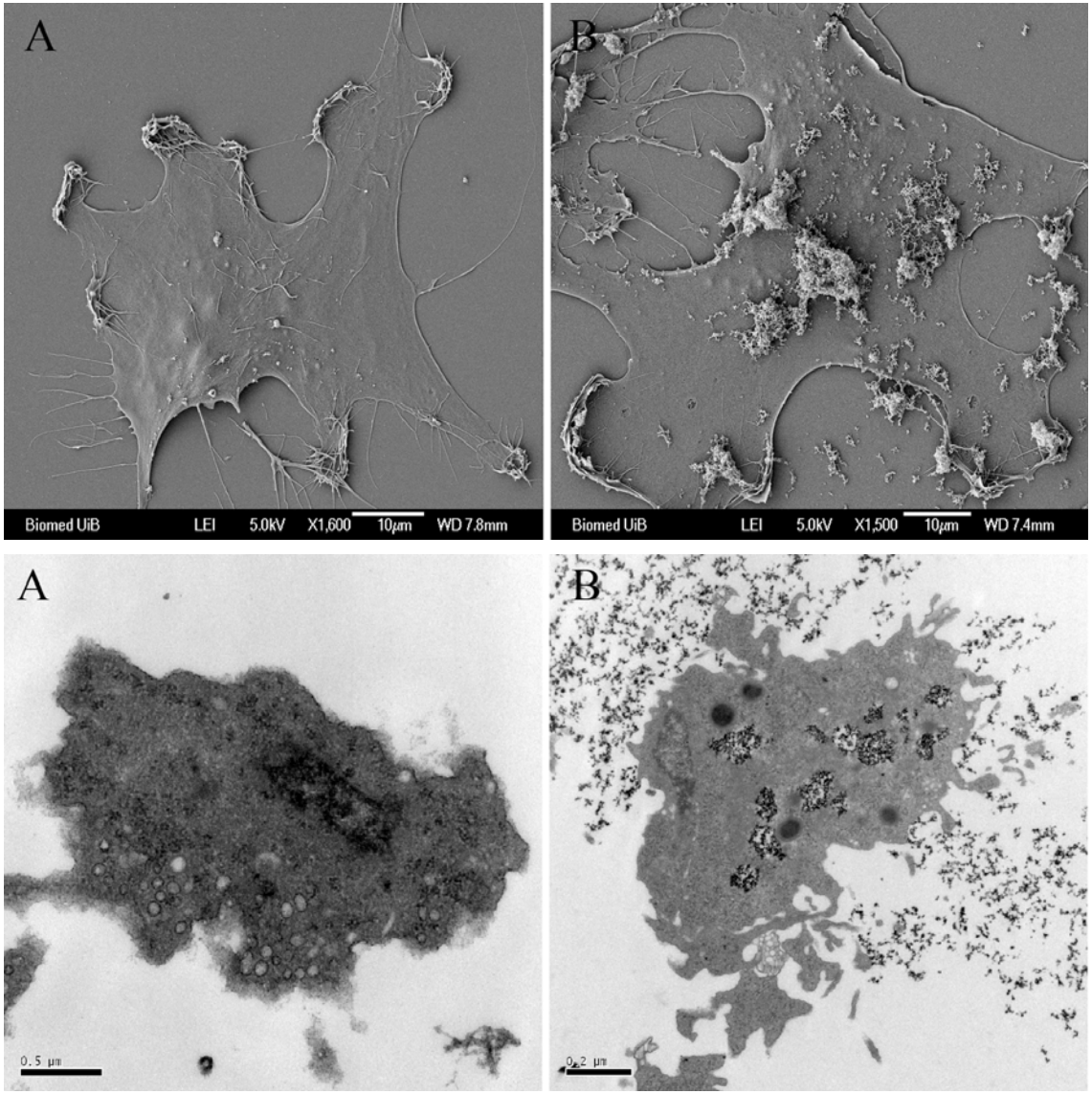
	G_0 phase	S phase	G_2M phase
U251 control #1	47.0 %	49.7 %	3.3 %
U251 control #2	50.4 %	48.5 %	1.1 %
U251 Fedex	51.2 %	45.0 %	3.8 %

In vitro Fedex labeling of tumor cells

Labeling of U251 and U373 glioma cells, and H1 metastatic melanoma cells.

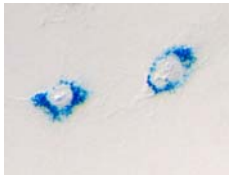


SEM and TEM study of labeled tumor cells

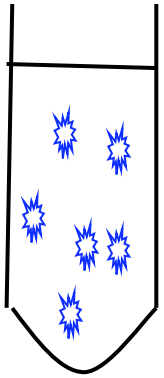


ndorf tube

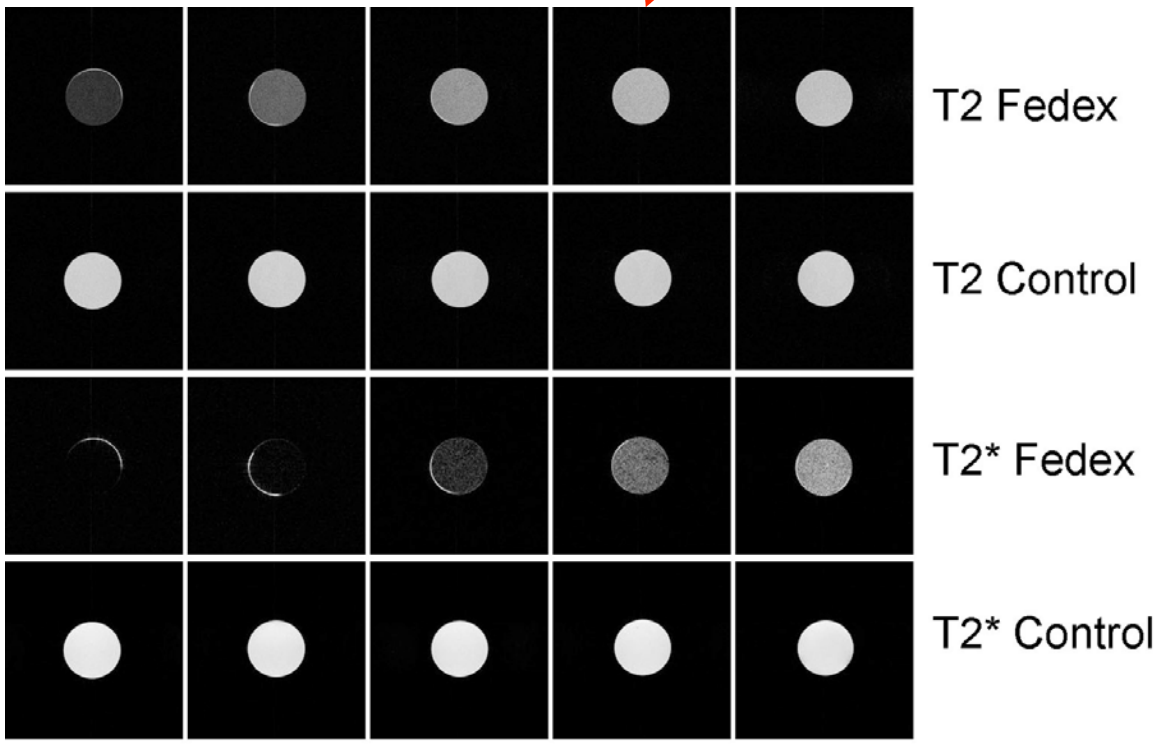
In vitro T2 Mapping of Tumor cells



24 hrs labeling

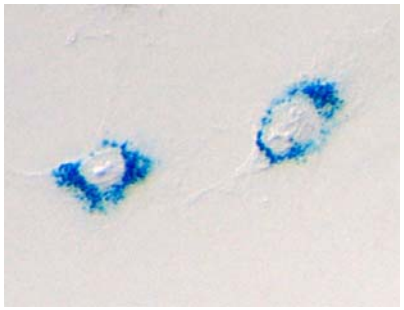


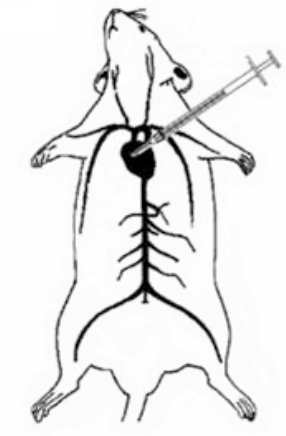
#cells	T_2 NC	T_2 Fedex
4000	68,5	13,4
2000	67,3	20,2
1000	62,8	31,2
500	61,9	42,4
250	61,5	51,5
150	63,9	48,9
100	65,4	54,2
50	64,0	59,7



In vivo MRI at single-cell level in a mouse model of melanoma cancer metastases to the brain

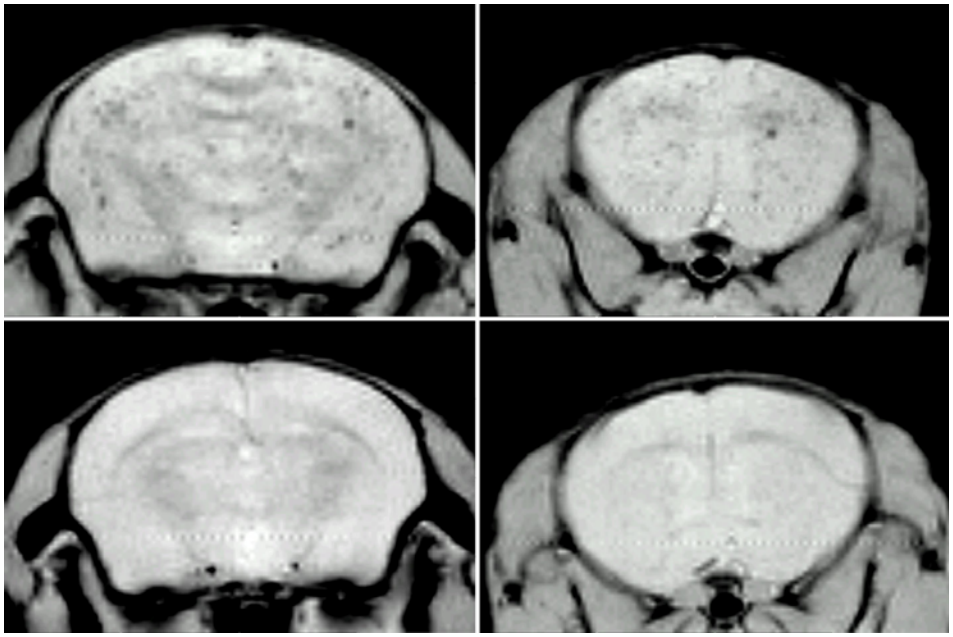
10 μg/ml Fedex for 24 hrs





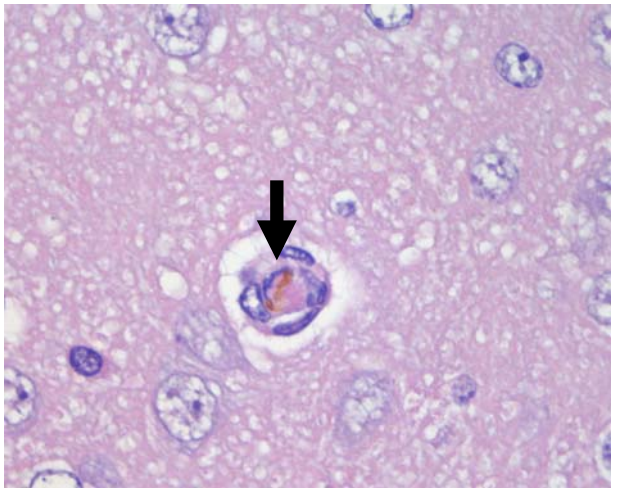
Intracardial injection of 1 mill cells

Detection of single cells 24 hrs after injection

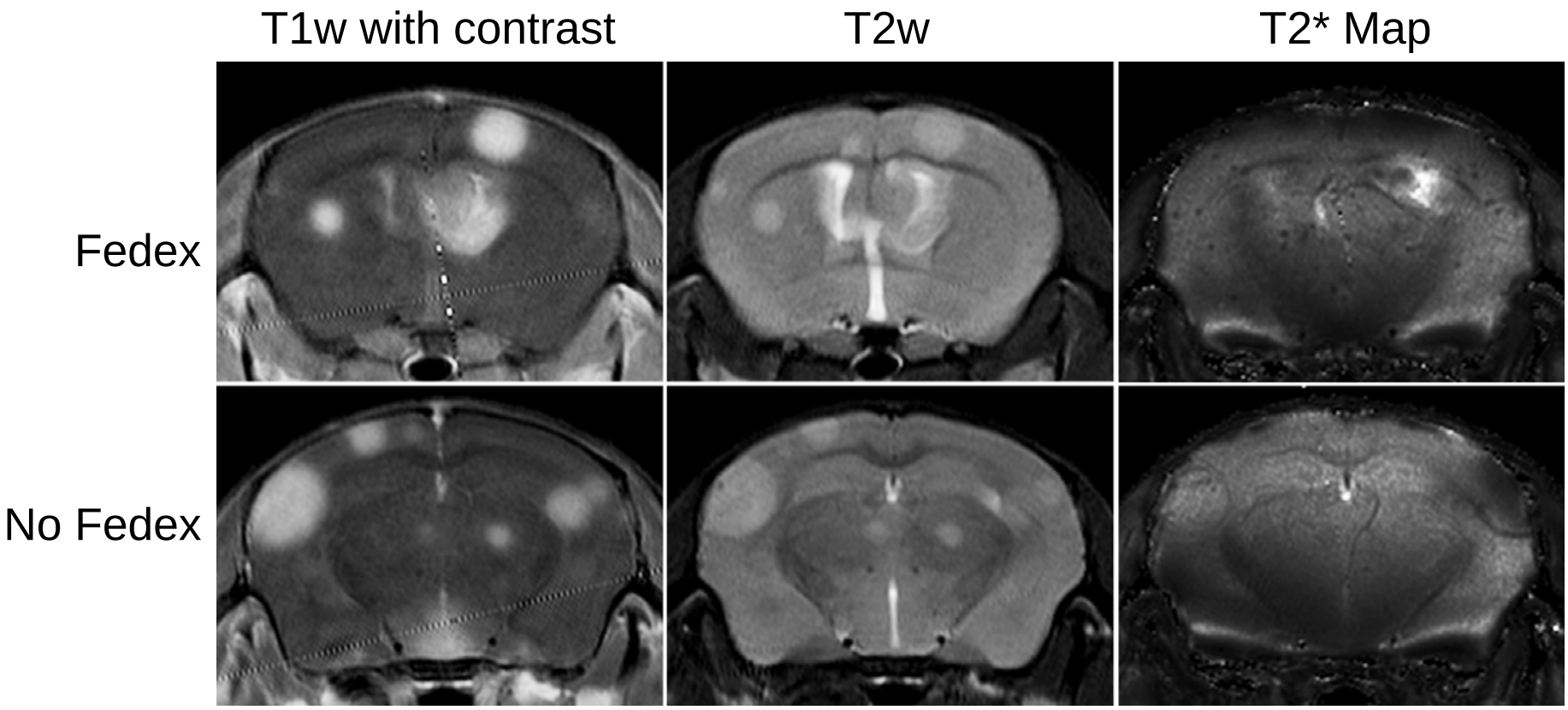


Fedex

No Fedex



Multiple brain metastases developed 7 weeks after injections



Between 20 and 30 metastases were observed by MR imaging
7 weeks after intracardial injections of the melanoma cells.

F. Thorsen, Unpublished 2010

Conclusions and future work

A new metastases growth model in the nude rat has been established, where human tumor tissue is operated directly into the animal brain.

The characterisation study shows that the animal brain metastases are similar to the patient brain metastases.

Future work: Treatment studies (Avastin, Gamma Knife)

We have established a model for studying the metastatic process of melanomas (H1 cell lines).

The H1 cell lines were tumorigenic when implanted intracranially, subcutaneously, or injected intracardially.

Future work: Repeat intracardial injections on larger number of mice. Genomic analysis, study of which ECM components are important for tumor cell migration and invasion.

Acknowledgements

Department of Biomedicine, UiB
Rolf Bjerkvig
Inderjit Daphu
Ingvild Wendelbo
Jian Wang
Jim Lorens
David Mickelm
Crina Tiron
Anja Torsvik
Agnete Svendsen
Audun Dahlberg

Department of Pathology,
Haukeland University Hospital
Heike Immervoll

Department of Internal Medicine
Haukeland University Hospital
Emmet Mc Cormack

Flow Cytometry Core Facility
MIC
Marianne Enger

Department of Neurosurgery,
Haukeland University Hospital
Paal-Henning Pedersen