

Orthotopic xenograft model why and how ?

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Inserm U 965 :

angiogenesis and translational research

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Second European Course of in vivo preclinical assays in cancer Therapy

Definition

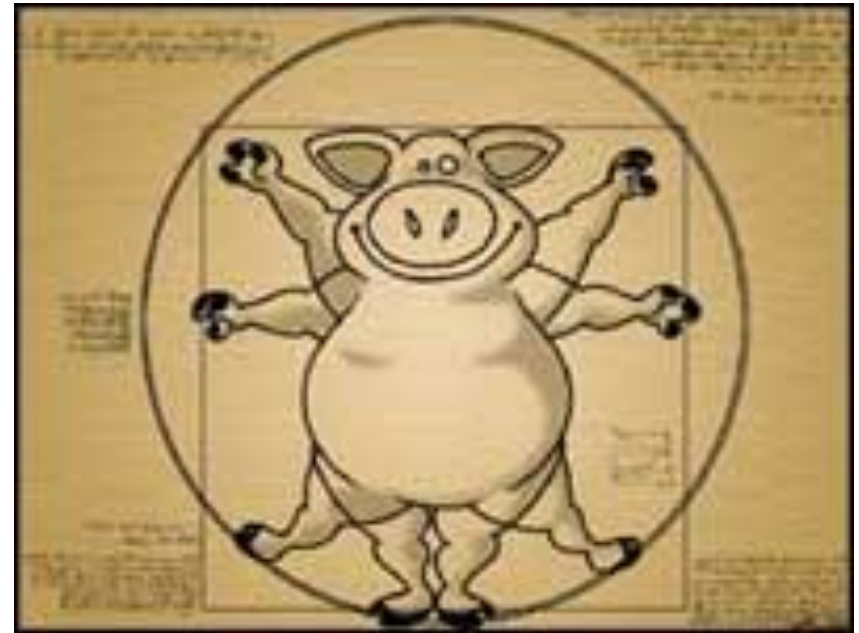
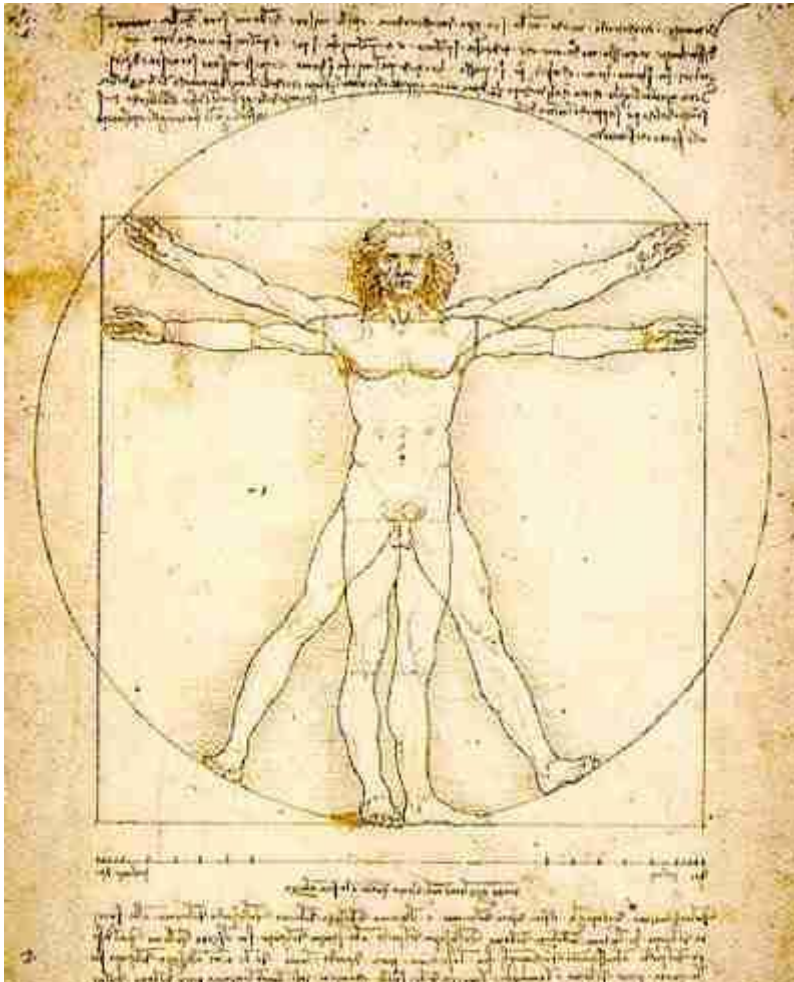
- **Orthotopic** : same place as in human
- **Xenograft** : cell or tumor fragment
 - Human cell and specific clones
 - Human tumor :
 - direct surgical or biopsy specimen
 - or derived from subcutaneous injection of cells
 - Animal cancer cell
 - Associated material (radioactive, fluorescent, nano technology, etc)



Correct for melanoma

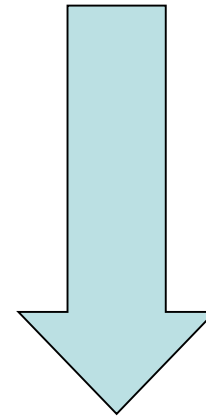
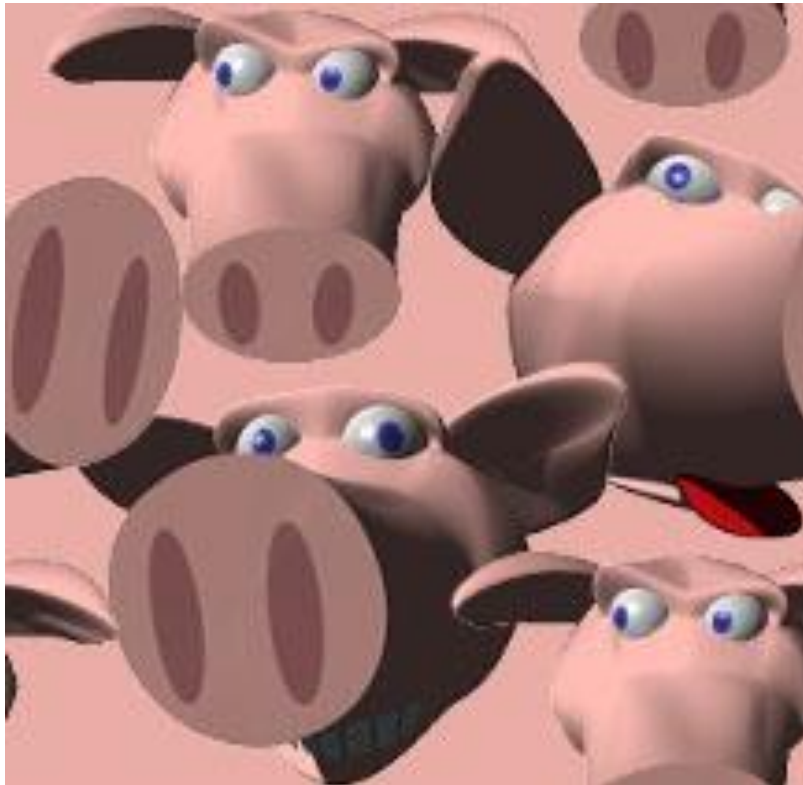
Exclusion of the stem cell procedure derived from a kidney graft

Definition



**Highly physiological mouse model
that closely resembles diverse cancer in humans**

Same cells, same animal, same surgical graft procedure



**Reproducibility
Standardization
Statistical analysis**

For all cancers ?

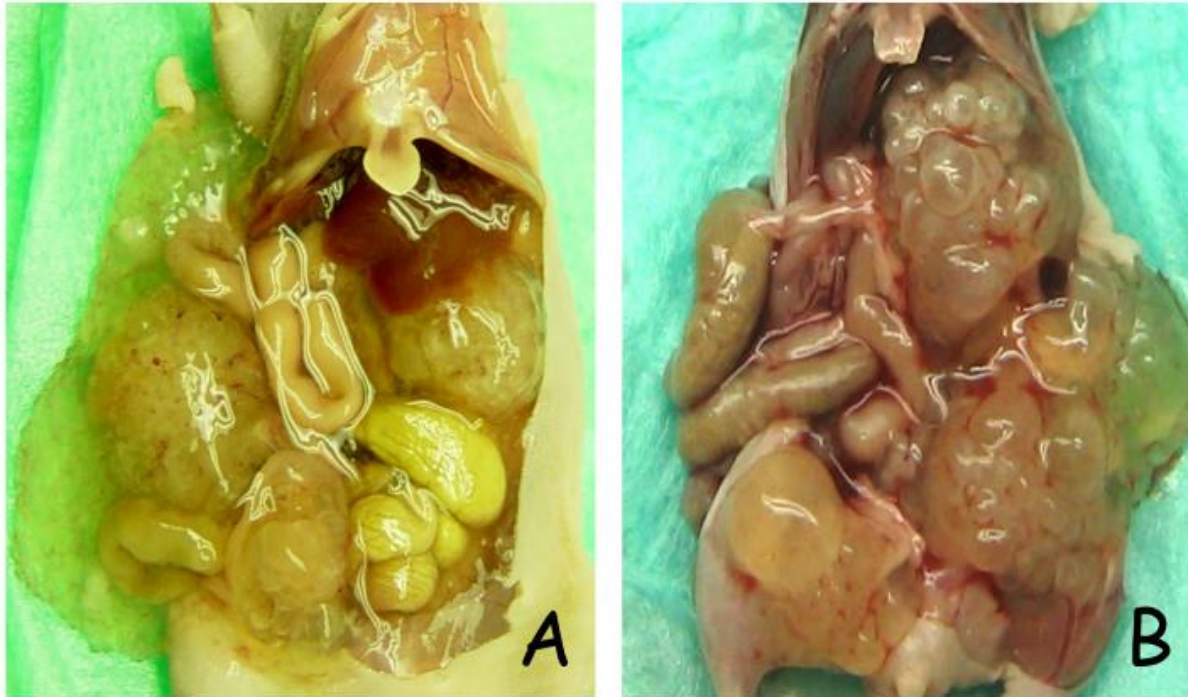
Liver
Brain
Chest
Bone
Colon
Rectum
Prostate
Kidney
Breast
Pancreas
Stomach
Bladder
Skin
Ovary

Create a primary tumor

Correlation between any biological specificity (gene function) and a specific tumor behavior

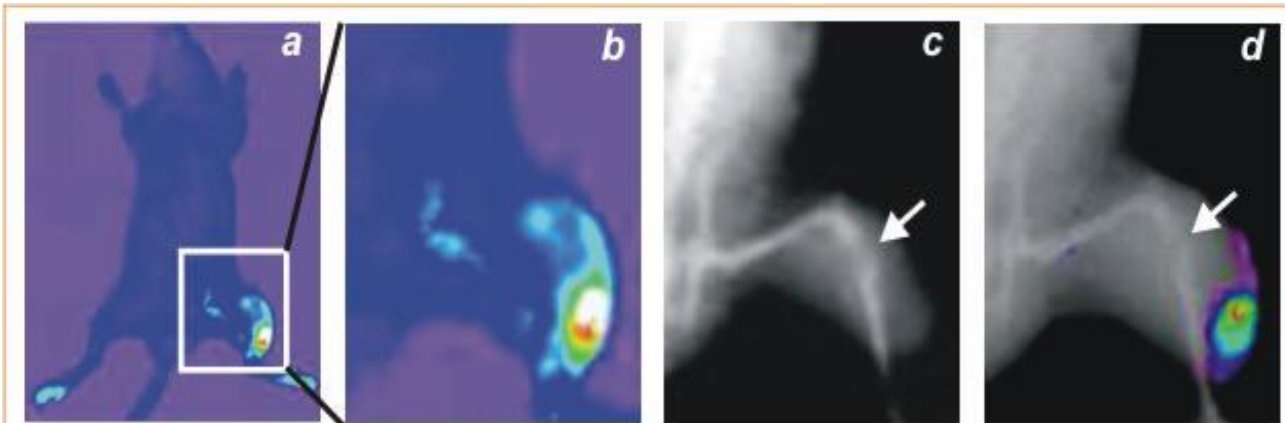
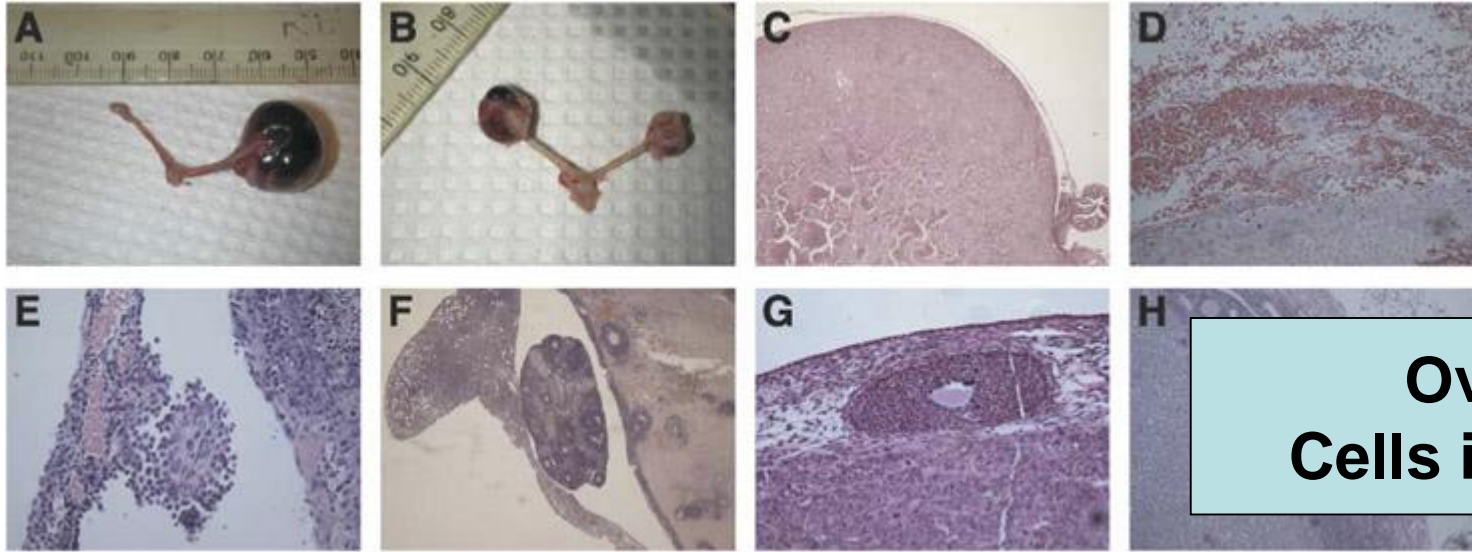
-Tumor growth

- metastasis process



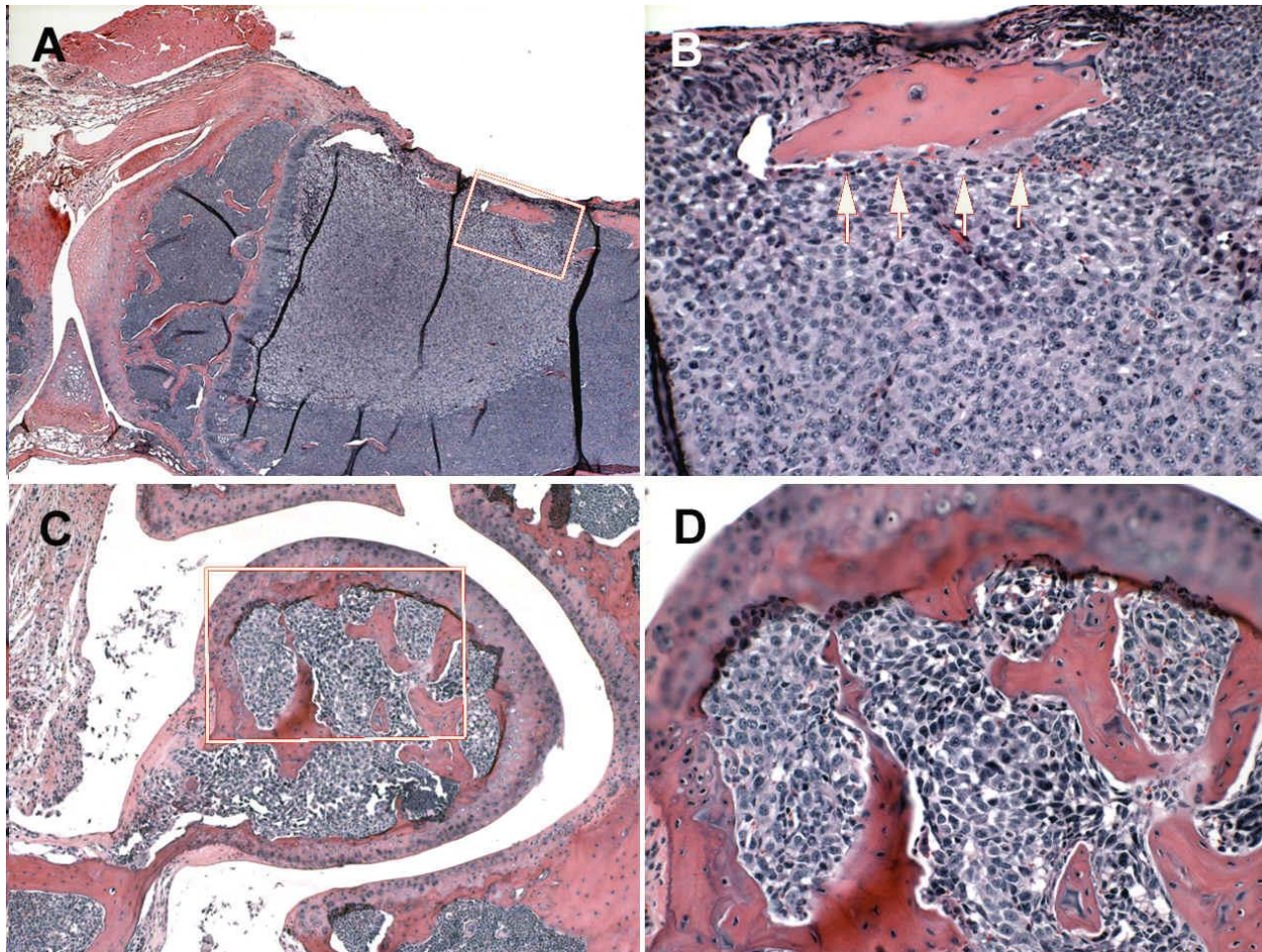
**The only solution to obtain tumor growth
– because in vitro cell culture failed
Rare carcinomatosis : pseudomyxoma**

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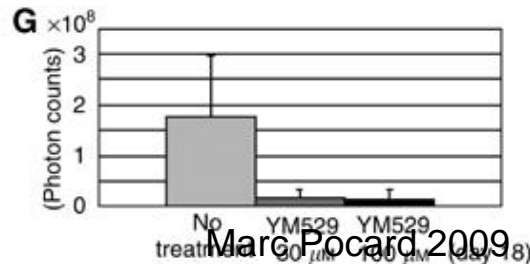
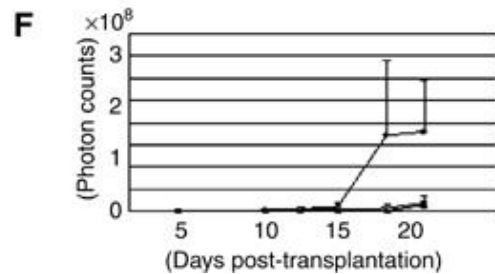
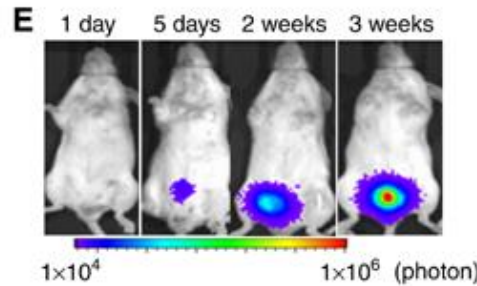
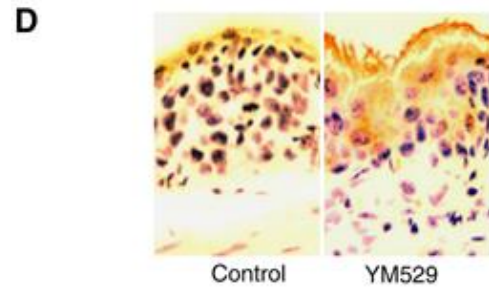
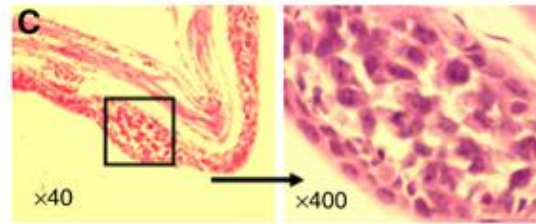
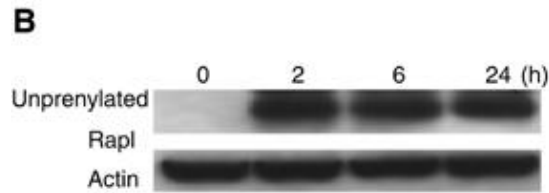
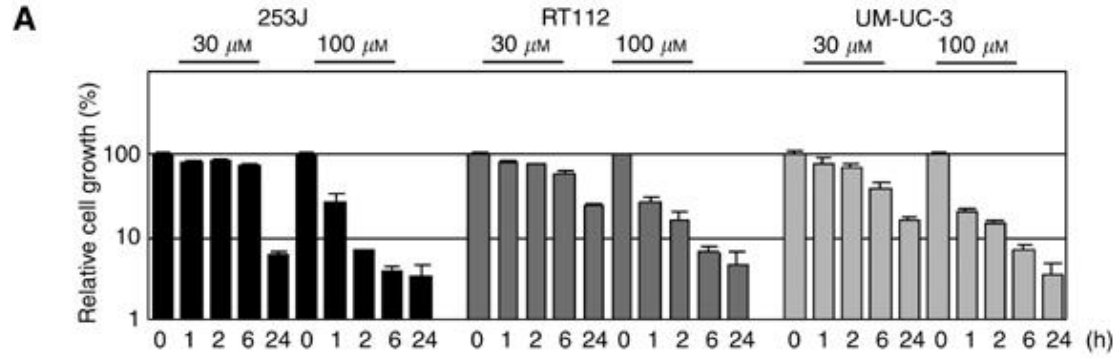
Osteosarcoma

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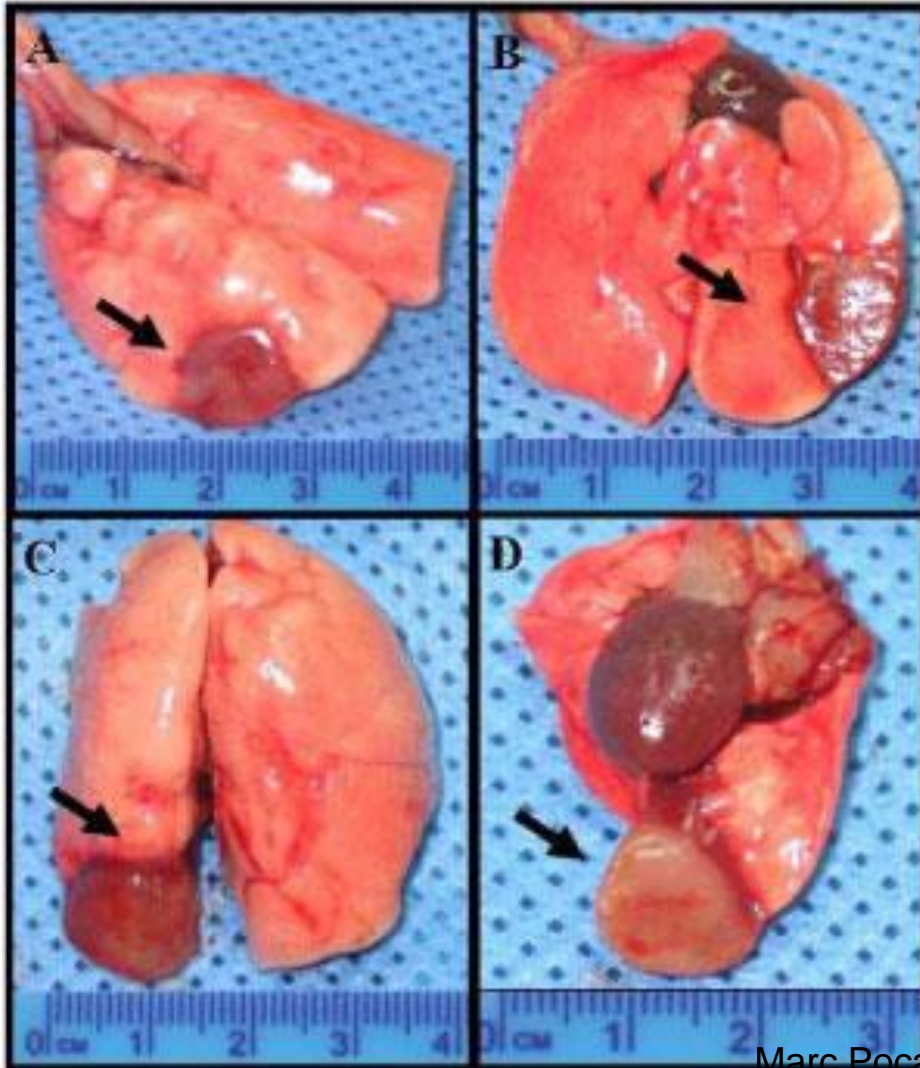


Osteolytic Bone Metastases (orthotopic injection):

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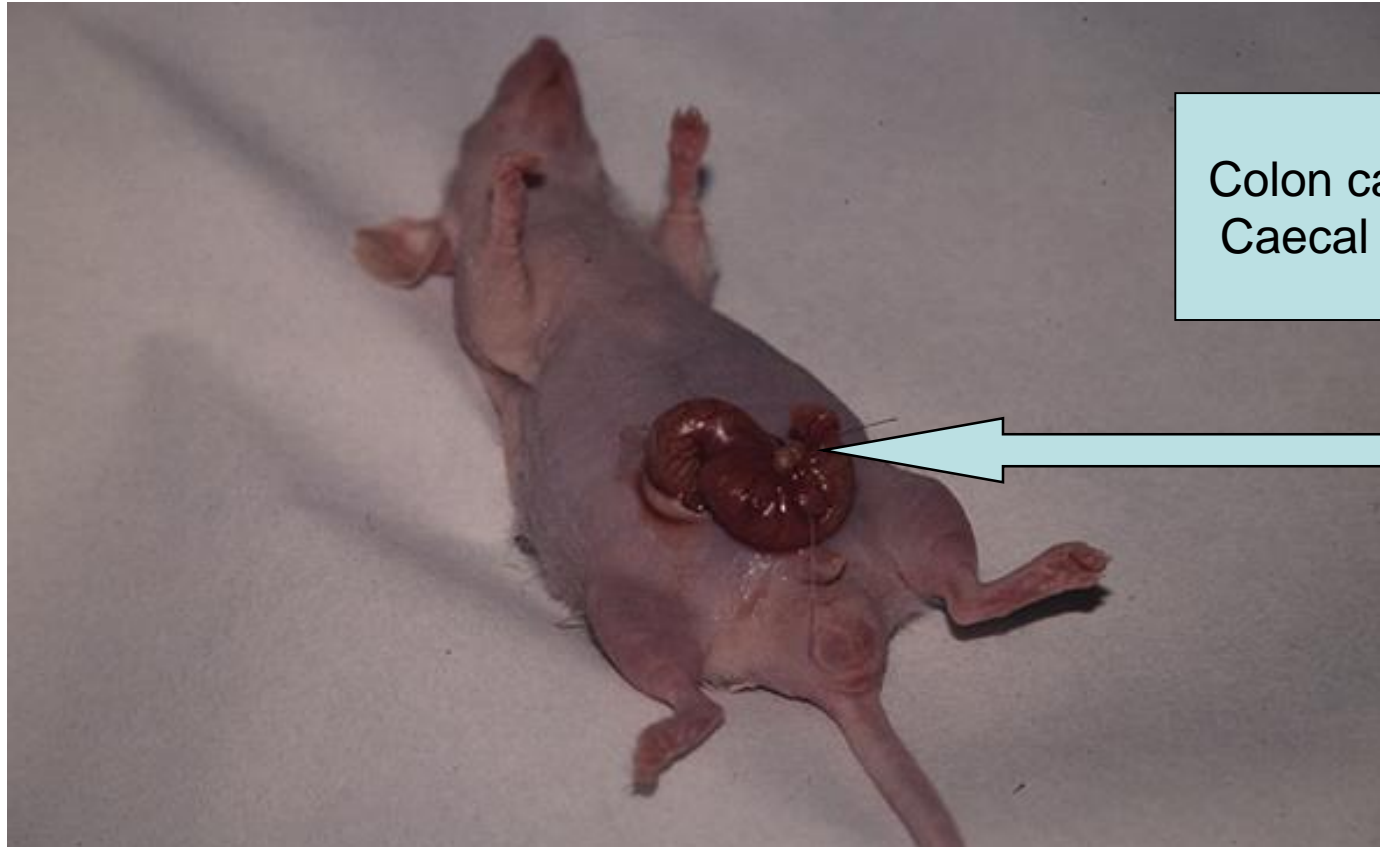


Bladder cancer
Transurethral injection



Fifth day following
Intrabronchial Implantation
with Walker's tumor cells.

Rat presented
different tumors location

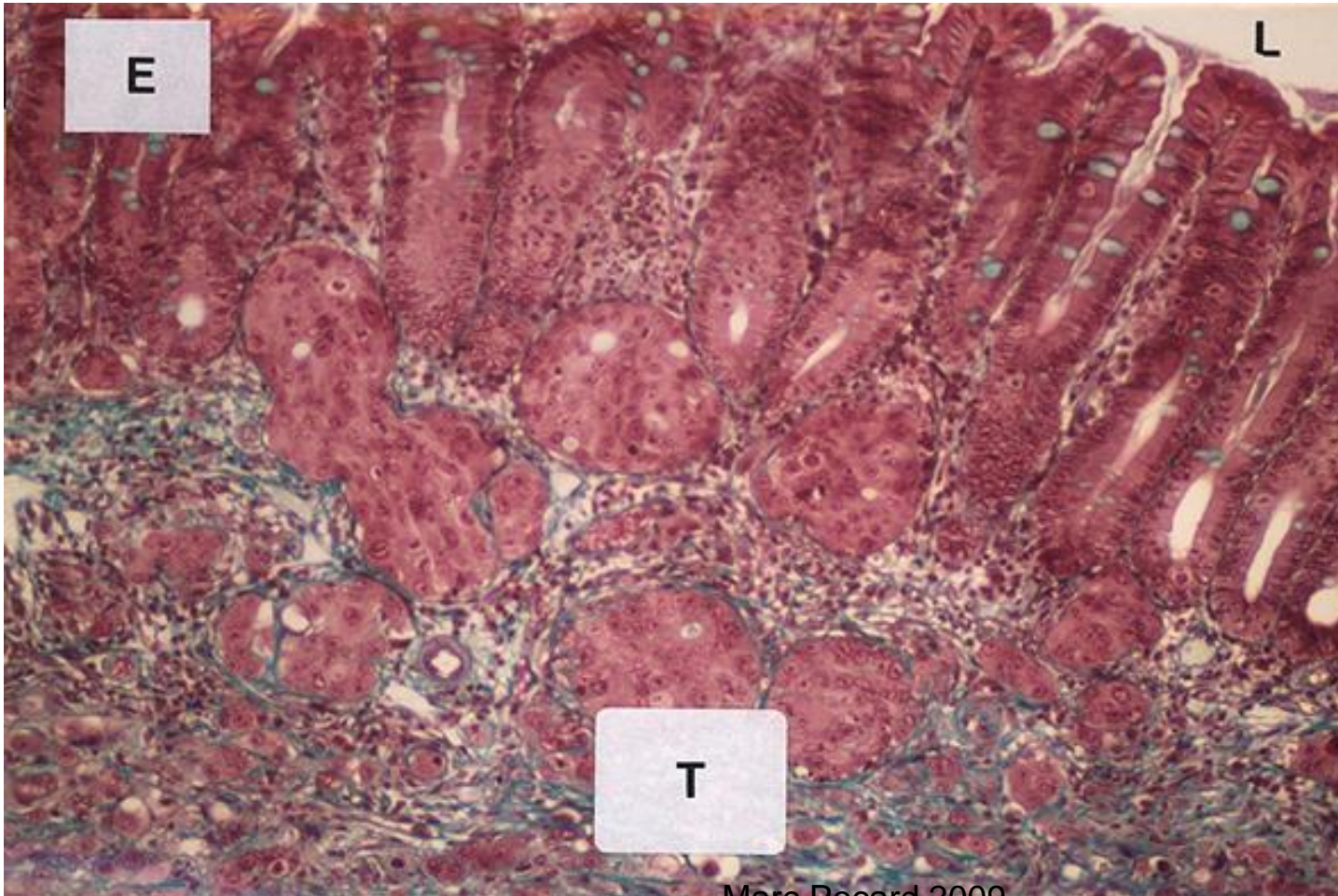


Colon cancer
Caecal graft



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Colon cancer
Caecal graft

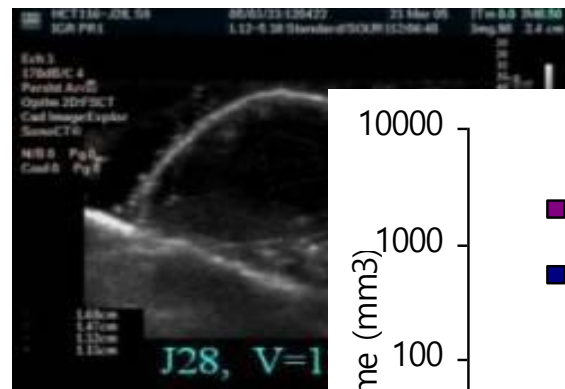
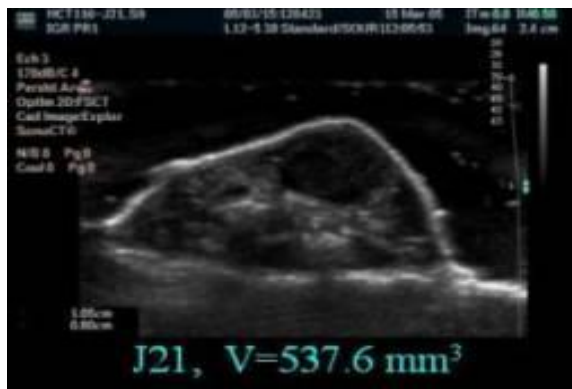
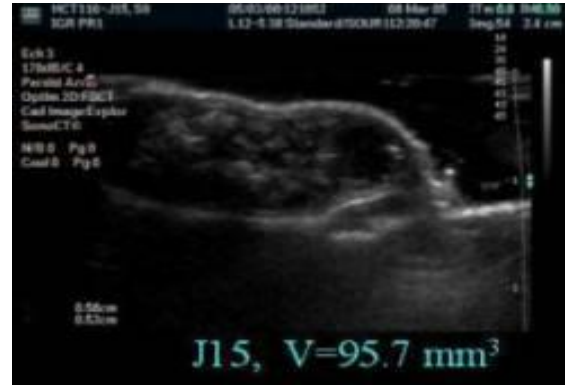
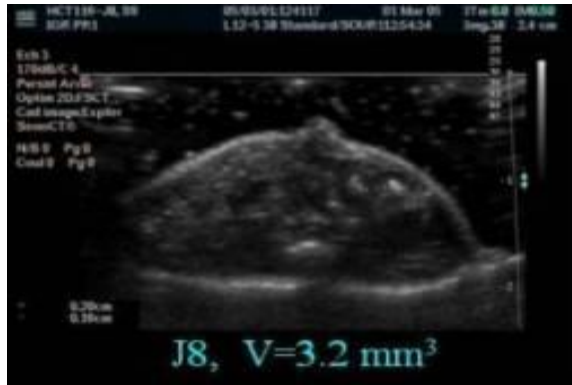


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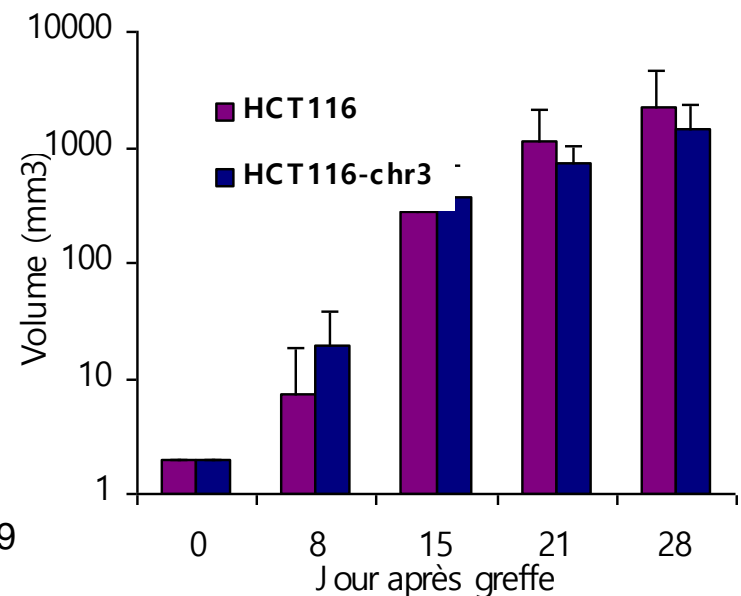
	Stage	volume Scut mm ³	Volume caecal mm ³	IC/S cut	p
TC-10	TxN0M0	343	691	2	0,05
TC-33	TxN0M0	647	2347	3,6	0,008
TC-71	TxN0M0	460	853	1,9	0,015
TC-37	TxN2M0	768	1322	1,7	0,01
TC-7	TxN2M0	569	483	0,85	ns
TC-70	TxN3M0	277	147	0,53	ns
TC-82	TxN3M0	215	256	1,2	ns
TC-118	M1	715	955	1,3	ns
TC-122	M1	761	679	0,9	ns
LoVo	M1	316	392	1,2	ns

Graft tumor fragment = 2 cells or tumor

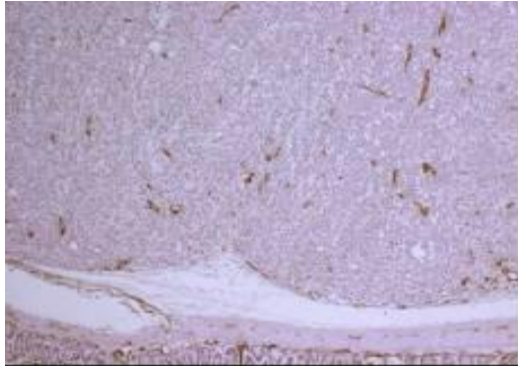
Tumor growth regarding divers cells lines



**HCT116 colon tumor –
Ultra-sonography.**



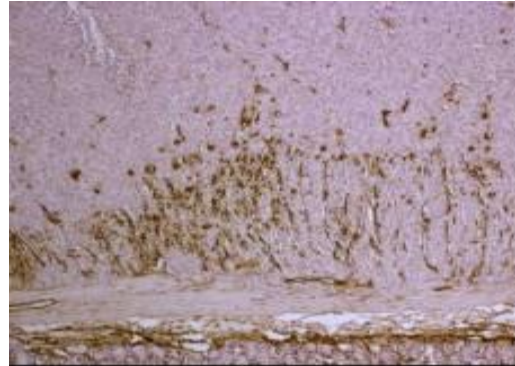
Local property : tumor and stroma : The stroma came from the murin tissue



**Histological aspect
of the parental cell
line HCT 116**

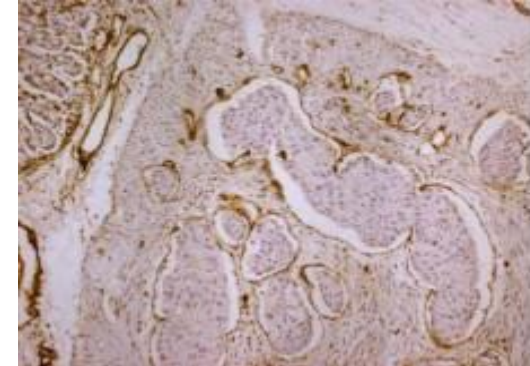
MSI tumor

Neo vessels



**HCT116 + chr3 after
a specific CD34
murin antigen**

MSS tumor



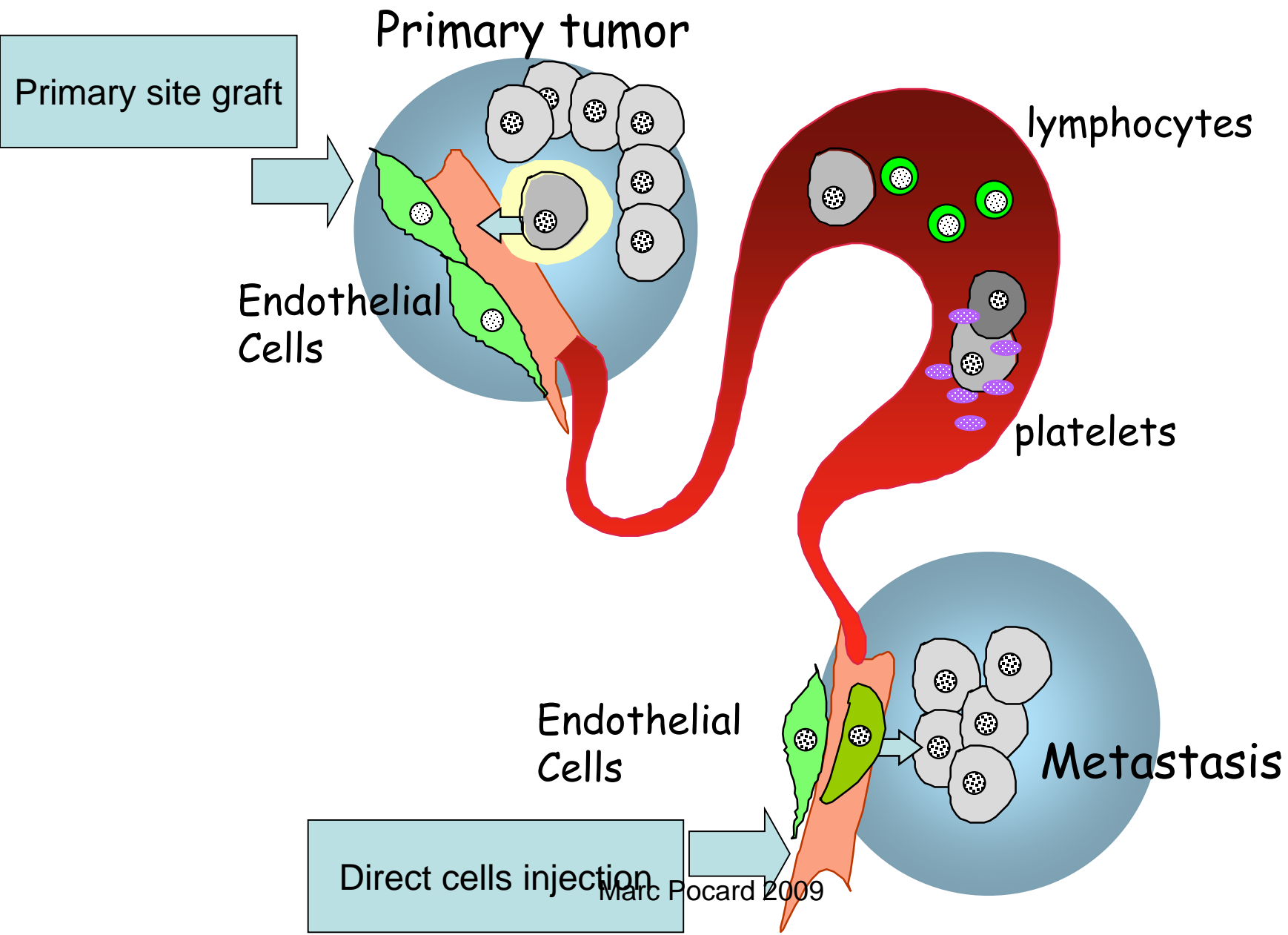
**Metastatic emboli
for a HCT116 + Chr3**

MSI stable tumor

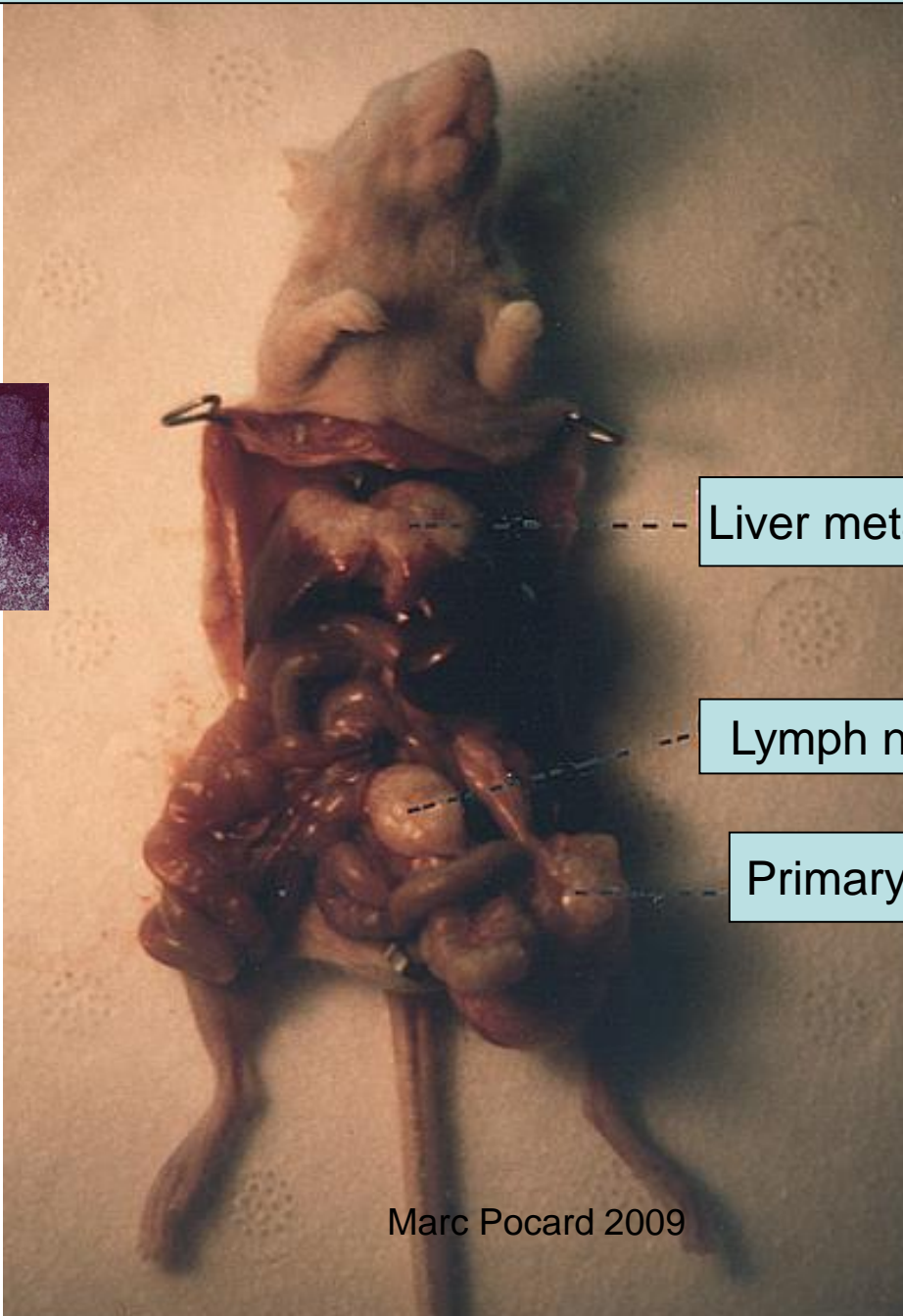
ANGIOGENESIS

Metastasis

- If cells or direct tumors fragment are at the primary sites, did they can create a metastatic process, mimicking the human behavior ?
- Yes but it is inconstant and take time
- Secondary solution : suppress the first part of the metastatic process – direct metastatic sites graft or injection



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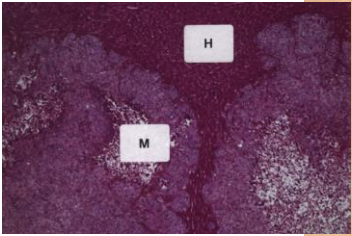


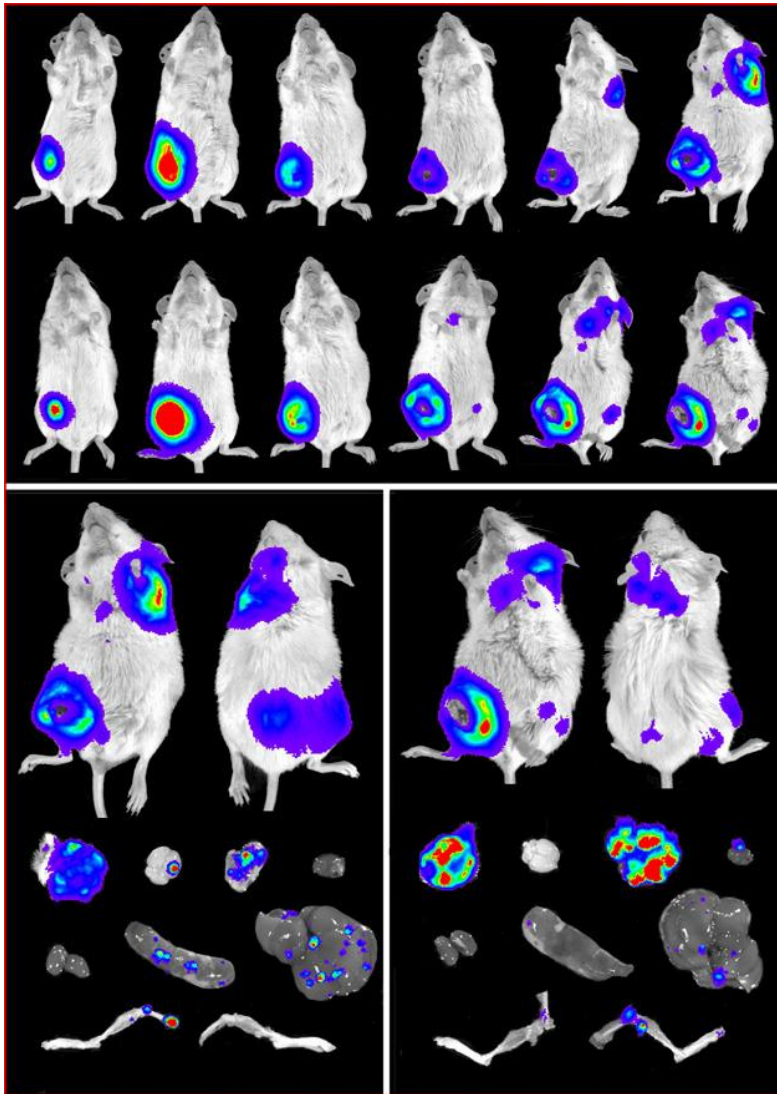
Colon cancer
Caecal graft

Liver metastasis

Lymph nodes

Primary colon cancer

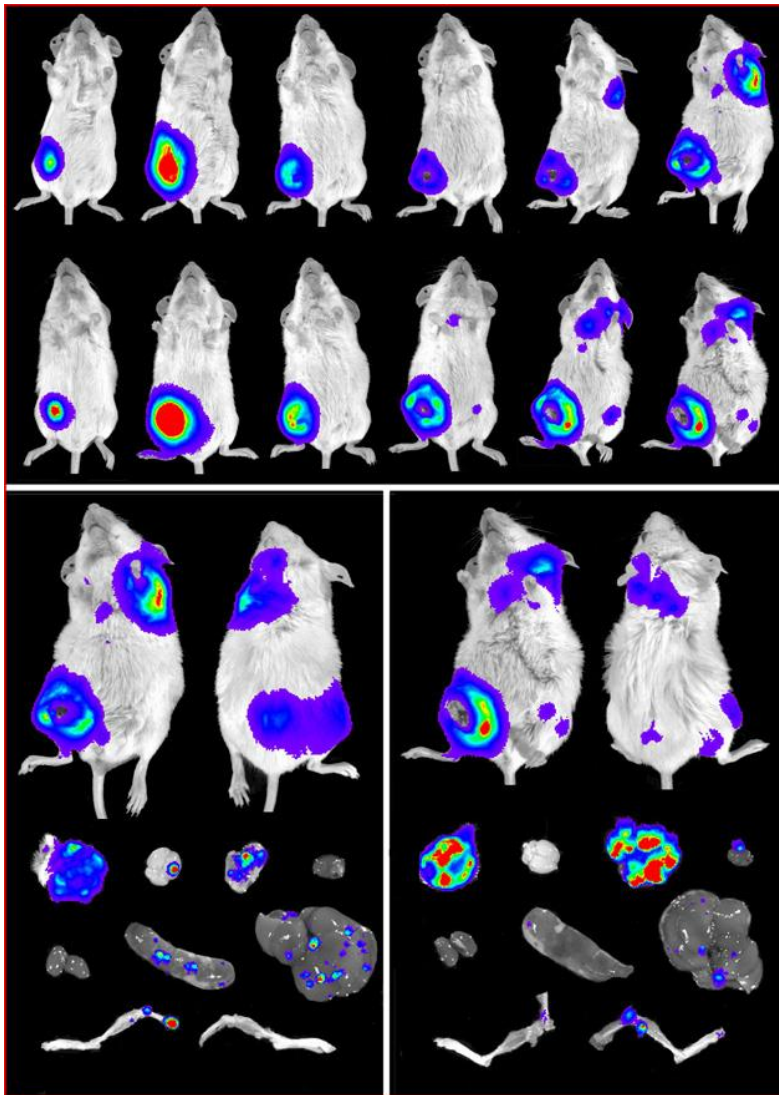




Breast cancer

The 4T1 metastatic breast cancer model is a syngeneic xenograft model based on 4T1-luc12B, a luciferase-expressing clone of the well characterized 4T1 mouse mammary tumor cell line.

Because the model is syngeneic in BALB/c mice, it can be used to study the role of the immune system in tumor growth and metastasis.



Breast cancer

The luciferase-expressing line can be introduced orthotopically into the mammary fatpad of nude, scid or normal BALB/c mice by surgery or direct injection, forms metastases in lungs, liver, bone over a period of

6 Weeks

When introduced into the tail vein of mice, metastases are observed in these same organs at

2 Weeks

General Concept

Early resection of primary orthotopically-growing human colon tumor in nude mouse prevents liver metastasis:
further evidence for patient-like hematogenous metastatic route.
Kuo TH, et al. *Anticancer Res.* 1993 Mar-Apr;13(2):293-7.

Surgical Concept

for the investigation of intraoperative tumor cell spillage during resection of the colon and the development of port site metastases.
Balague C, et al. *Surg Endosc.* 2001 Aug;15(8):833-6.

Treatment Concept

Prevention of hepatic and peritoneal metastases by the angiogenesis inhibitor fr-118487 after removal of growing tumor in mice.
Tanaka T, et al. *Jpn J Cancer Res.* 2001 Jan;92(1):88-94.

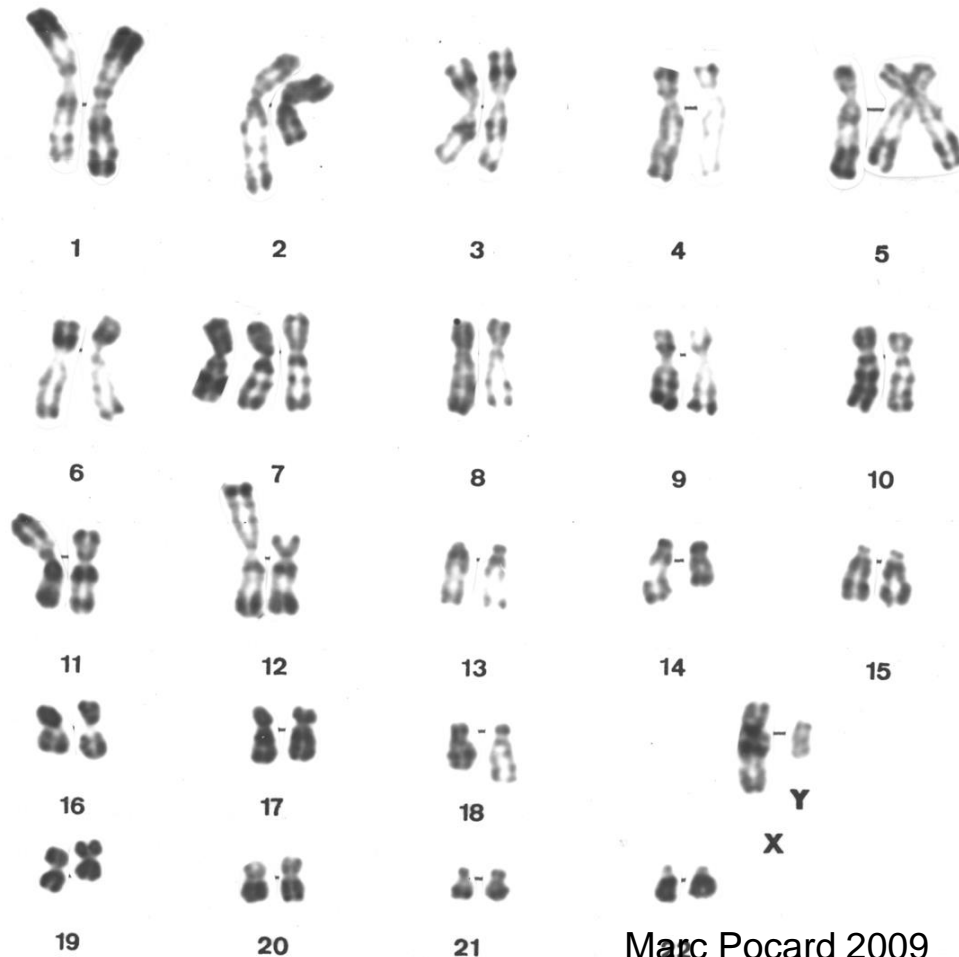
Tumoral graft at day 0, tumor resection at day 30 and results of surgery at day 100

Metastases

	Nbr animal	No tumor	Lymph nodes	liver	chest
HT-29	4	2/4	2/4	0/4	1/4
LoVo	5	5/5	0/5	0/5	0/5
TC-7	7	0/7	7/7	1/7	1/7
TC-82	4	0/4	2/4	2/4	0/4

The tumoral genetic property govern the surgical resection result :

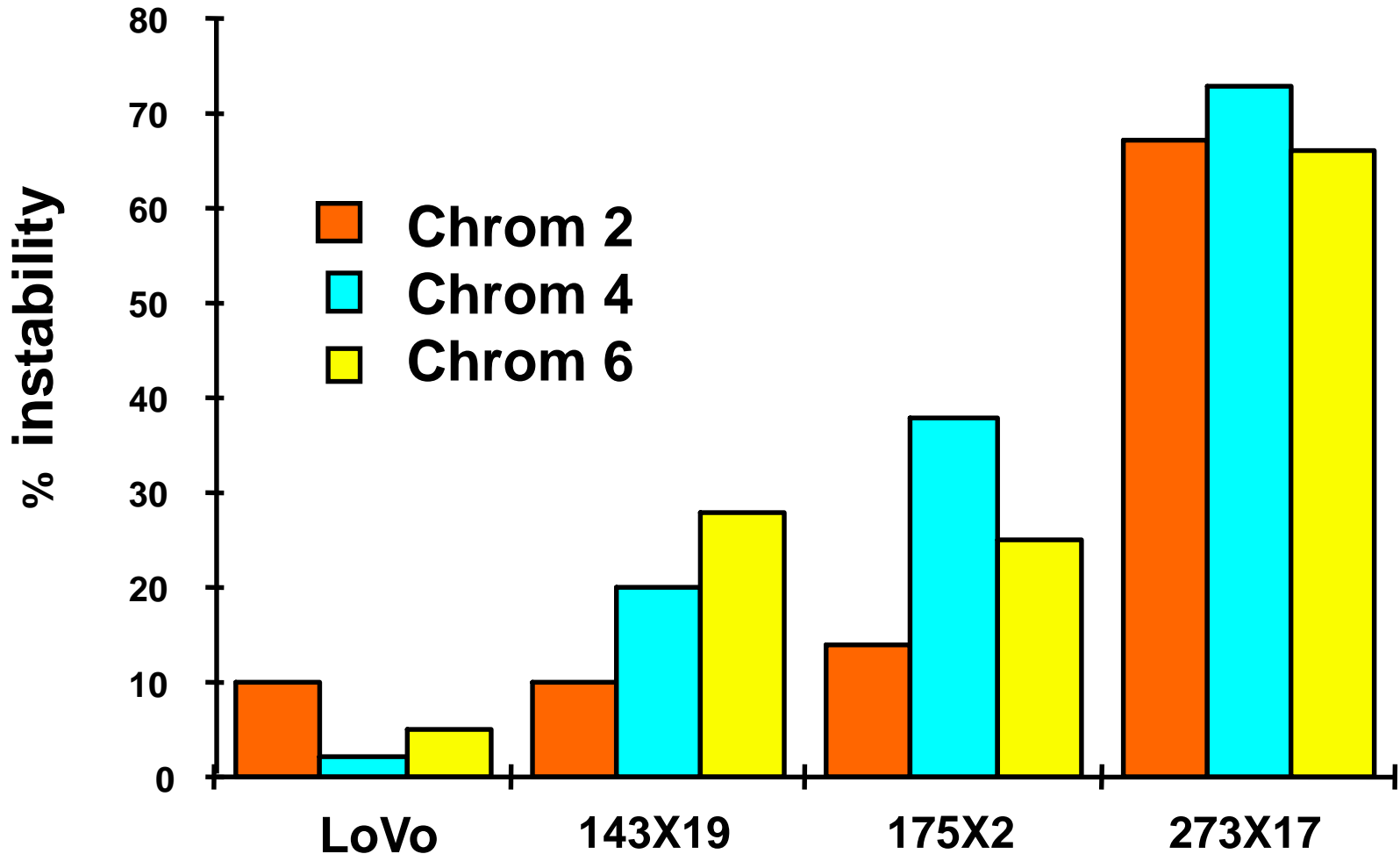
P53 - Chromosomique instability



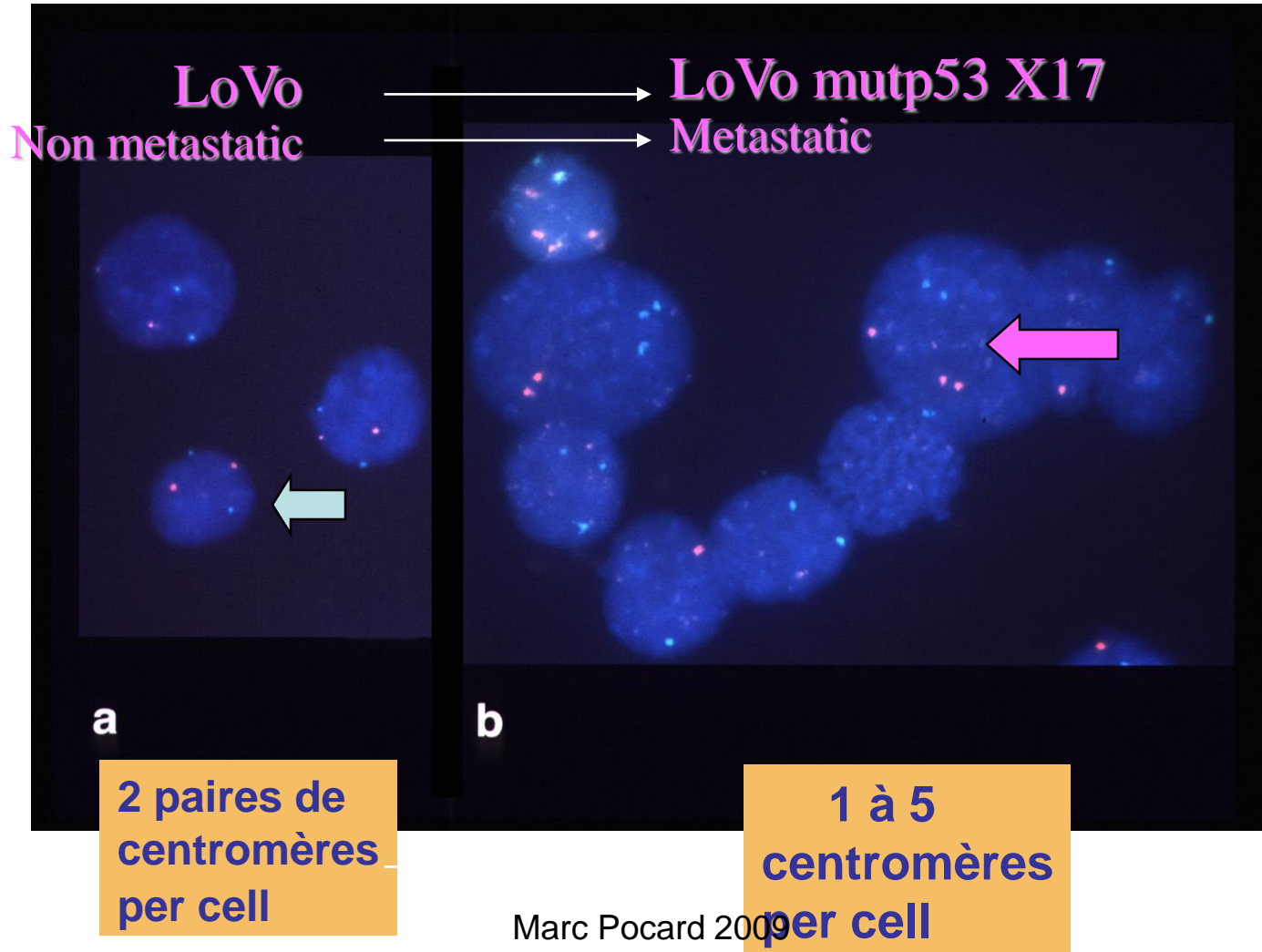
LoVo
Caryotype :
stability

P53 wild type

chromosomique instability induce by muted p53 transfection



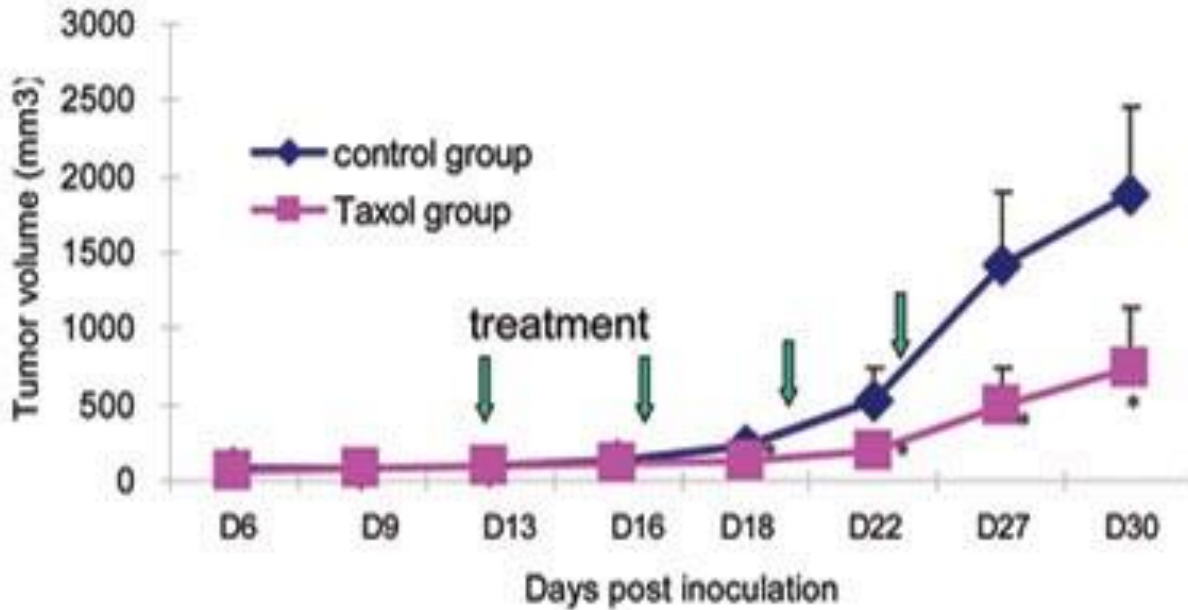
FISH analysis of LoVo Cells lines



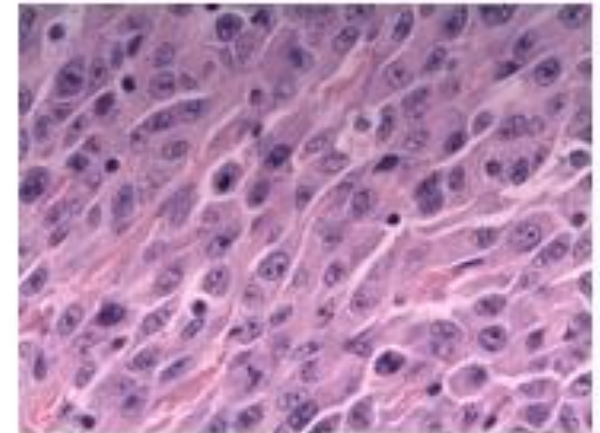
Surgical colon resection at day 15
results at day 45 regarding the p53 status

	LoVo	143 X19	175 X2	273 X17
tumoral volume	65 ±05	87 ±13	103 ±08	109 ±03
% cure	100	100	50	0
local recurrence	0	0	50	100
Lymph nodes	0	0	30	100
Liver	0	0	50	100
Peritoneum	0	0	40	80

Inhibition of U87MG gliomablastoma growth by Paclitaxel



Tumor tissue



H&E, 400×

- Female Balb/c nu mice were inoculated with 1×10^6 U87MG cells on D0
- Mice were randomized into two groups (n=10 for each group) when average tumor size reached 100-150 mm³
- Mice were treated with either saline or Paclitaxel via i.p. injection
- Significant inhibition on tumor growth was observed one week days after the first drug treatment (#p<0.05), and the effect maintained throughout the rest of the experiment (*p<0.01)

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Claudin-1 regulates cellular transformation and metastatic behavior in colon cancer

Punita Dhawan,¹ Amar B. Singh,² Natasha G. Deane,^{1,3} YiRan No,¹ Sheng-Ru Shiou,¹
Carl Schmidt,¹ John Neff,¹ M. Kay Washington,⁴ and R. Daniel Beauchamp^{1,5,6}

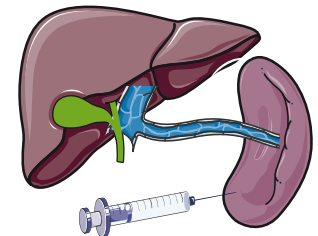
¹Surgical Oncology Research Laboratories, Department of Surgery, ²Department of Medicine, ³Department of Radiology and Radiological Sciences, ⁴Department of Pathology, ⁵Department of Cell & Developmental Biology, and ⁶Department of Cancer Biology, Vanderbilt University Medical Center, Nashville, Tennessee, USA.

The Journal of Clinical Investigation <http://www.jci.org> Volume 115 Number 7 July 2005

Hypothesis : overexpression of ... is related with metastasis

To assess the impact of overexpression or inhibition of claudin-1 on metastasis in vivo, female athymic/nude mice (7 weeks old; $n = 5$) were injected in the spleen with $5 \cdot 10^6$ cells of either SW480control and SW480claudin-1 cells or SW620 parental, SW620control, and SW620siRNA cells.

Choosing the right animal model



The spleen was removed after tumor cell injection, and a small hemoclip was applied to the splenic vessels. Nude mice in the SW480 group were sacrificed at 4 weeks, and the number and size of metastatic tumor foci on the surface of the livers were documented.

Quantification to obtain statistical analysis

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For the SW620 group,

small-animal microPET imaging was used to screen for nonpalpable lesions in the liver, using 100–150 μ Ci of 18F-deoxyglucose (18FDG) injected i.p to detect metabolically active foci in the abdomen.

When the tumors were visible by microPET (7 weeks), the mice were subjected to autopsy.

Timing is essential

The number and size of metastatic tumor foci on the surface of the livers were documented.

Always confirm with pathological exam

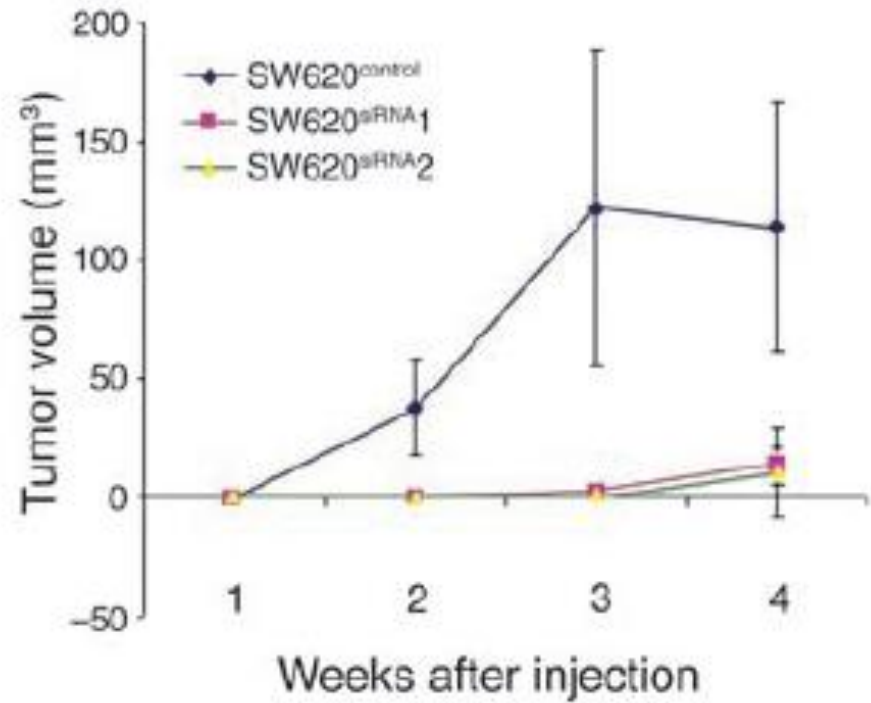
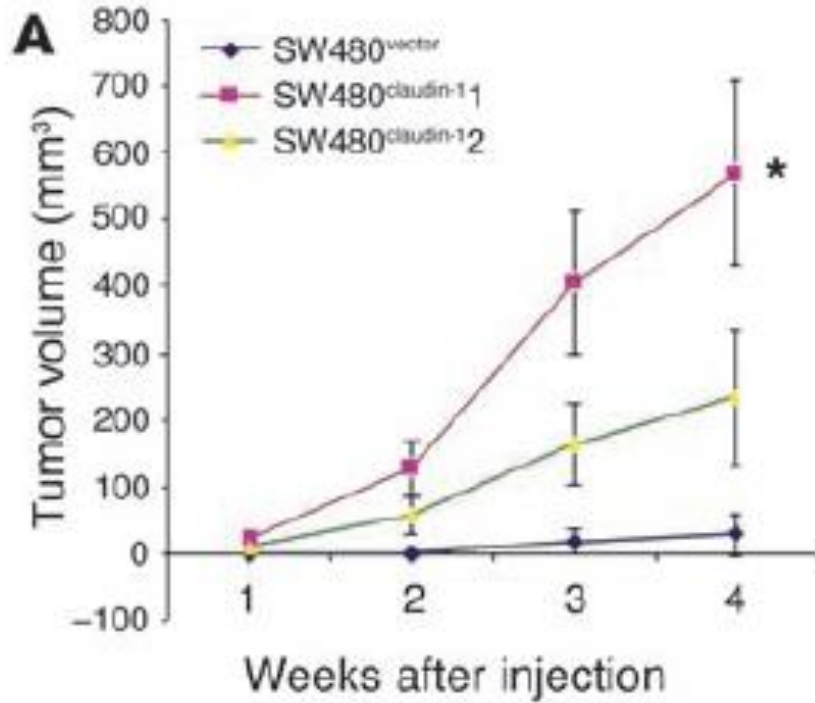


Table 2

Effect of overexpression of claudin-1 on liver metastasis

Group	No. of mice with liver metastasis (%)	No. of foci (mean ± SE)
SW480 ^{control}	0/4 (0%)	0
SW480 ^{claudin-1}	4/4 (100%)	12 ± 0.89 ^A
SW480 ^{claudin-12}	3/3 (100%)	10 ± 0.95

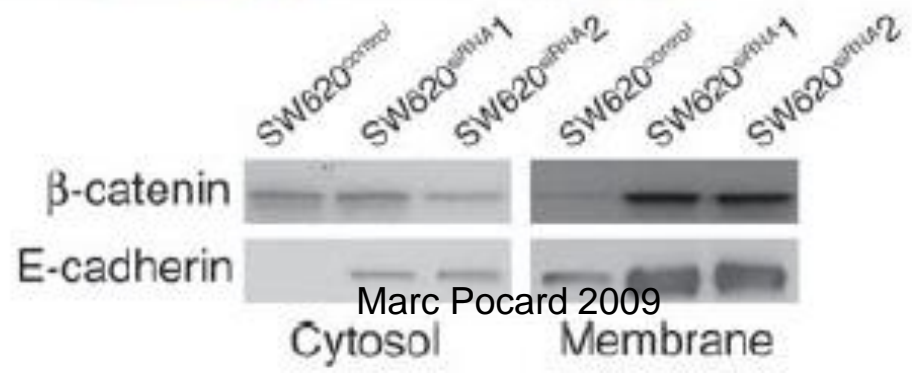
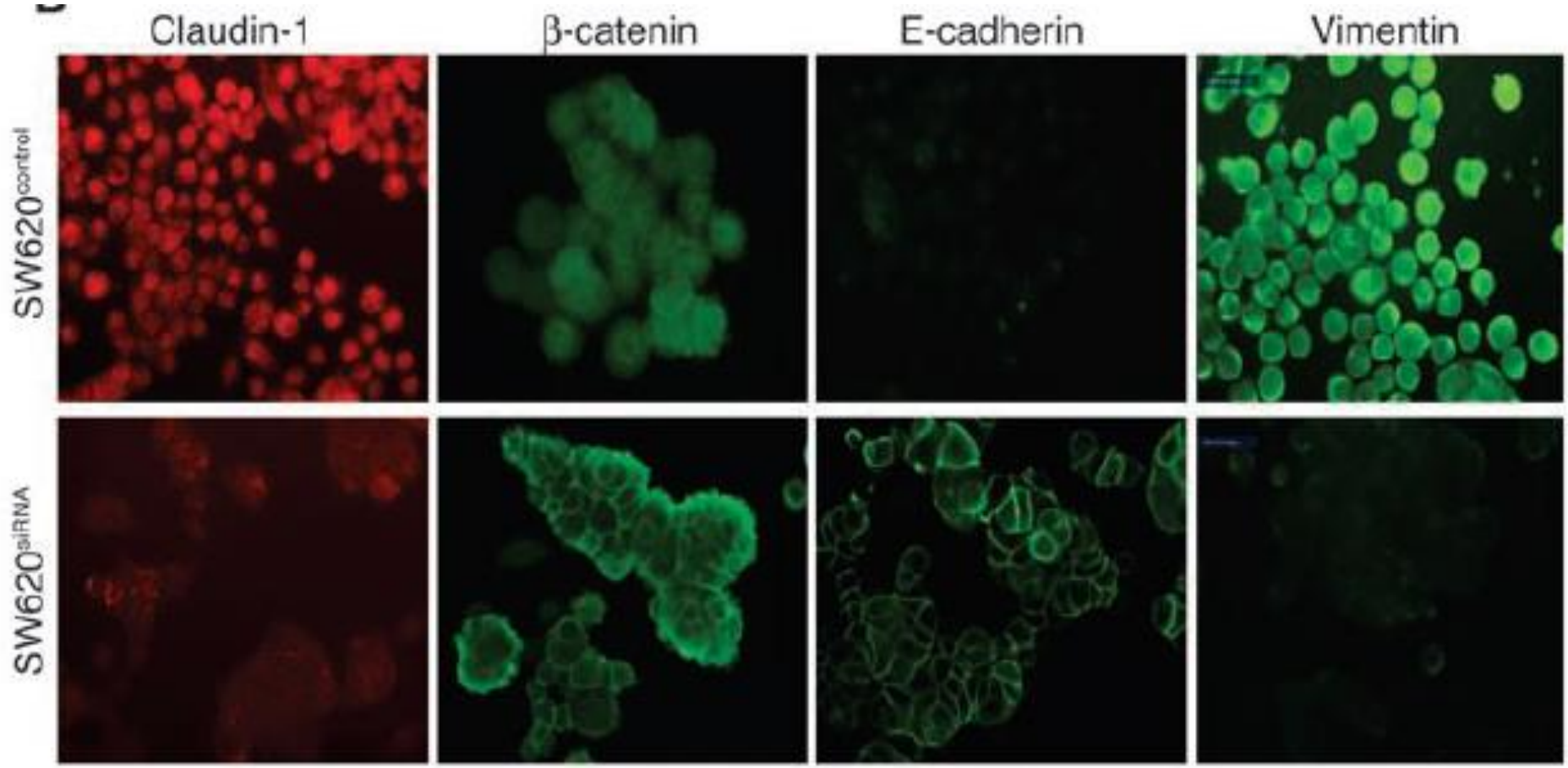
AP < 0.05 compared with parental or control.

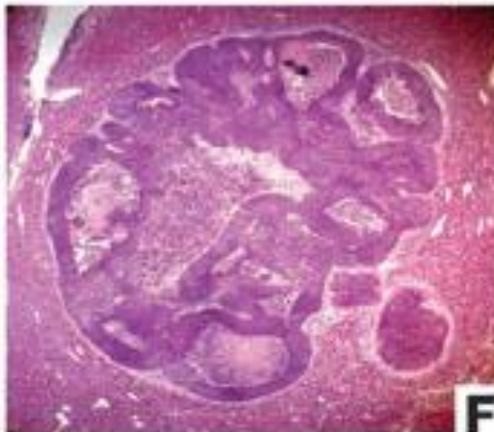
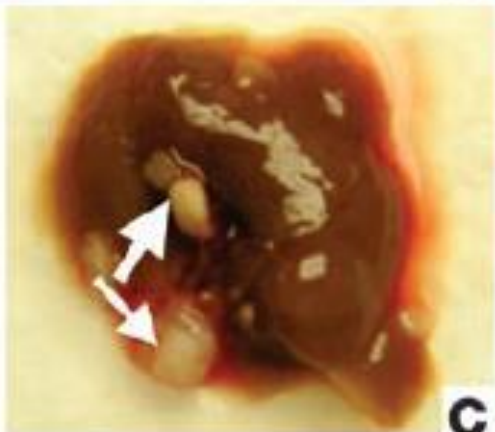
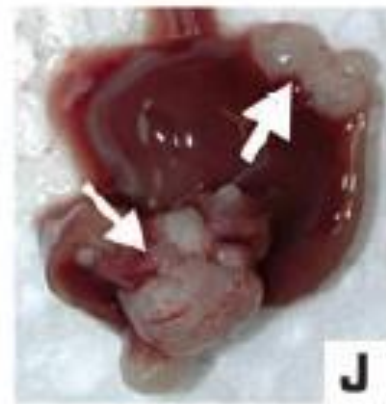
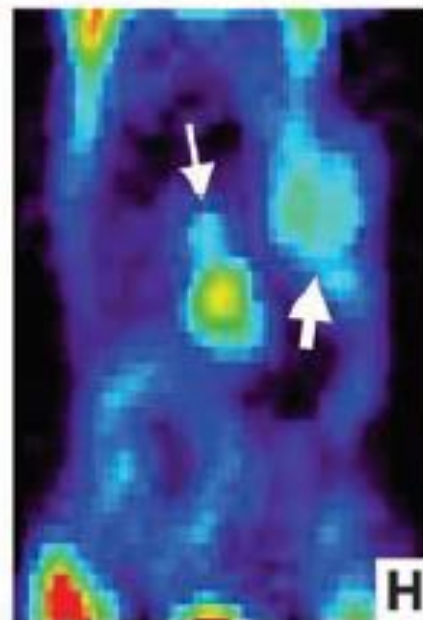
Table 3

Effect of inhibition of claudin-1 on liver metastasis

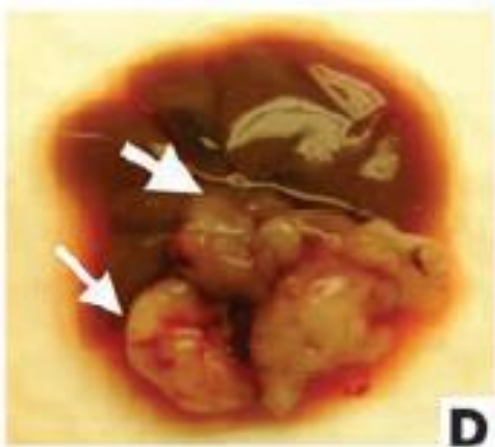
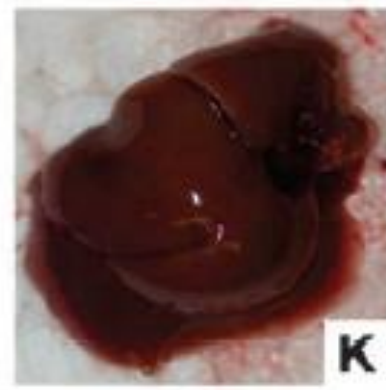
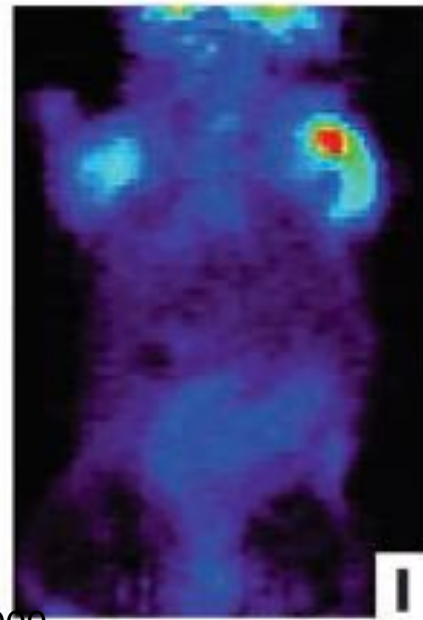
Group	No. of mice with liver metastasis (%)	No. of foci (mean ± SE)
SW620	5/5 (100%)	3.8 ± 0.71
SW620 ^{control}	4/4 (100%)	3.25 ± 0.57
SW620 ^{sIRNA1}	2/4 (50%)	0.75 ± 0.48 ^A
SW620 ^{sIRNA2}	2/4 (50%)	1.25 ± 0.95
SW620 ^{sIRNA3}	1/5 (20%) ^A	0.4 ± 0.40 ^A

^A*P* < 0.05 compared with parental or control.



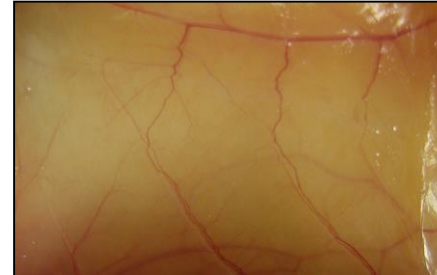
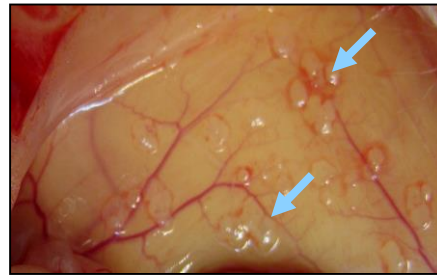


SW620^{control}



SW620^{siRNA}

Nétrine 4 mutated expression reduce the carcinomatosis in an orthotopic model of human colon cancer carcinomatosis



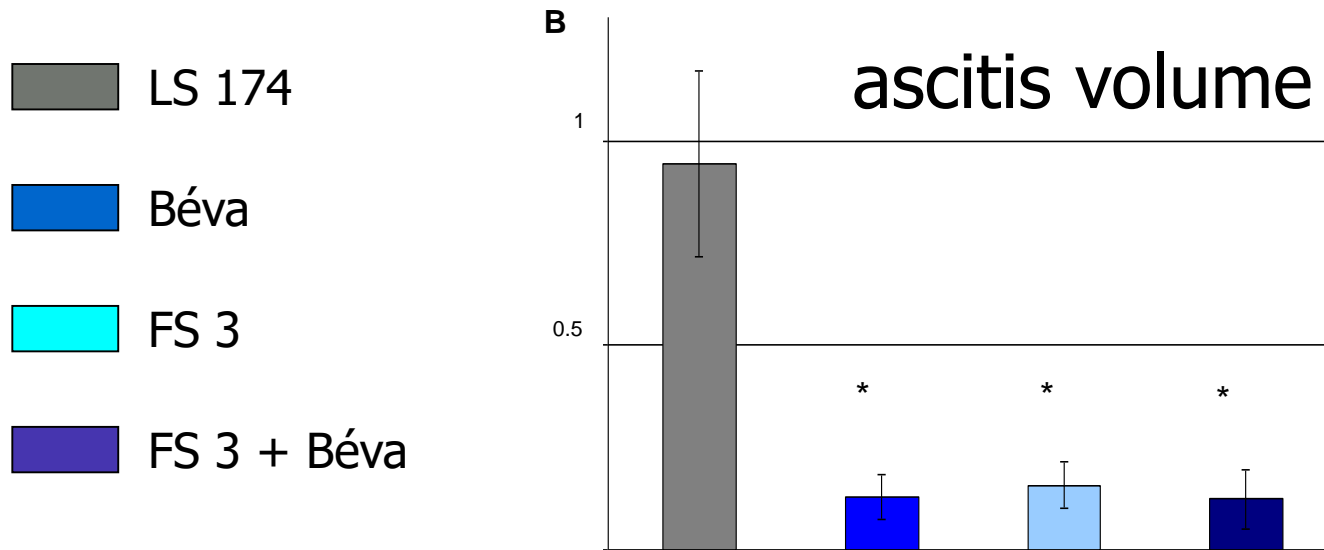
LS174

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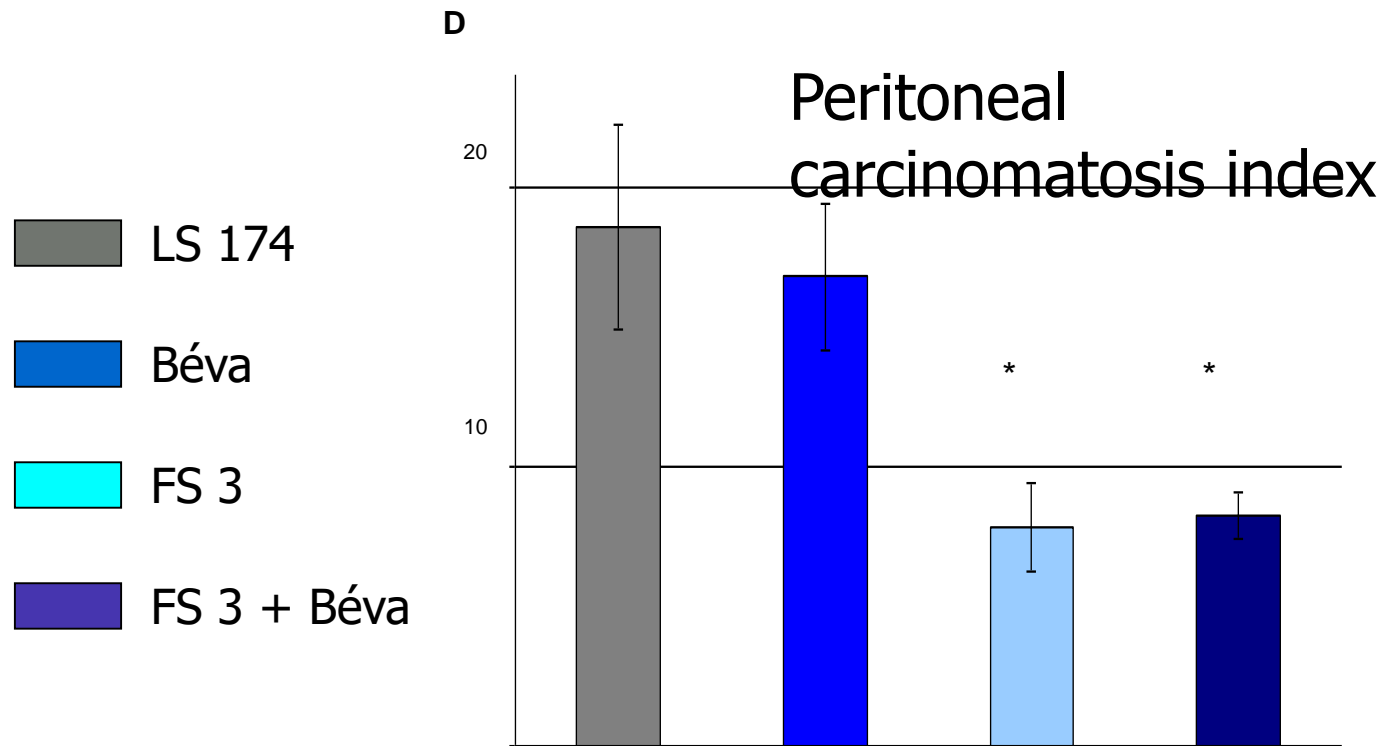
FS3

transfected

Mutated Nétrine 4 expression Change the tumor growth



Bevacizumab did not change the tumoral growth in this model



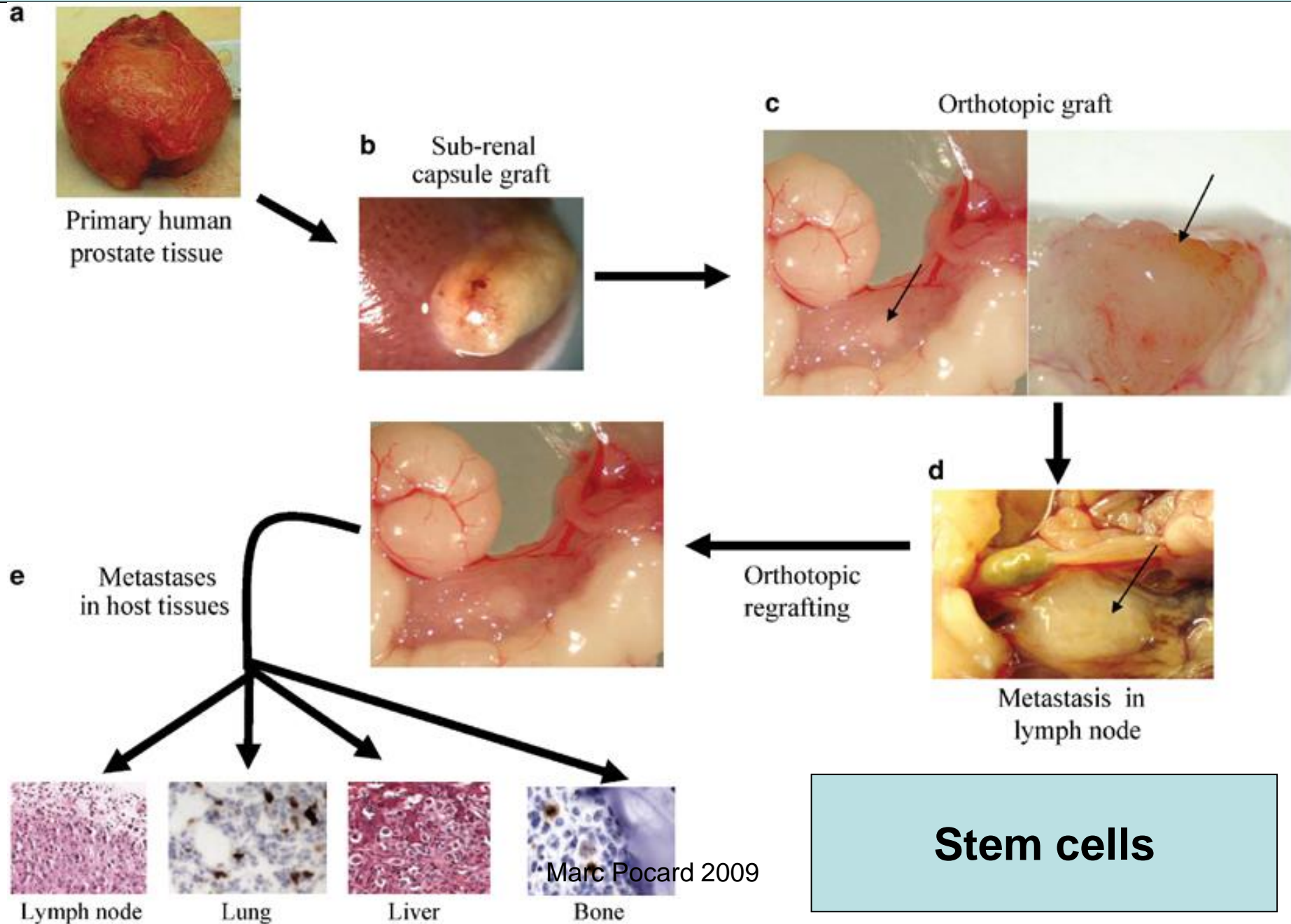
limitations

stem cells – specific interest

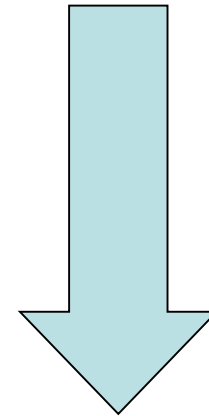
animal right

coast

uveal melanoma

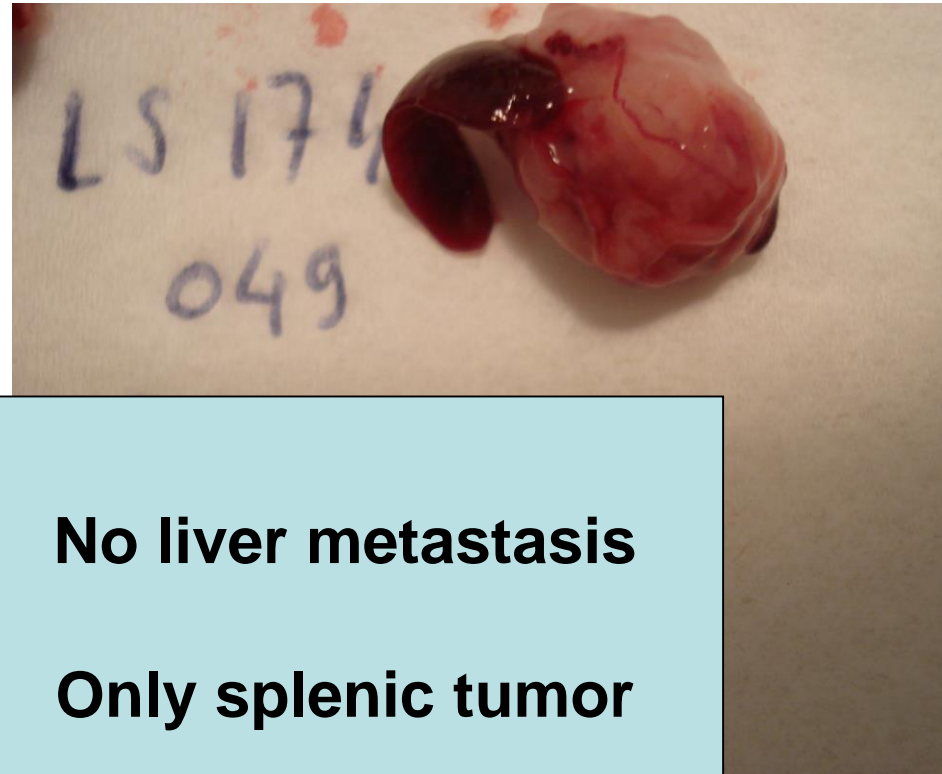
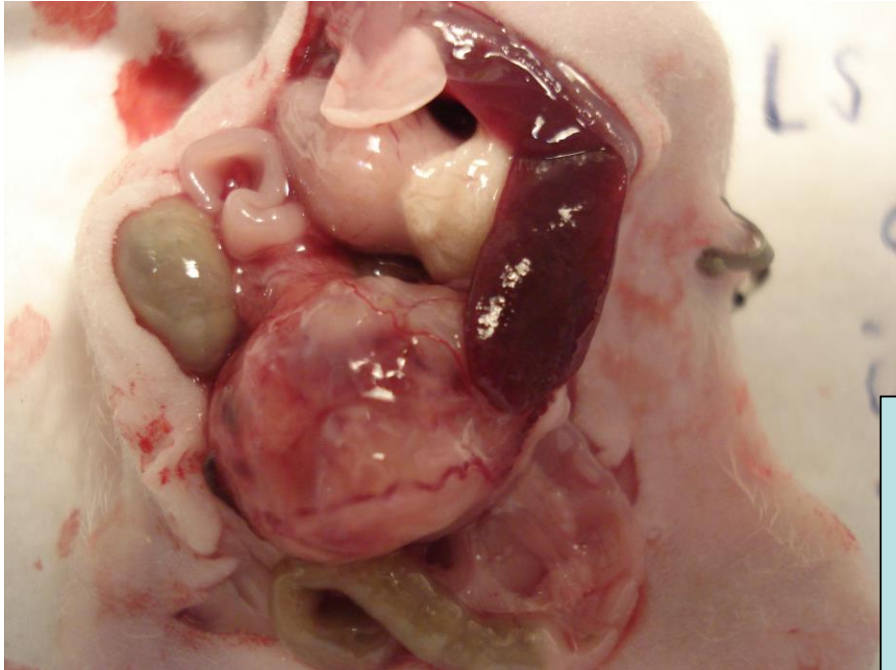


Animal experimentation – pain



Animal Procedure
Ethic consideration

experimentation failure

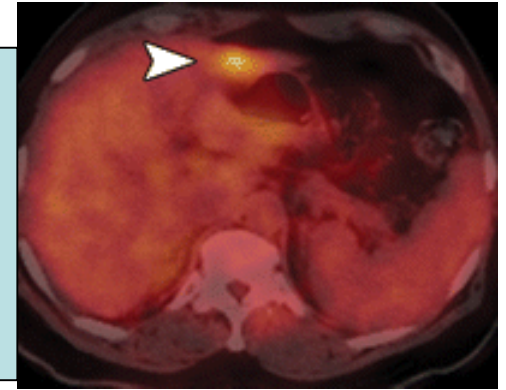


No liver metastasis

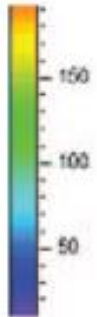
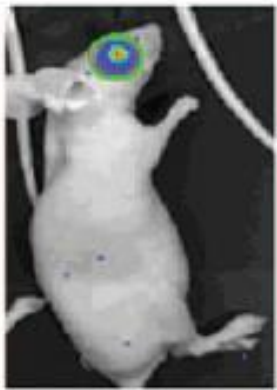
Only splenic tumor



**Uveal melanoma :
pure hematogenous
dissemination
One specific metastatic sites**



Nobody is perfect



**Human uveal melanoma cell line
(OCM-1 FRT/Luc) was inoculated
Into the anterior chamber of murines eyes
or intracardiac injection**

**4 to 6 weeks : bone – lung – mediastinum
No liver metastasis**



50 days

Orthotopic cancer model

- **Orthotopic** : Able to mimic any human cancer
- **Offer specific possibility**
 - Proof of concept regarding : metastasis – genetic instability – angiogenesis hypothesis
 - Phase 0 study
 - Testing divers therapeutic plan (surgery – radio – chemo)
- **Limitations**
 - Ethic – Coast - Material
 - Time consuming & can require surgical environment

Orthotopic cancer model

- **Nude mice possibility**

- Correlation between any genetic abnormality limited to a clone cells versus parental cell line and any specific evolution (volume, chemo sensitivity, surgical or radiotherapy efficiency, metastasis)
- Correlation between any genetic pathway and specific tumor evolution (sub groups identification)

- **Immunocompetent mice possibility**

- Testing particular therapeutic algorithm including immune function, or recovery after aggressive treatment
- Testing particular way of drugs delivery