THE BIOLOGY AND THERAPY OF BRAIN CANCER METASTASIS

Richard V. Smalley, M.D., Memorial Lecture
October 29, 2009

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DEPARTMENT OF CANCER BIOLOGY
CANCER METASTASIS RESEARCH CENTER
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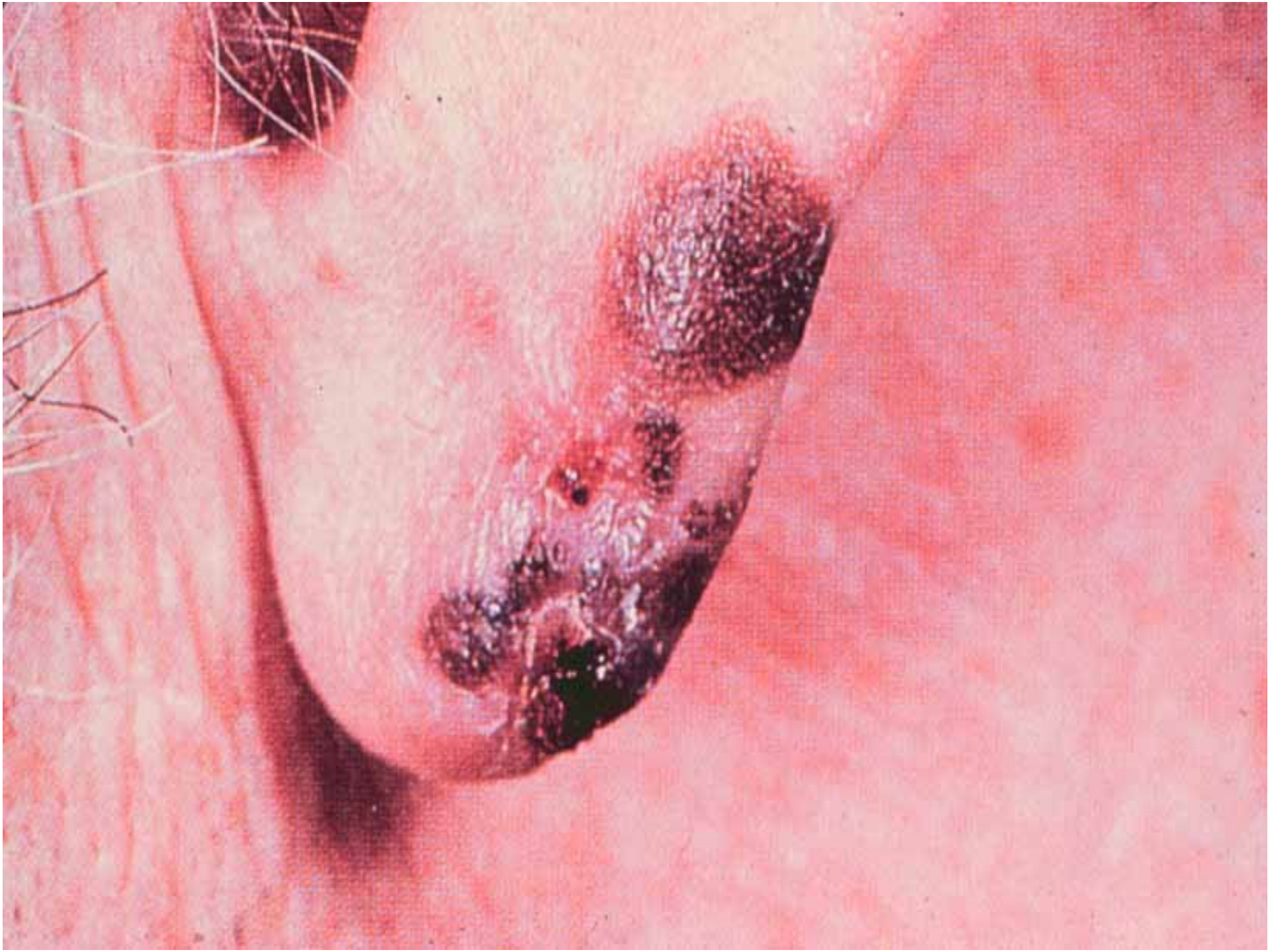
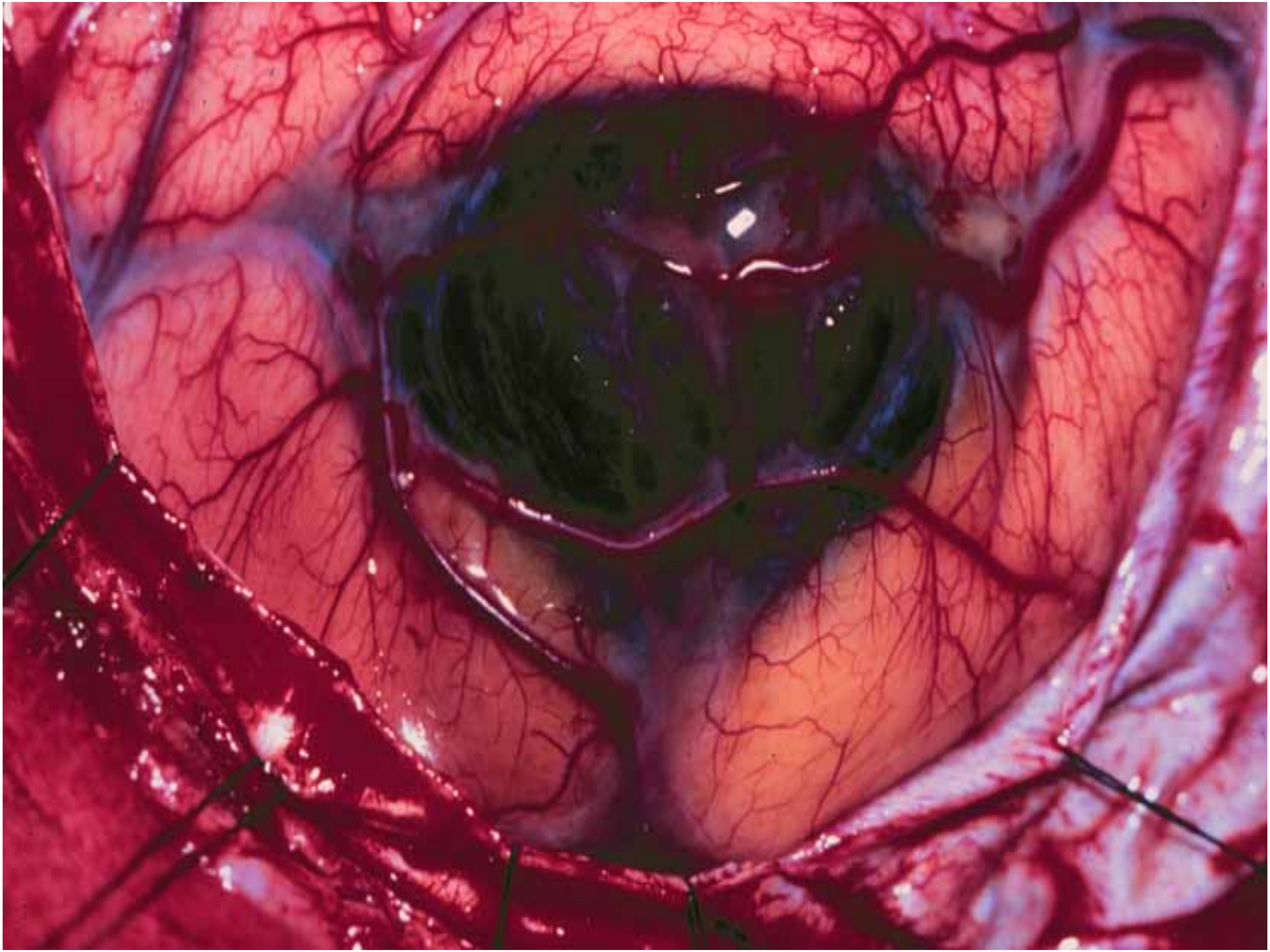
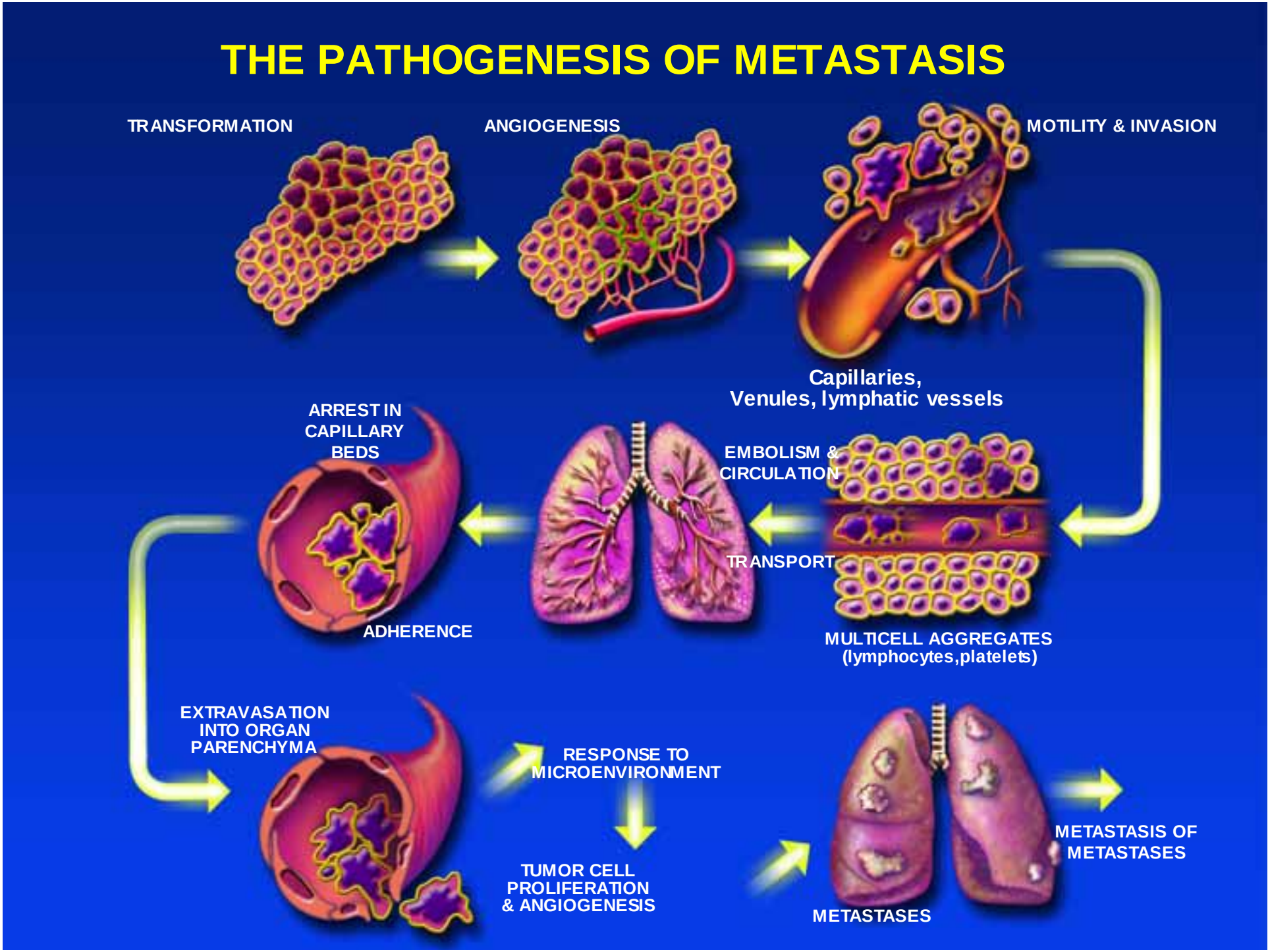
HOUSTON , TEXAS





THE MAJOR CAUSE OF DEATH FROM CANCER IS DUE TO METASTASES THAT ARE RESISTANT TO CONVENTIONAL THERAPY

THE PATHOGENESIS OF METASTASIS



METASTATIC INEFFICIENCY

LESS THAN 0.01% OF CELLS THAT ENTER THE CIRCULATION SURVIVE TO ATTACH IN CAPILLARY BEDS OF DISTANT ORGANS

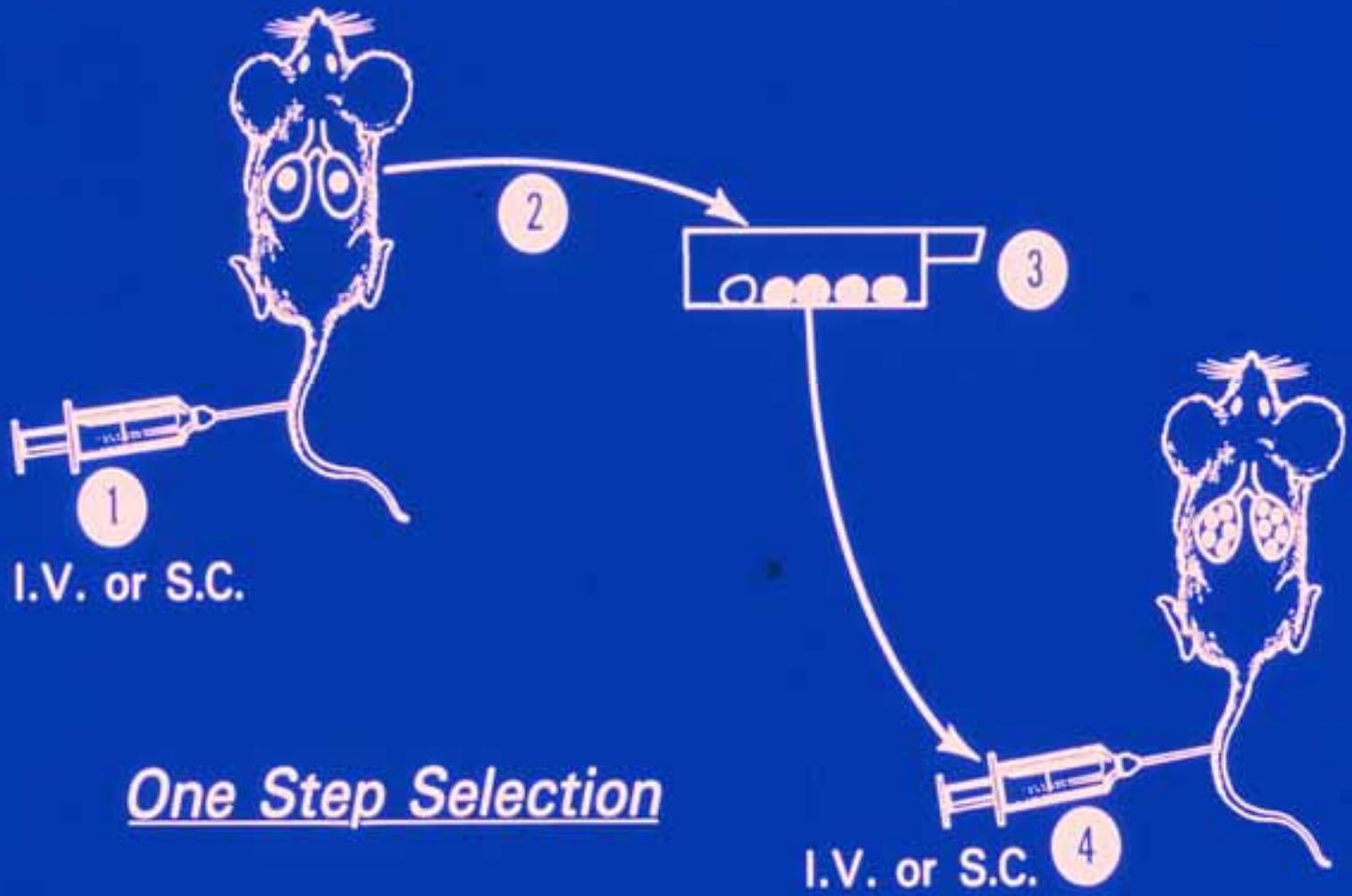
DOES THE DEVELOPMENT OF METASTASES REPRESENT

CHANCE SURVIVAL OF TUMOR CELLS

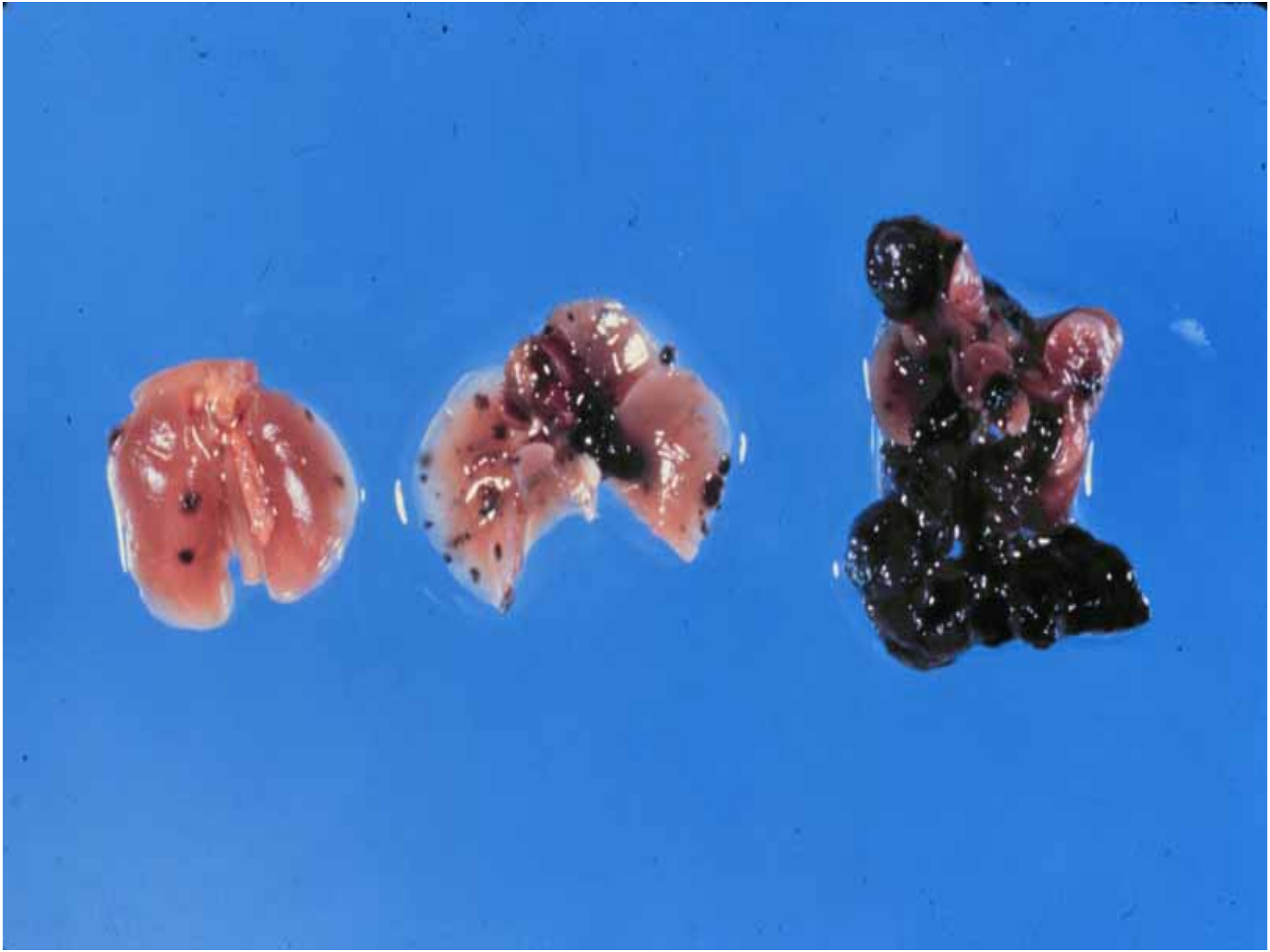
OR

SELECTIVE GROWTH OF SPECIALIZED TUMOR CELLS?

in vivo Enrichment for Metastatic Cells



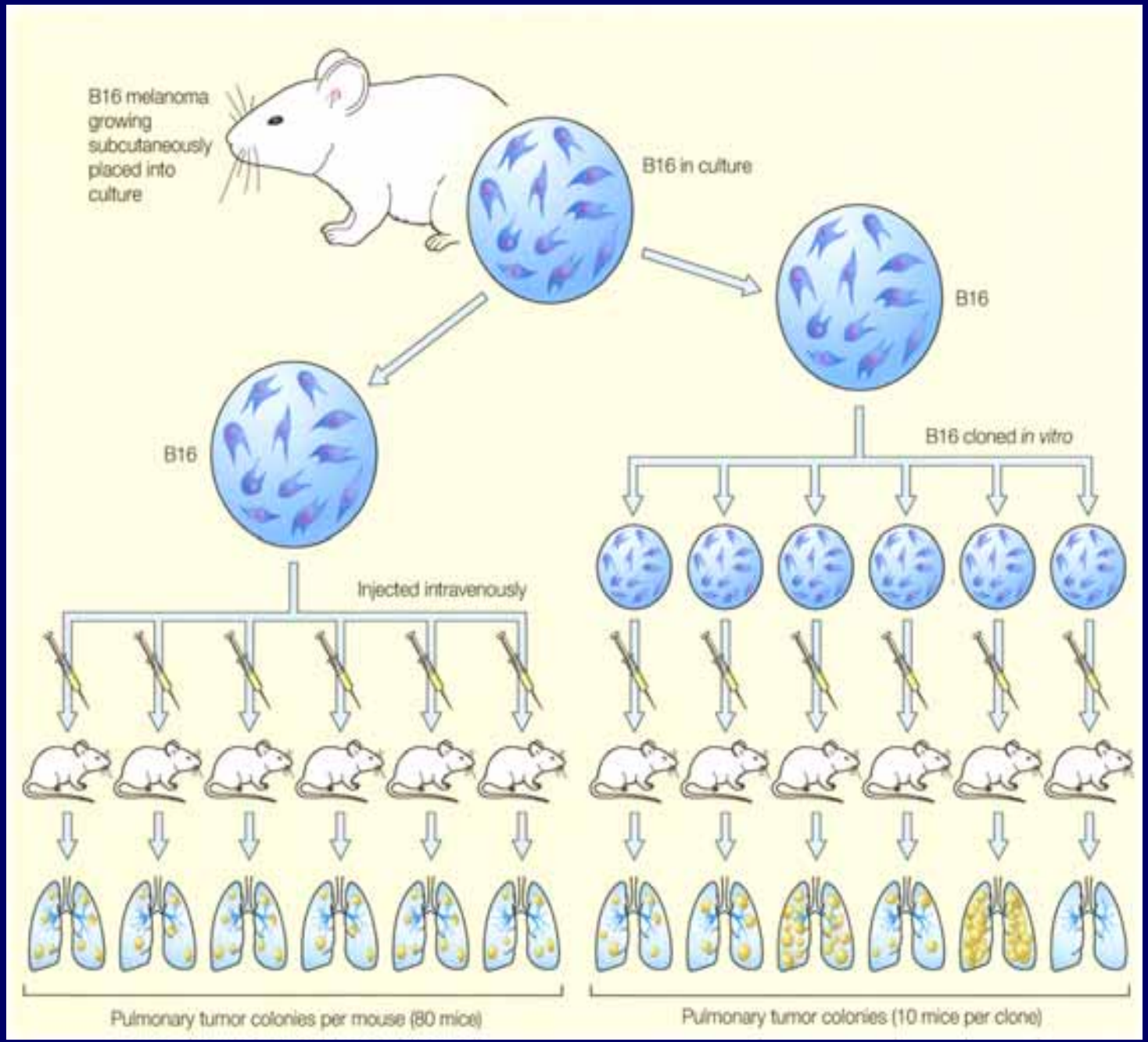
One Step Selection



METASTATIC TUMOR CELL VARIANTS:

1. ARISE DURING METASTASIS BY THE PROCESS OF ADAPTATION.

2. PREEXIST IN THE PARENTAL NEOPLASM.


B16 melanoma growing subcutaneously placed into culture
B16 in culture
B16
B16
B16 cloned in vitro
Injected intravenously
Pulmonary tumor colonies per mouse (80 mice)
Pulmonary tumor colonies (10 mice per clone)

CANCER METASTASIS

THE PROCESS IS HIGHLY SELECTIVE FOR PREEXISTING METASTATIC CELLS

METASTASES ARE CLONAL IN ORIGIN

TUMOR CELLS IN GENERAL AND METASTATIC CELLS IN PARTICULAR ARE GENETICALLY UNSTABLE

CANCERS ARE BIOLOGICALLY HETEROGENEOUS

METASTASIS CAN NOT BE A RANDOM PROCESS BECAUSE PATTERNS OF METASTASIS ARE PREDICTABLE.

'SEED AND SOIL' HYPOTHESIS

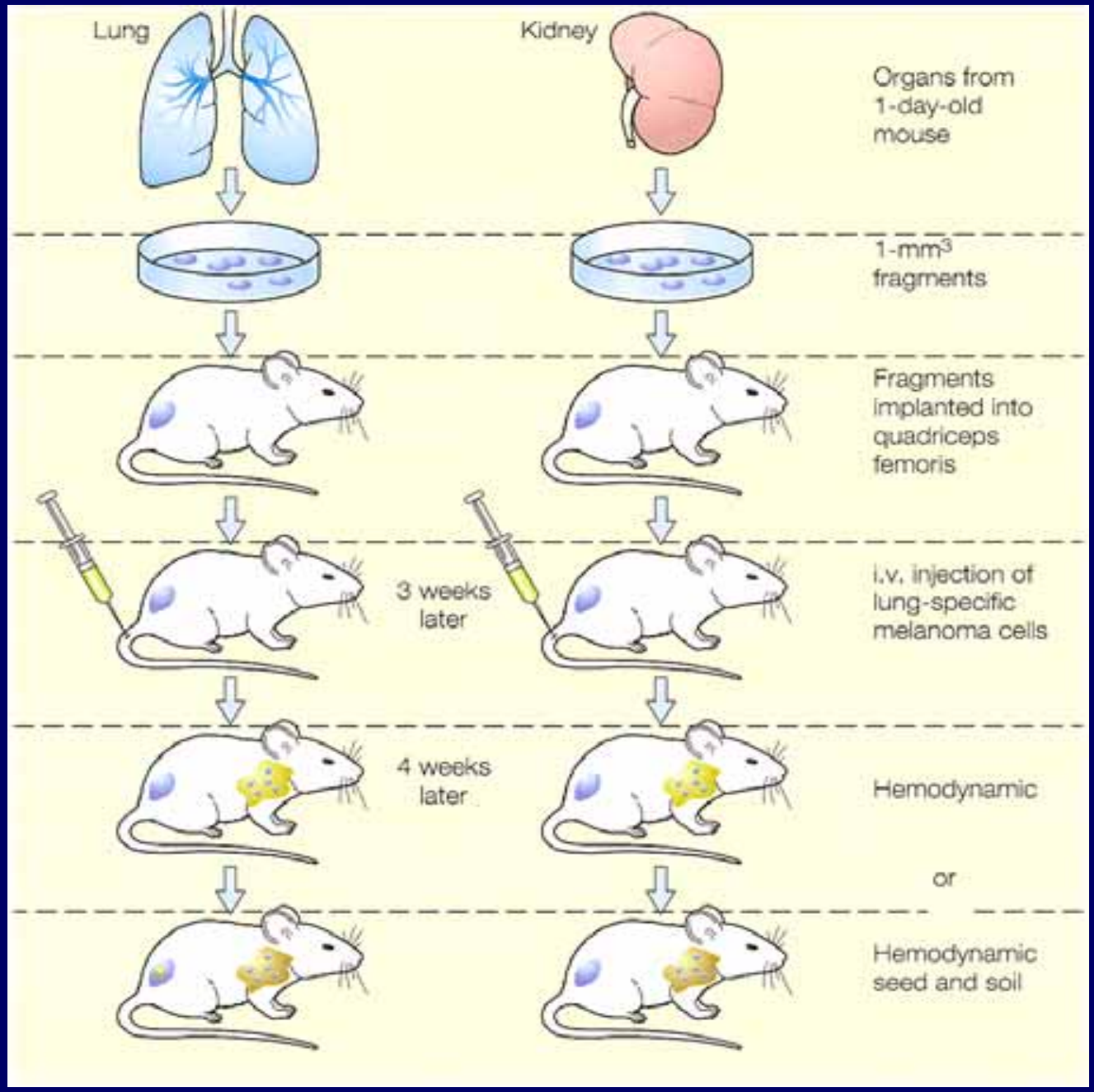
- 735 Breast Cancer Patient Records Analyzed
- Discrepancy Between Blood Supply and Frequency of Metastases in Various Organs

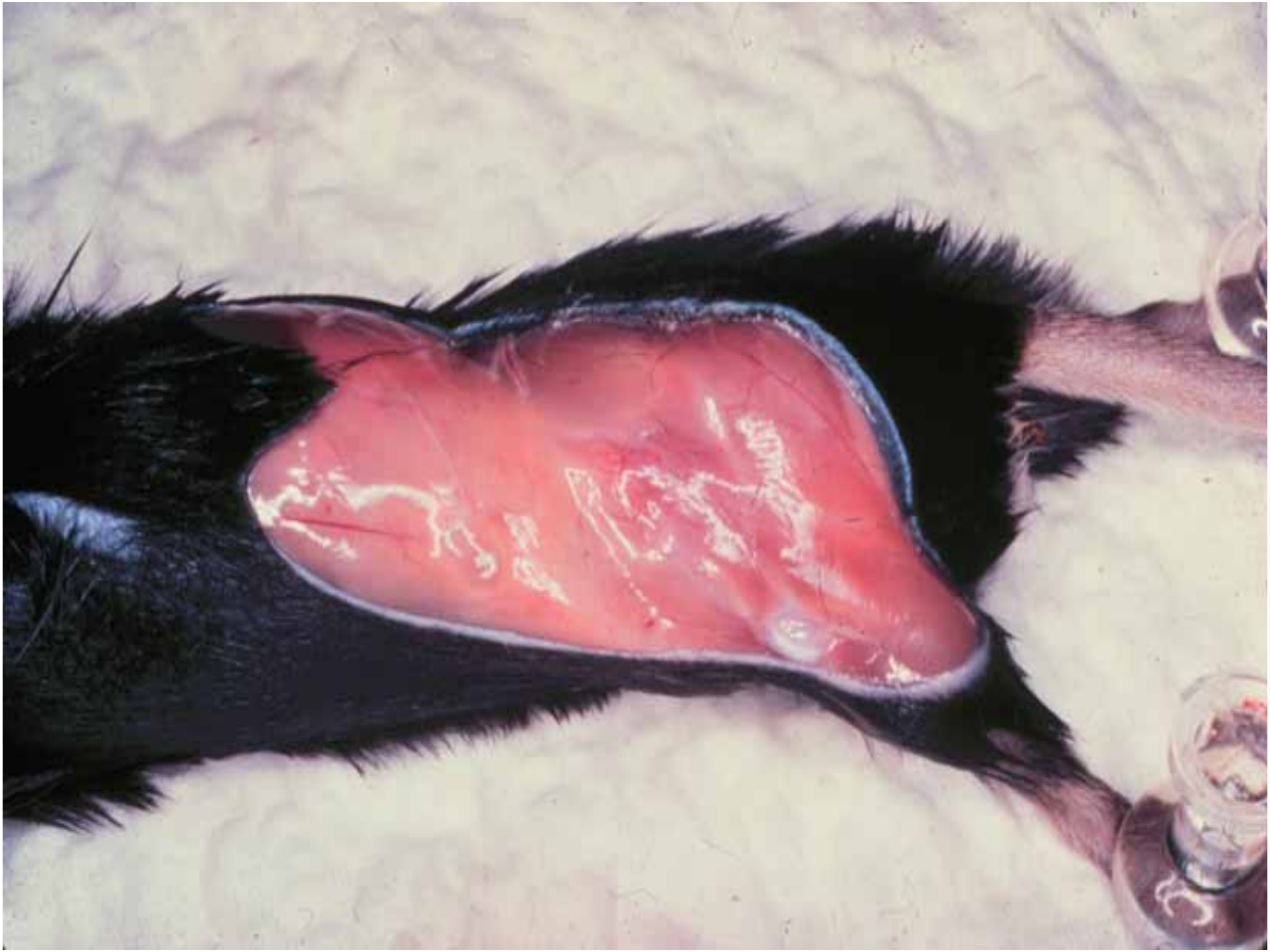
Stephen Paget, M.D.
Lancet 1: 571-573, 1889

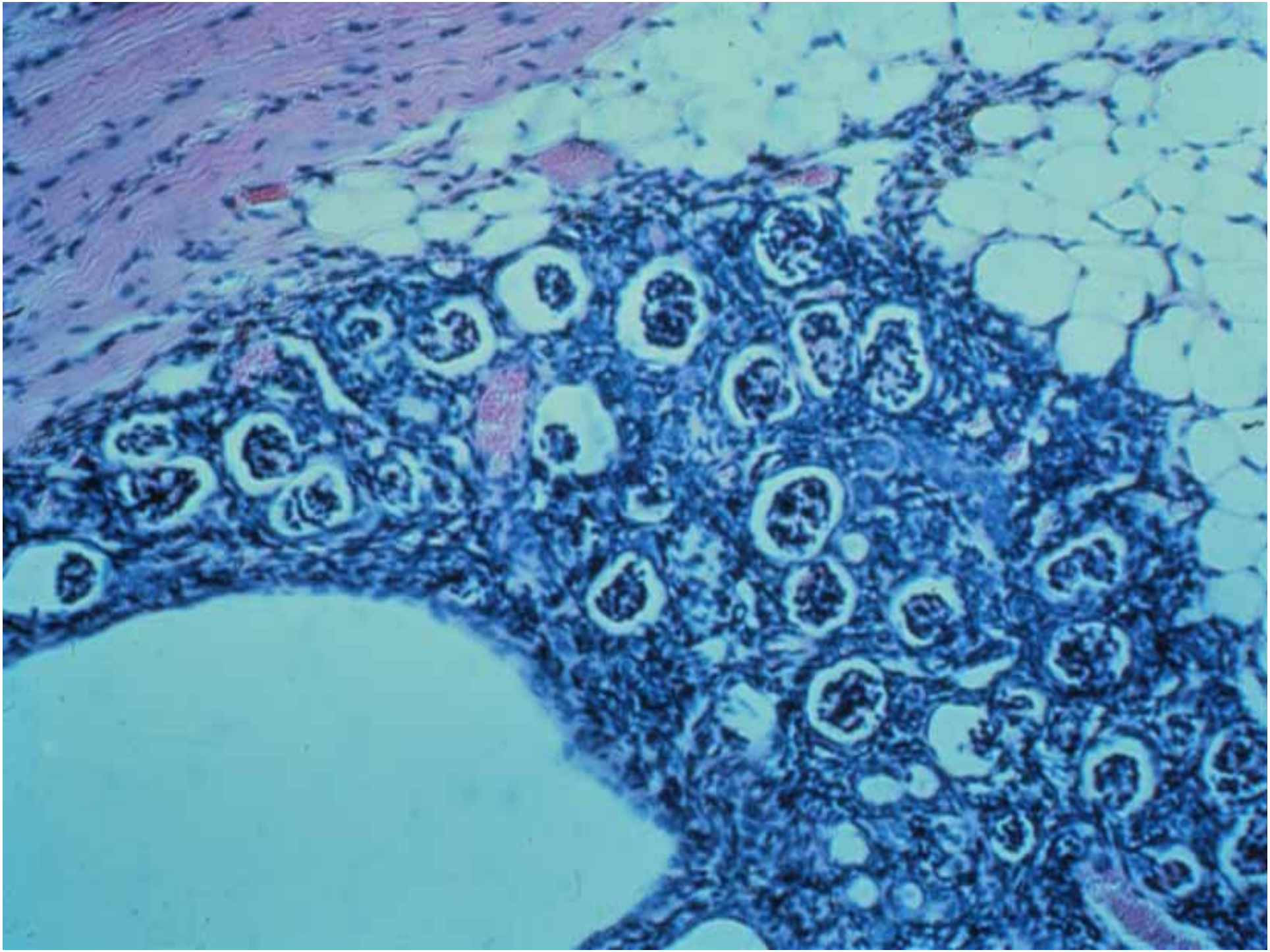
SEED AND SOIL HYPOTHESIS

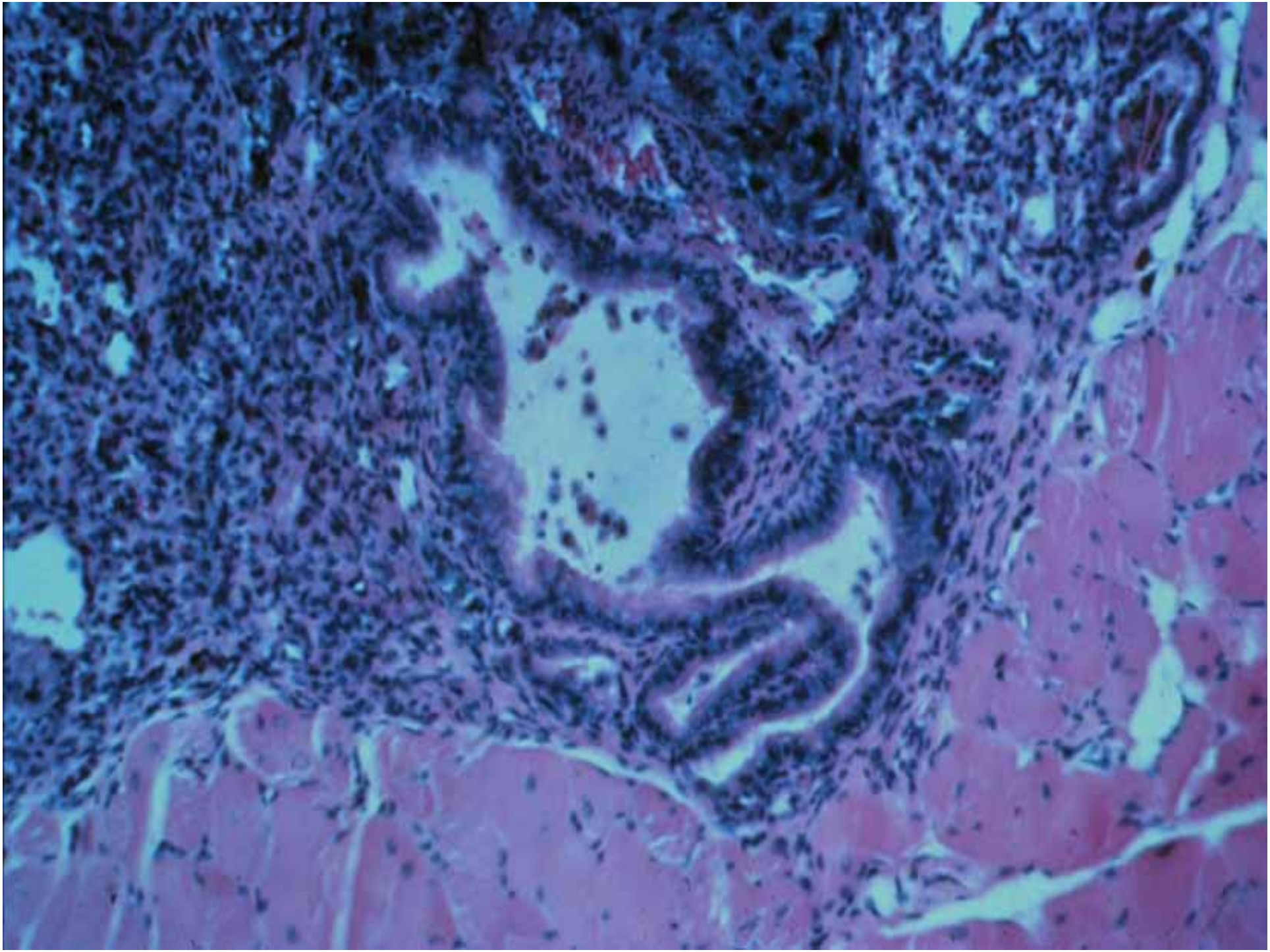
- PATTERNS OF METASTASIS ARE PREDICATABLE.
- CERTAIN TUMOR CELLS (SEED) HAVE AN AFFINITY FOR CERTAIN ORGANS (SOIL).
- METASTASIS OCCURS ONLY WHEN THE SEEDS AND THE SOIL ARE COMPATIBLE.

DR. STEPHEN PAGET, 1889


Lung
Kidney
Organs from 1-day-old mouse
1-mm3 fragments
Fragments implanted into quadriceps femoris
3 weeks later
i.v. injection of lung-specific melanoma cells
4 weeks later
Hemodynamic
or
Hemodynamic seed and soil



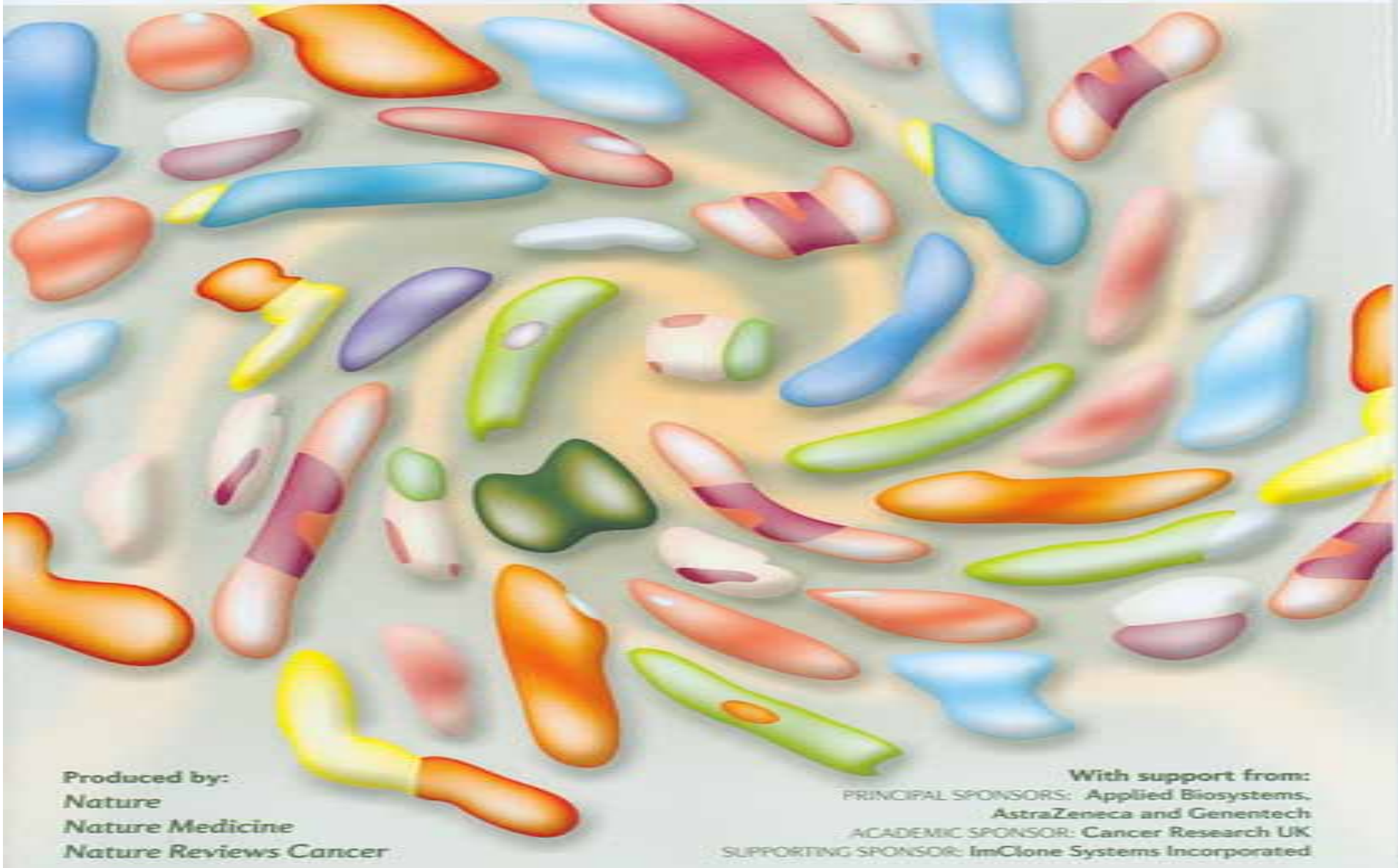




April 2006 http://www.nature.com/milestones/cancer
Supplement to Nature Publishing Group

nature MILESTONES

CANCER



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Observations from a ploughman

"What is it that decides what organs shall suffer a case of disseminated cancer?" This question intrigued Stephen Paget, assistant surgeon to the West London hospital and the Metropolitan hospital, whose self-effacing paper of 1889 records his careful analyses of case histories that led to the visionary 'soil and seed' hypothesis of metastasis.

"When a plant goes to seed, its seeds are carried in all directions," he wrote. "But they can only live and grow if they fall on congenial soil." This idea was at odds with one prevalent theory of the time, which stated that cancer cells, having been spread through the body in the blood or lymph, could lodge in a tissue and persuade the surrounding cells to grow similarly. However, Paget followed the school of thought that all cancer cells could continually develop wherever they settled, but grew only in certain organs that were somehow predisposed to a secondary cancer.

Paget reasoned that if the organs where secondary tumours arose were 'passive' in the process, then these cancers would be distributed randomly. By analysing 735 case histories of fatal breast cancer, he found that metastases formed in the liver far more often than in any other organ — even those such as the spleen that could be considered to have the same exposure to the cancer cells because of similar blood flows.

This was enough to persuade Paget that sites of secondary growths are not a matter of chance, and that some organs provide a more fertile environment than others for the growth of certain metastases. "The best work in the pathology of cancer is now done by those who .... are studying the nature of the seed," he noted. "They are like scientific botanists; and he who turns over the records of cases of cancer is only a ploughman, but his observation of the properties of the soil may also be useful."

This proved to be the case and, although it languished in the shadows for many years, the seed and soil hypothesis was revived fully in 1980 by Ian Hart and Isaiah Fidler. By this time, clinical observations had established that certain organs were, indeed, more susceptible to metastasis, even after specific properties of the tumour cells and other host factors had been accounted for.

So, Hart and Fidler examined whether the locations of metastases exist merely because tumour cells tend to come to rest in particular organs — for instance, because the blood capillaries are more narrow — or because the distributed cells can only grow at particular sites, in accordance with the Paget hypothesis. Using mice, they grafted kidney, ovary and lung tissue under the skin or into the muscle, and showed that the transplanted tissues established their own blood supply. They then injected the mice with melanoma cells. Metastases developed in the grafted lung and ovary tissue but not in the renal tissue, thereby showing a distinct preference.

Notably, radioactive labelling of the injected cells showed that they were equally likely to be trapped in the kidney tissue as in either of the other transplants. So, just landing in a tissue is not sufficient for cancer cells to develop a secondary tumour; rather, some property of the tissue itself must sustain the new growth. The idea that cancer cells require some 'nourishment' from their environment to develop still motivates research today, with the focus now being on unravelling the molecular mechanisms that bring seed and soil together to promote metastases.

Nikki Dell, Nature, Locum Associate
News and Views Editor

URL
http://www.cancer.gov/cancertopics/types/melanoma

References and links

ORIGINAL RESEARCH PAPERS Paget, S. The distribution of secondary growths in cancer of the breast. *Lancet* **1**, 571–573 (1889) | Hart, I. R. & Fidler, I. J. Role of organ selectivity in the determination of metastatic patterns of B16 melanoma. *Cancer Res.* **40**, 2281–2287 (1980)

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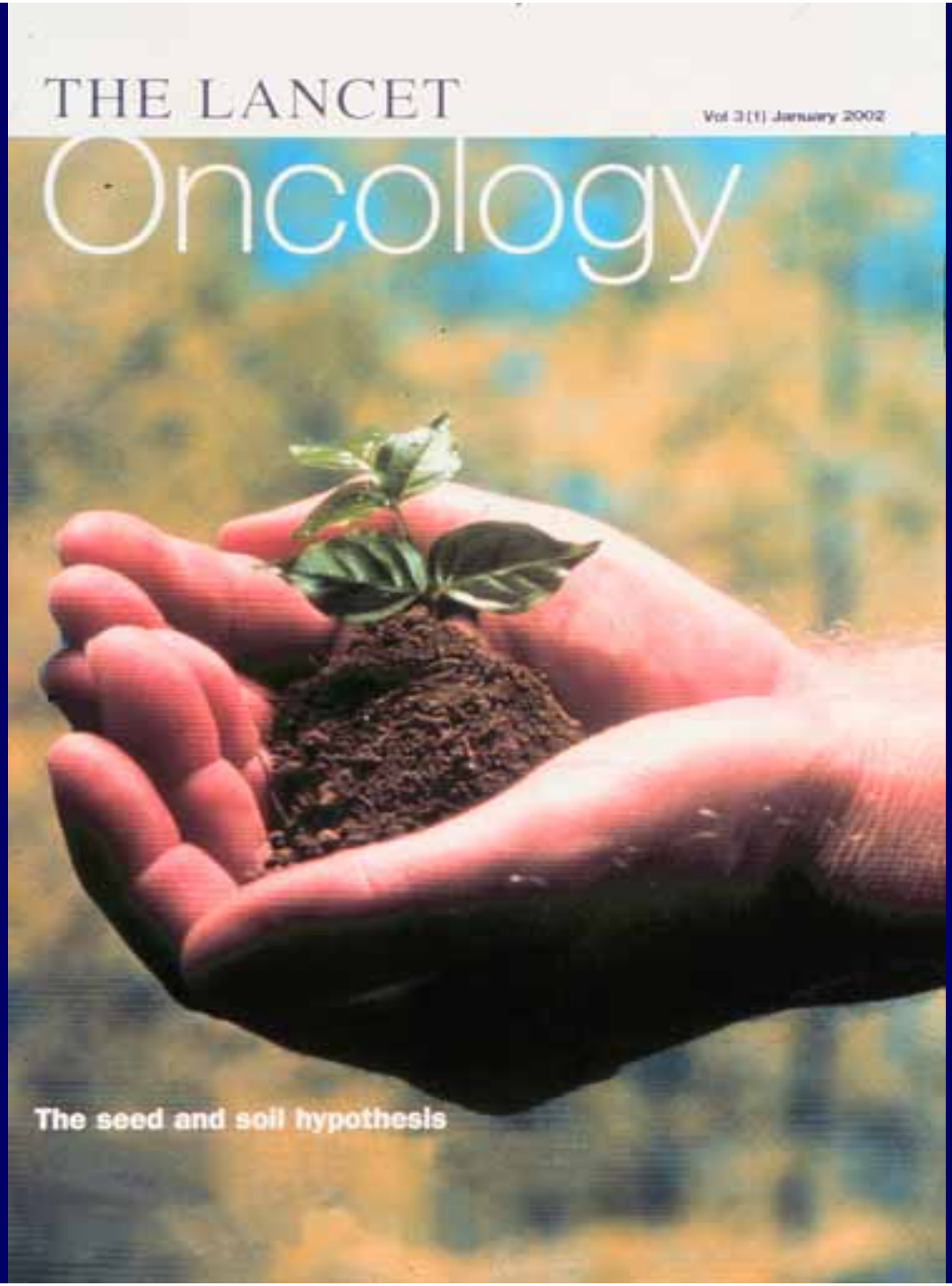

THE LANCET
Vol 3 [1] January 2002
Oncology
The seed and soil hypothesis

CANCER METASTASIS

PRIMARY NEOPLASMS ARE HETEROGENEOUS.

THE PROCESS IS HIGHLY SELECTIVE FOR PREEXISTING METASTATIC CELLS.

THE PROCESS DEPENDS ON THE INTERACTION OF TUMOR CELLS WITH HOST FACTORS.

Brain metastasis

20-40% of all cancer patients develop CNS metastasis

In adults the primary tumors are:

Lung (50-60%),

Breast (15-20%),

Melanoma (8-10%)

GI tract (5-7%)

Landis SH, et al. Cancer statistics, 1998.

Brain metastasis

For untreated patients the median survival is 1-2 months.

Conventional treatment (radio-chemotherapy) can extends the median survival to 4-6 months.

Zimm S, et al. Cancer 1981

Kehrli P et al Neurochirurgie 1999;

Sawaya R, et al. Brain tumors. Churchill-Livingstone; 2001.

BRAIN METASTASES ARE RESISTANT TO CHEMOTHERAPY

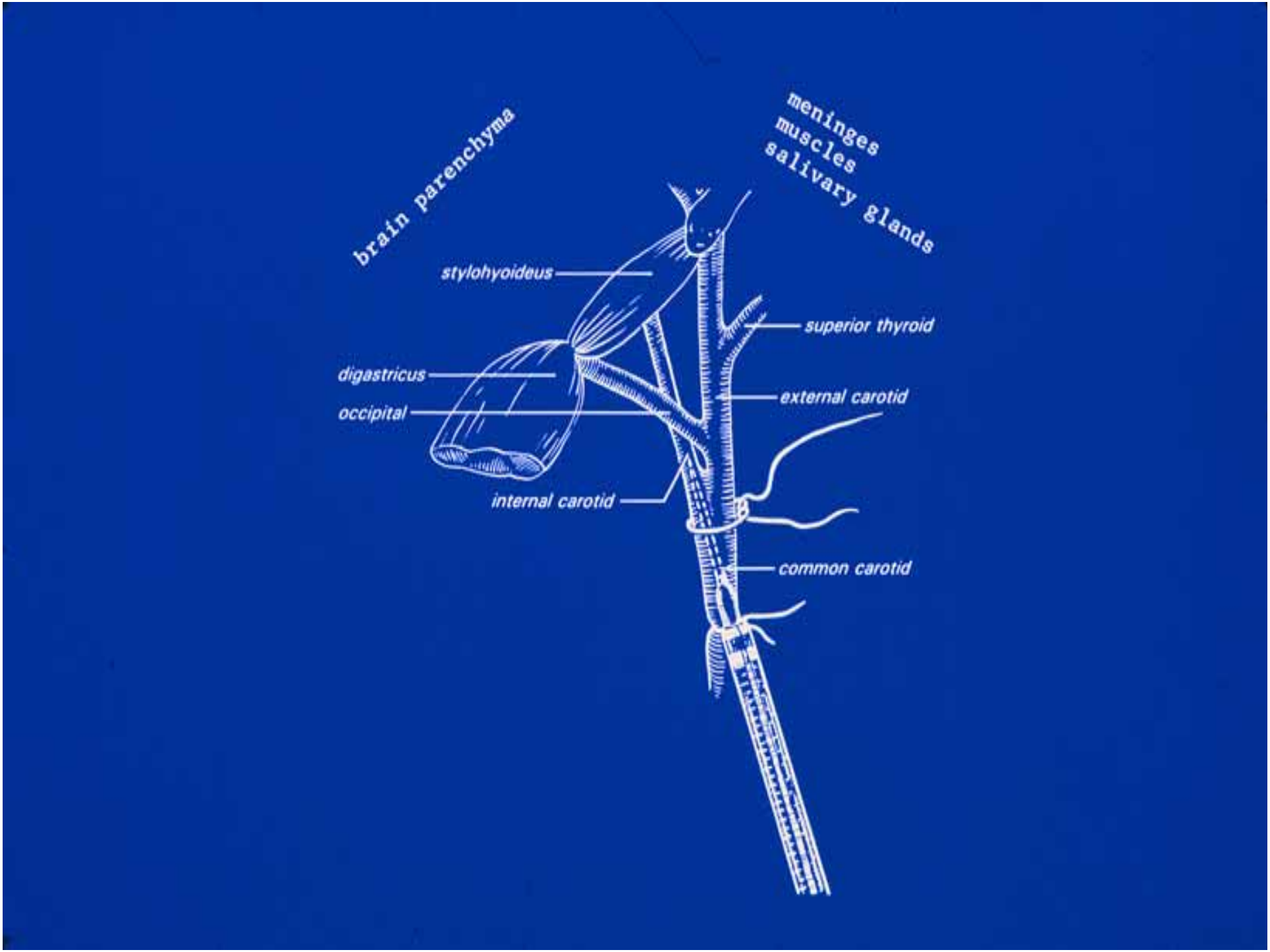
BLOOD – BRAIN – BARRIER

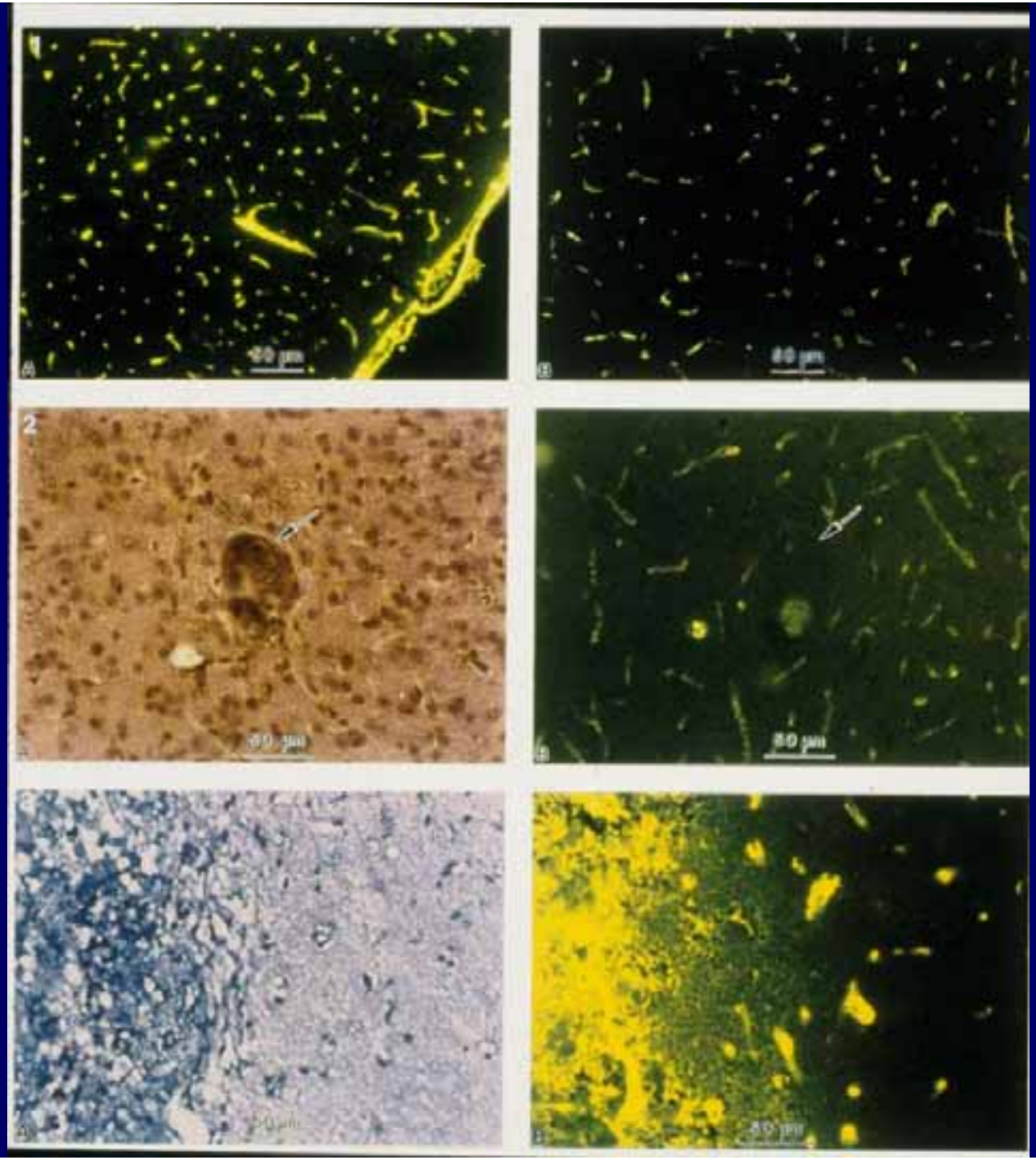
MODELS FOR HUMAN CANCER METASTASIS MUST EMPLOY:

RELEVANT TUMOR CELLS (SEED)

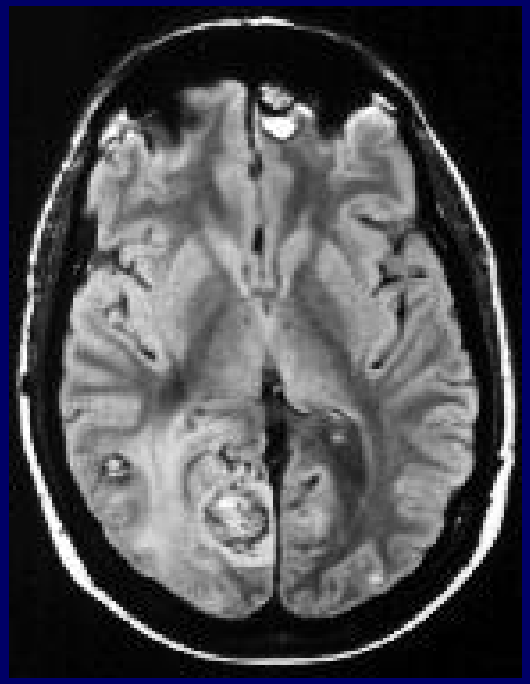
RELEVANT ORGAN MICRENVIRONMENT(SOIL)

Orthotopic implantation


brain parenchyma
meninges
muscles
salivary glands
stylohyoideus
superior thyroid
digastricus
external carotid
occipital
internal carotid
common carotid

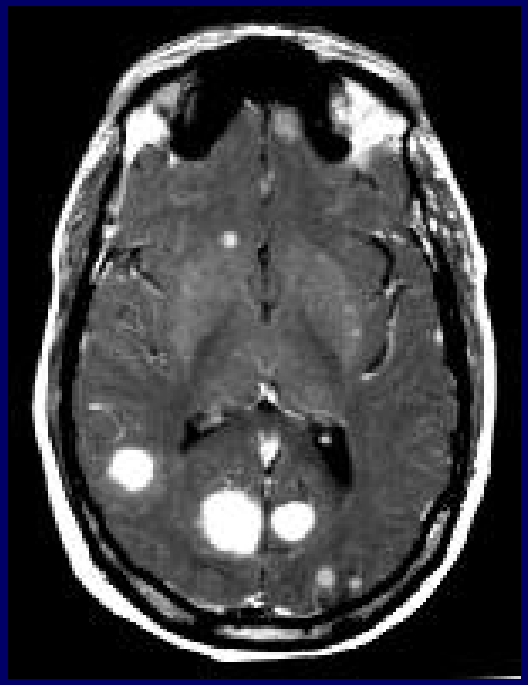


Imaging of melanoma brain metastasis



MRI T1 Weighed Image

Without contrast agent (gadolinium)

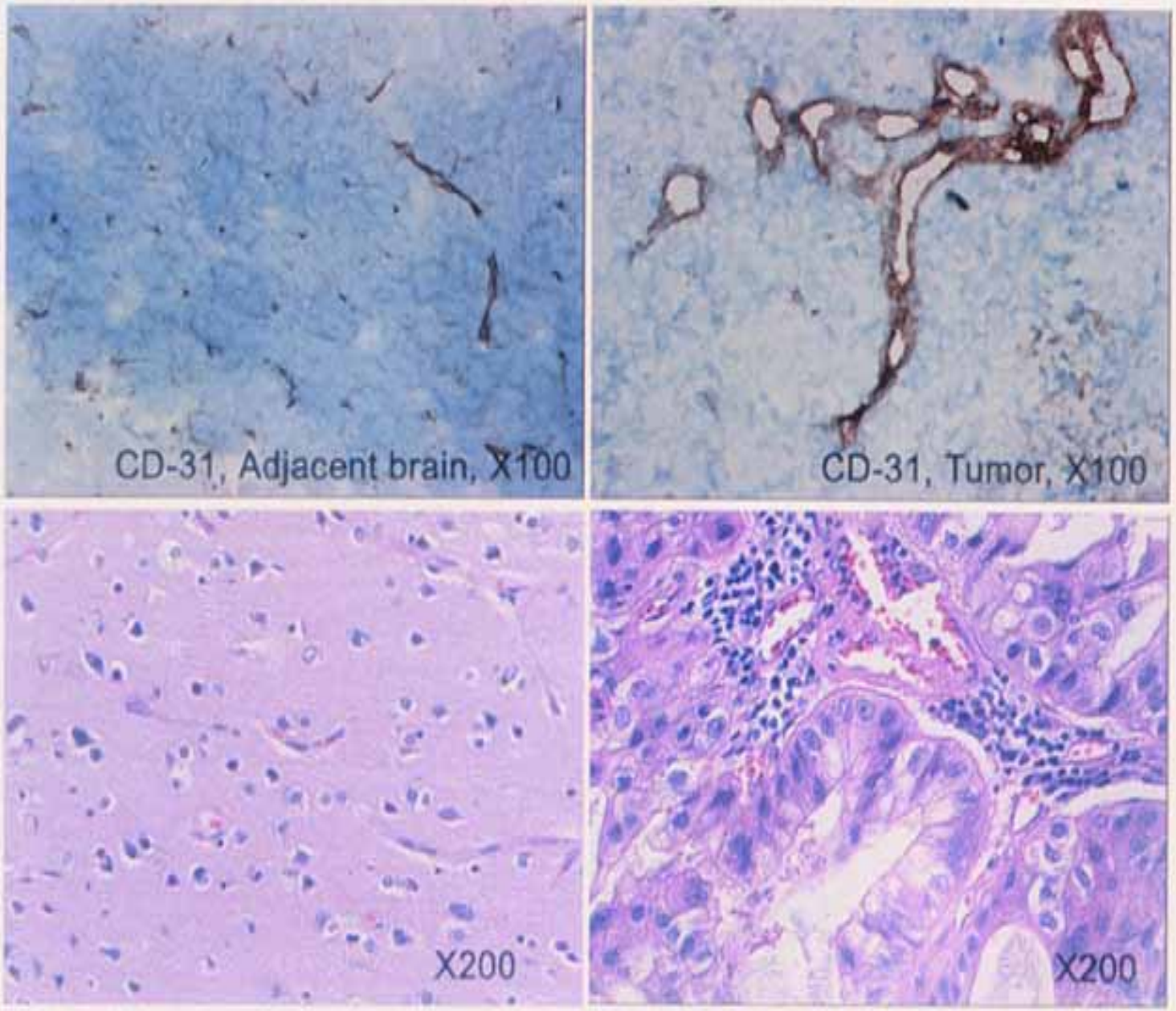


MRI T1 Weighed Image

With contrast agent (gadolinium)

UHrad.com (University Hospitals of Cleveland)

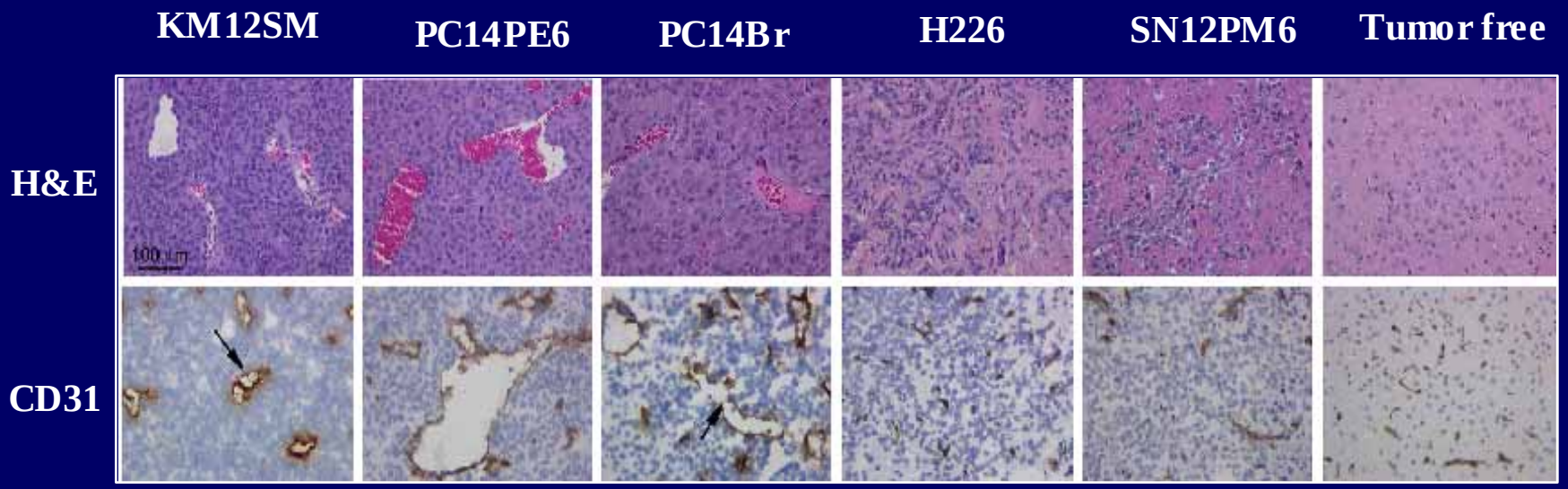
Human Lung Adenocarcinoma Brain Metastasis



EXPRESSION OF VPF-VEGF

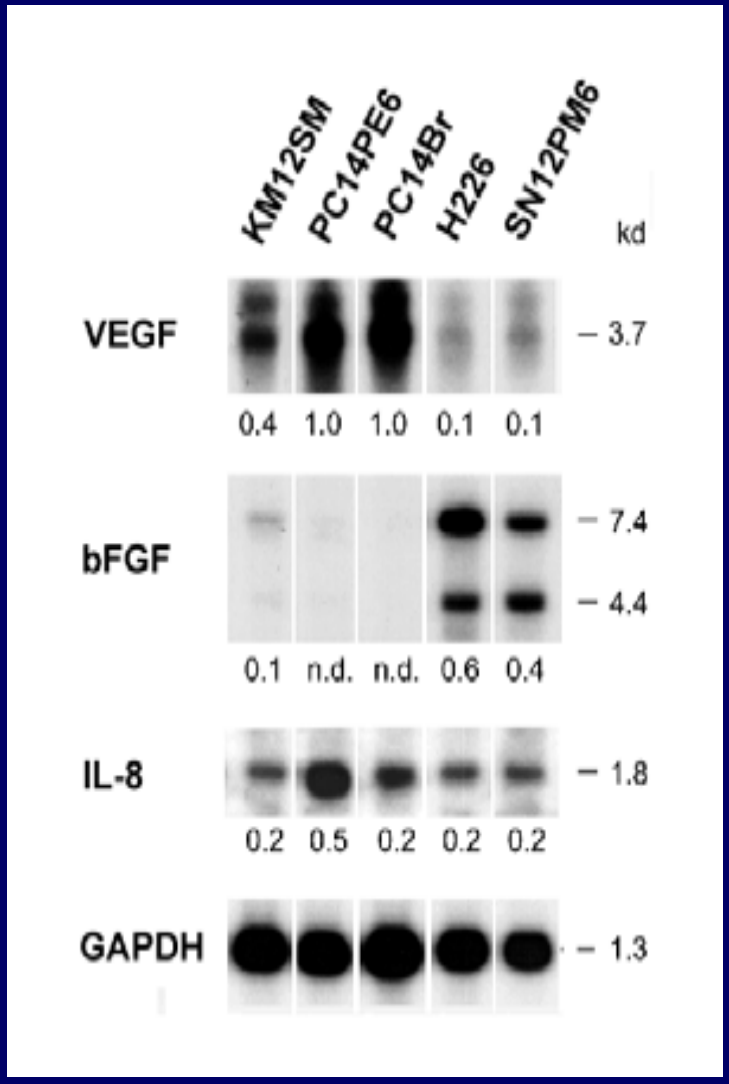
Brain metastasis and vascularization

Cell line	Brain mets. Incidence	Survival (days)	MVD		Large vessels (%)	
			Tumor	NT	Tumor	NT
KM12SM	12/14	30-42	10±1	45±5	75±20	1±1
PC14PE6	11/14	30-53	12±3	35±6	58±14	1±1
PC14Br	10/10	27-52	16±5	42±7	69±21	2±3
H226	3/10	92-110	25±4	37±5	4±1	1±1
SN12PM6	3/7	104-108	20±4	49±13	9±4	1±1

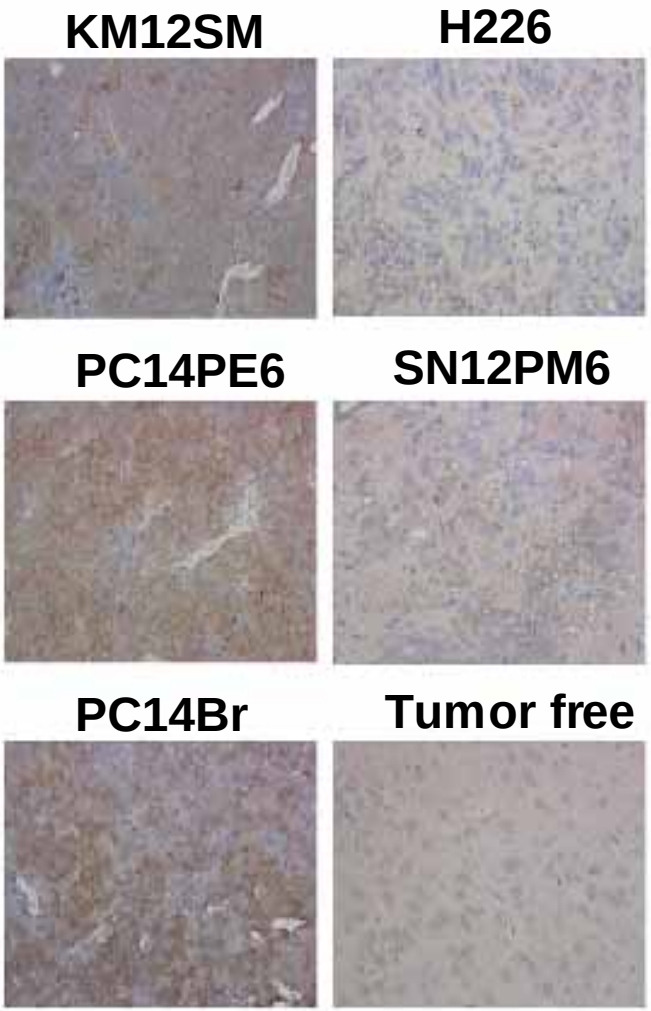


Expression of angiogenic cytokines

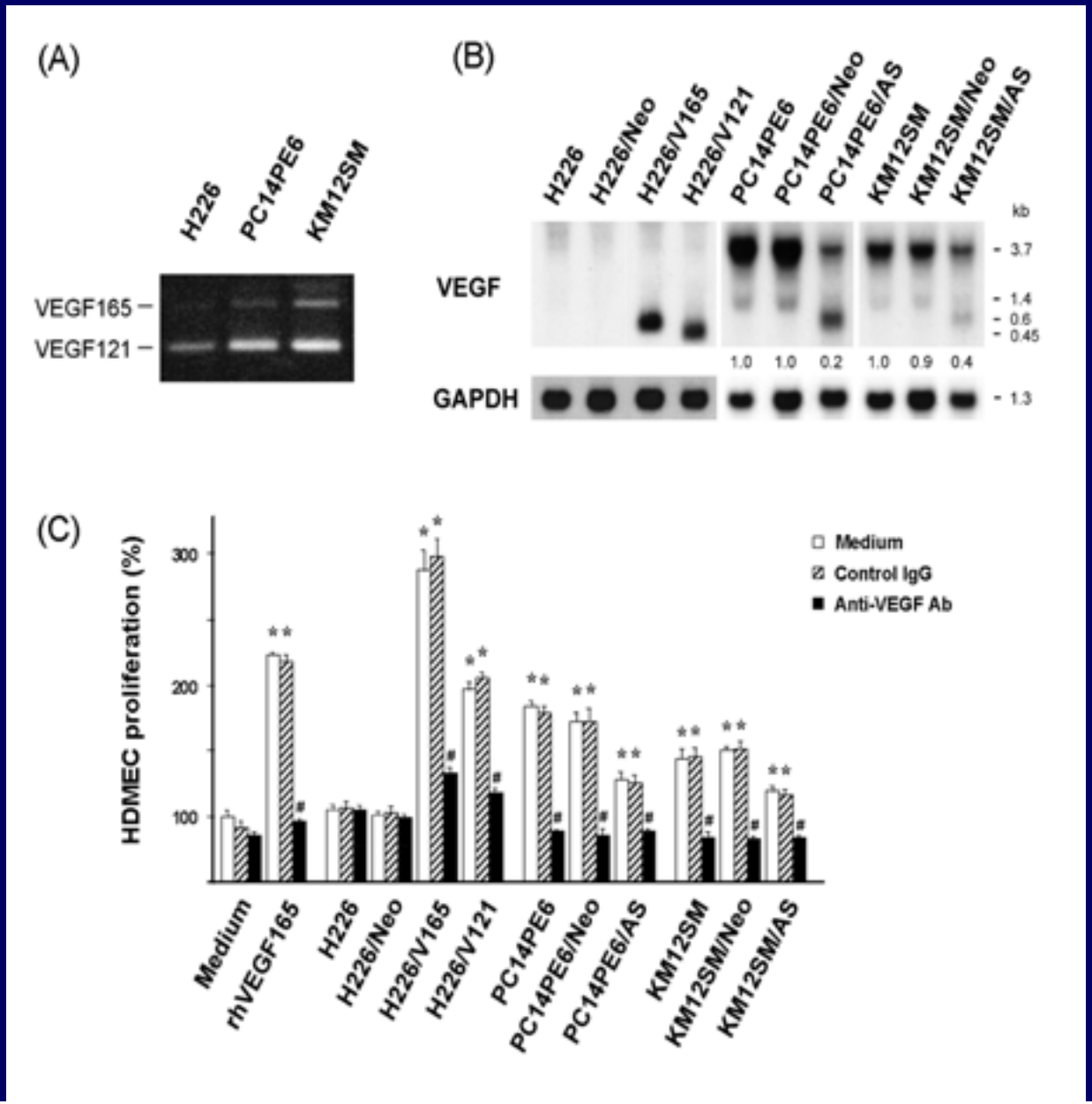
mRNA expression *in vitro*



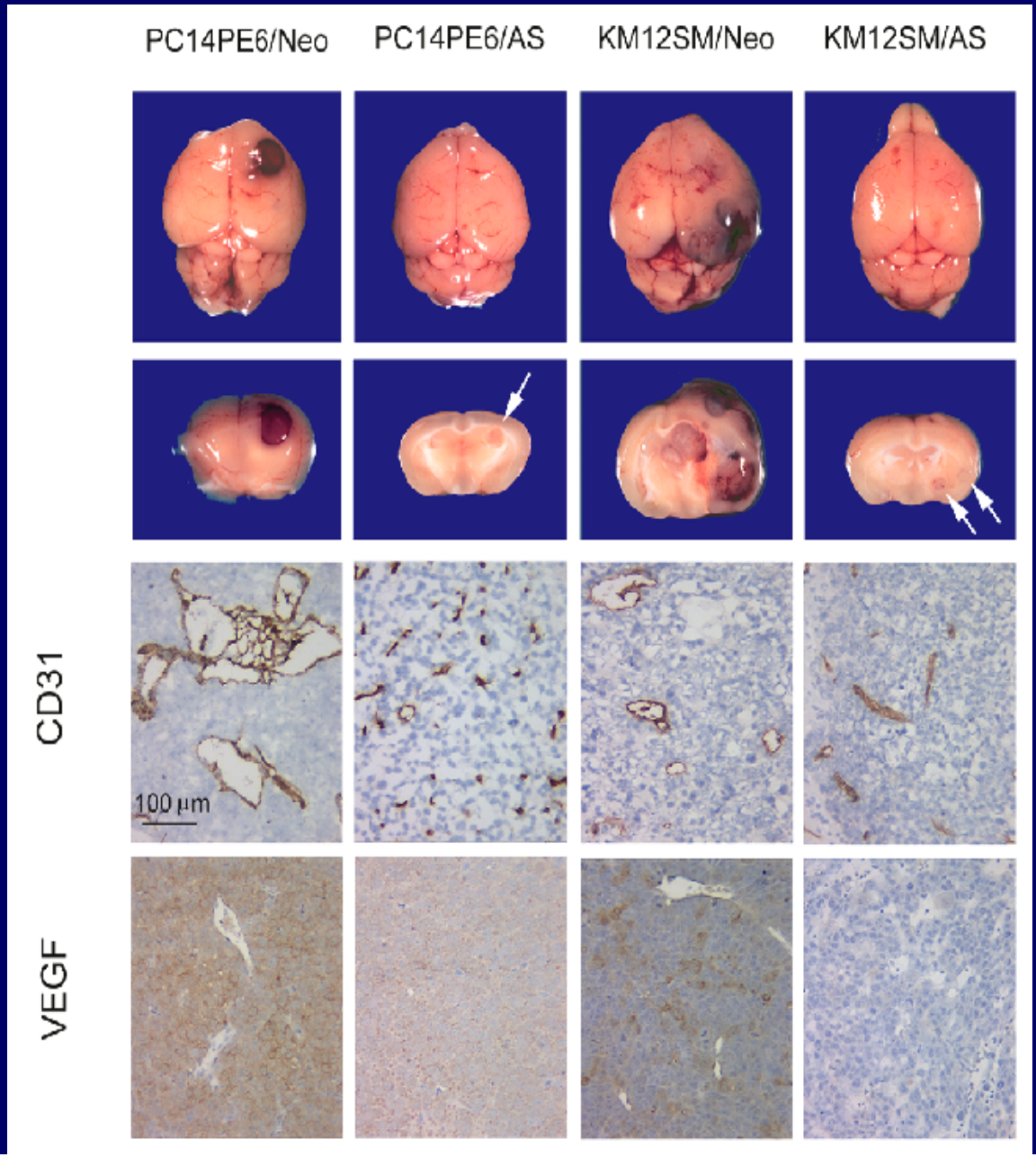
VEGF expression *in vivo* (brain)



Transfection of sense or antisense VEGF gene



Antisense-VEGF165 gene transfection



TRANSFECTION OF THE VEGF121 OR 165 GENES INTO H226 CELLS DID NOT RESULT IN PRODUCTION OF BRAIN METASTASIS

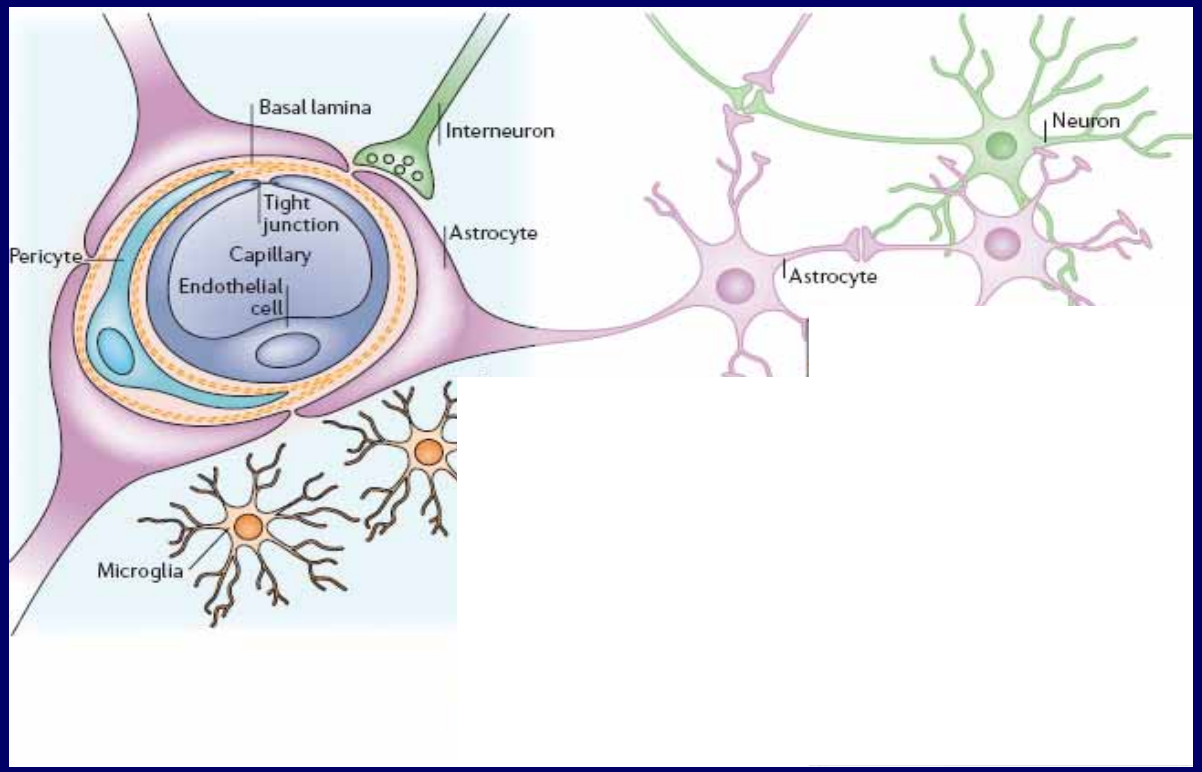
EXPRESSION OF VPF-VEGF IS ESSENTIAL BUT NOT SUFFICIENT FOR PRODUCTION OF BRAIN METASTASIS

BRAIN METASTASES ARE RESISTANT TO CHEMOTHERAPY

THE BRAIN MICROENVIRONMENT:

ASTROCYTES

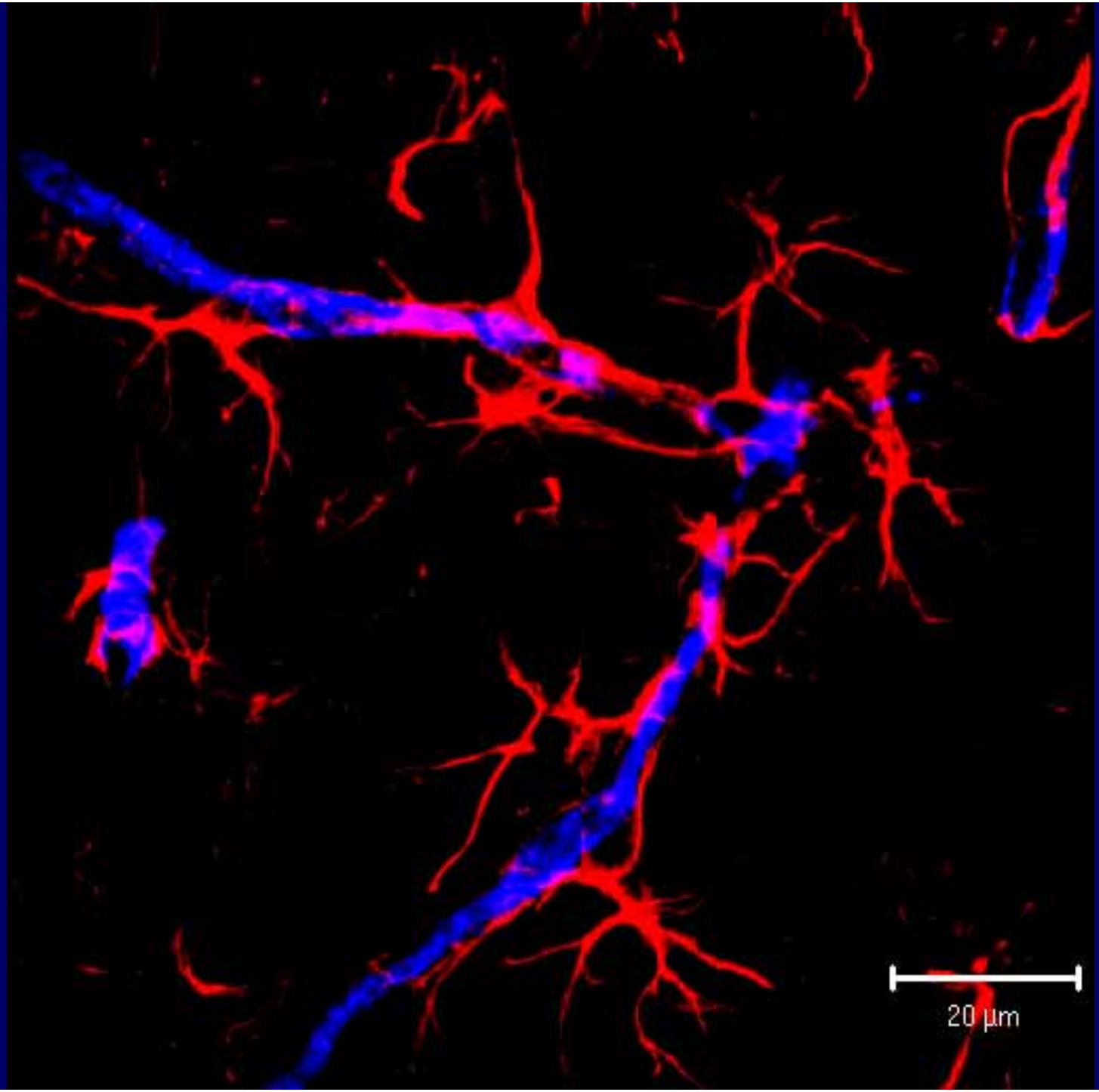
Astrocytes in the Brain microenvironment

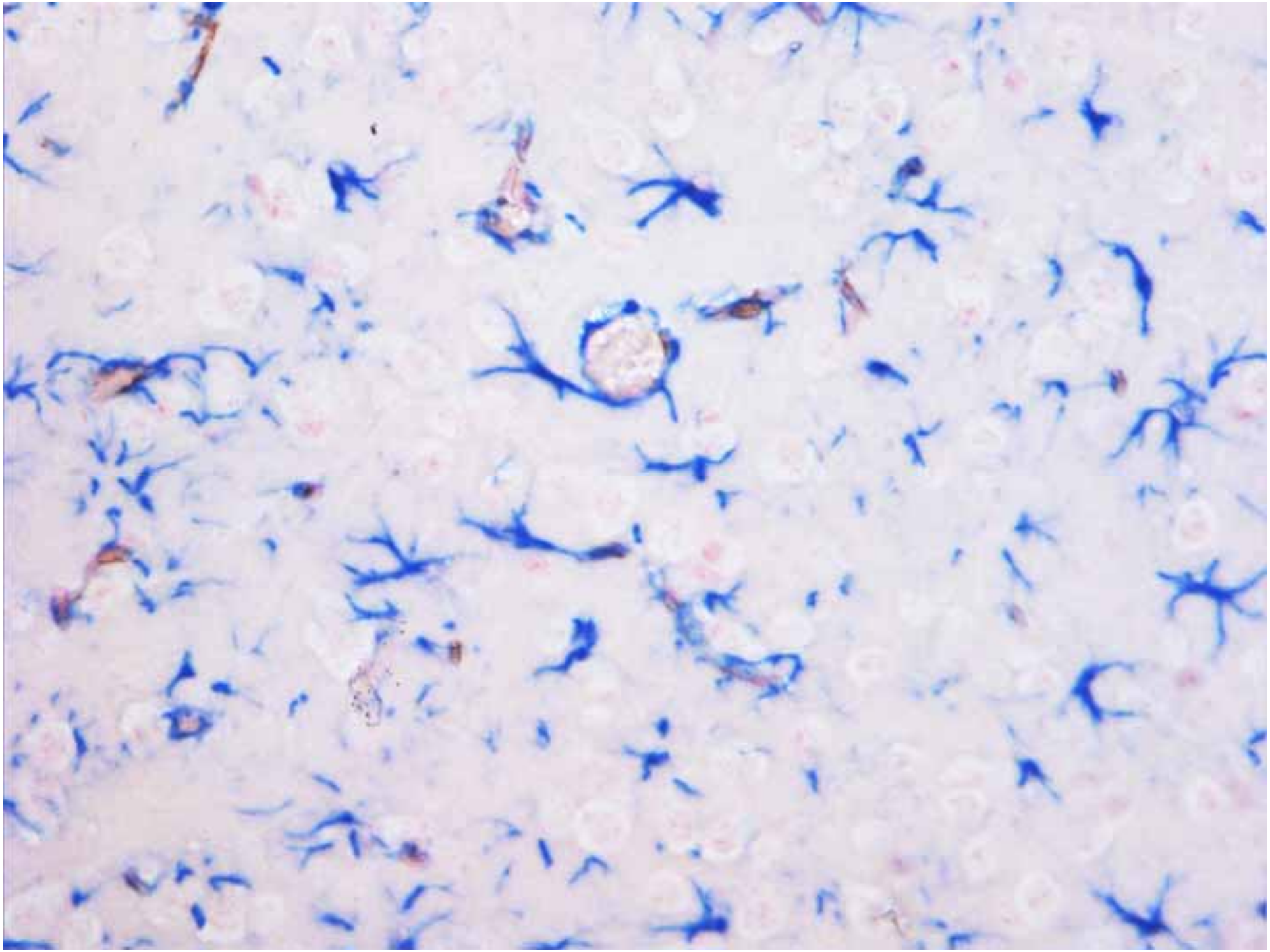


Functions of astrocytes:

- **Transport nutrients from blood to neurons**
- **Protect neurons**
- **Participate in neuronal signal transmission**
- **Maintain homeostasis: [k]+ [Na]+, [PH], H_2O**

Abbott NJ, et. al. Nat Rev Neurosci. 2006.


20 µm



Activated Astrocytes Protect Neurons

- Faulkner JR, Herrmann JE, Woo MJ, et al. Reactive astrocytes protect tissue and preserve function after spinal cord injury. J Neurosci. 2004 Mar 3;24(9):2143-55.
- Wang XF, Cynader MS. Pyruvate released by astrocytes protect neurons from copper-catalyzed cysteine neurotoxicity. J Neurosci. 2001 May 15;21(10):3322-31.
- Desagher S, Glowinski J, Premont J. Astrocytes protect neurons from hydrogen peroxide toxicity.J Neurosci. 1996 Apr 15; 16(8):2553-62.
- Van Damme P, Bogaert E, Dewil M, et al. Astrocytes regulate GluR2 expression in motor neurons and their vulnerability to excitotoxicity.Proc Natl Acad Sci U S A. 2007 Sep 11; 104(37):14825-30.

ASTROCYTES IN PHYSIOLOGY

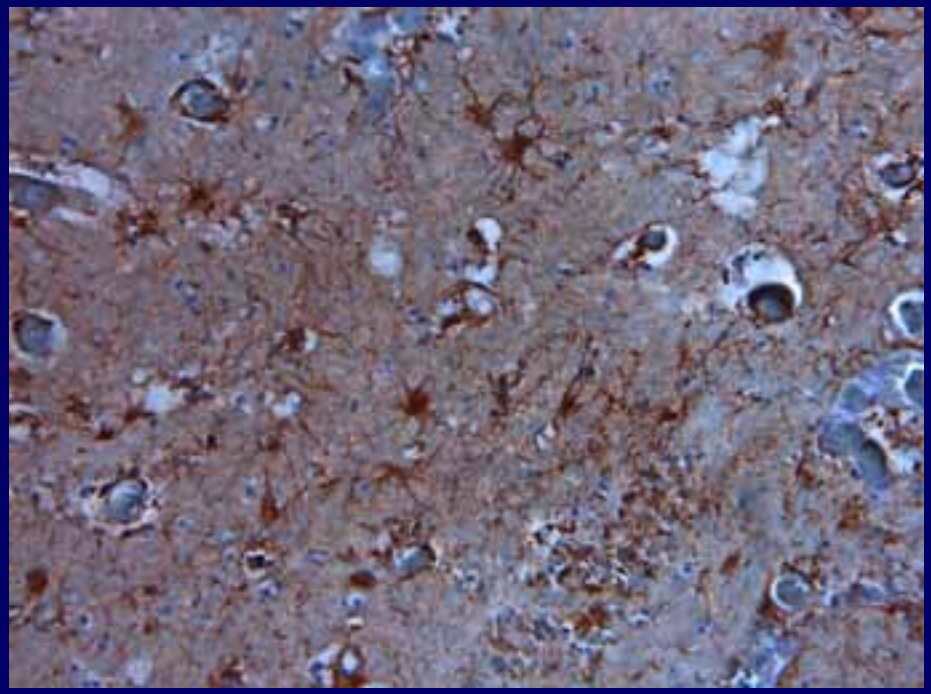
SUPPLY GLUCOSE AND OXYGEN TO NEURONS

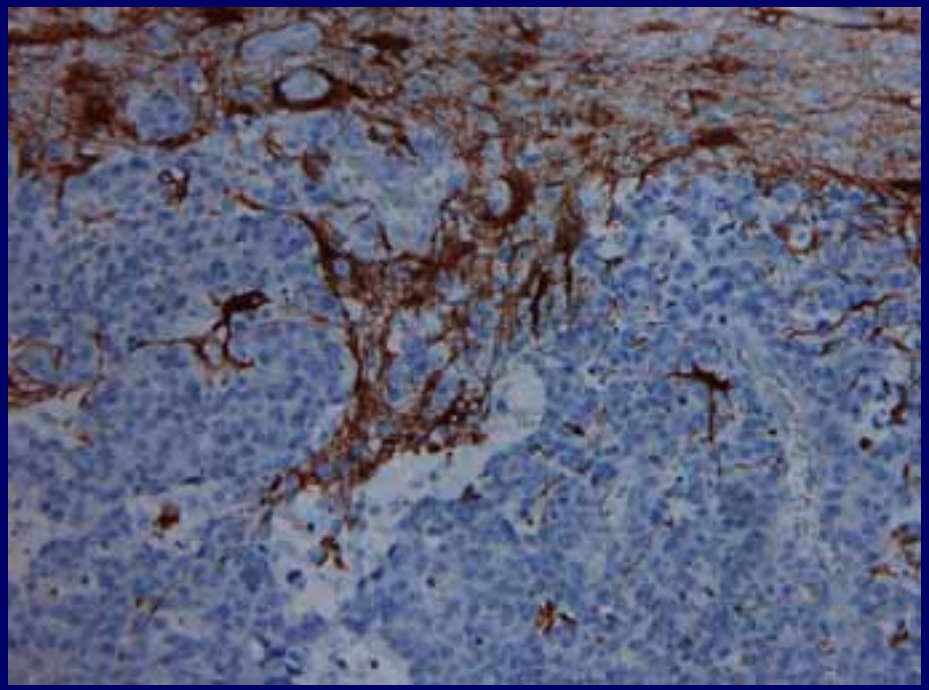
SURVIVAL OF NEURONS
AND ENDOTHELIAL CELLS

ASTROCYTES IN PATHOLOGY

?

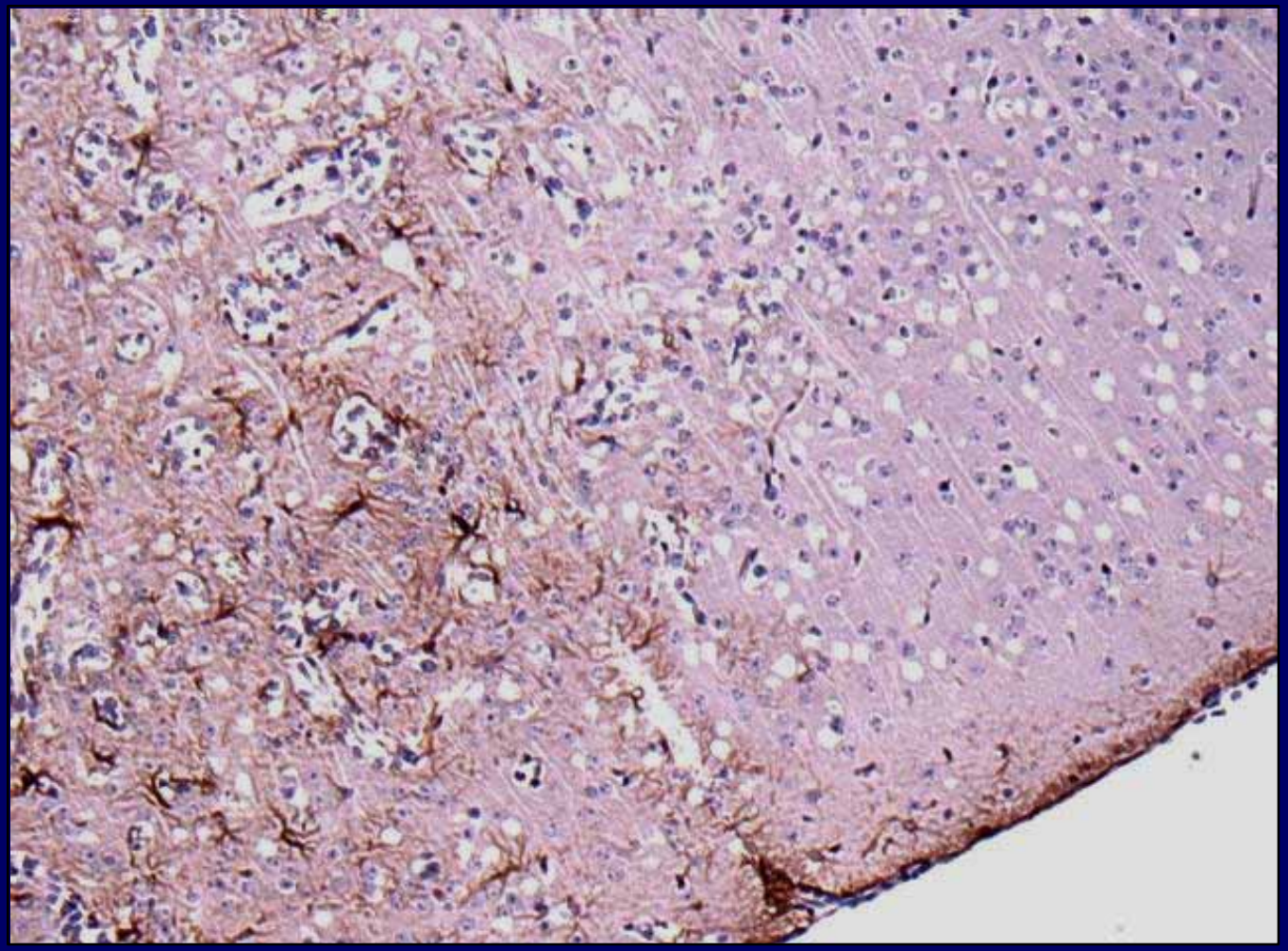
Lung cancer brain metastasis (clinical specimen)





Astrocyte staining with GFAP (glial fibrillary acidic protein)

Lung Cancer



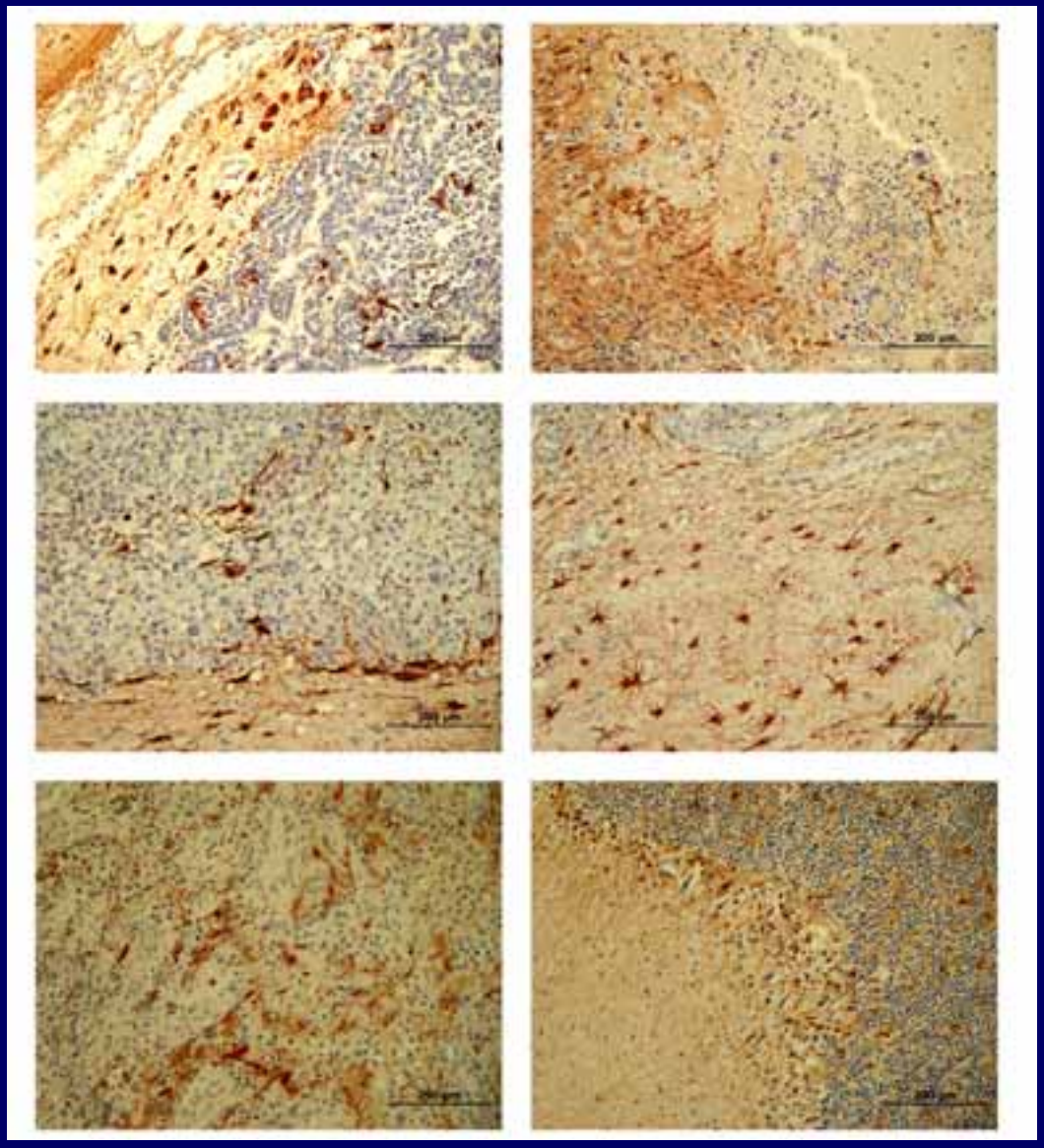
Astrocytes/tumor region Tumor free

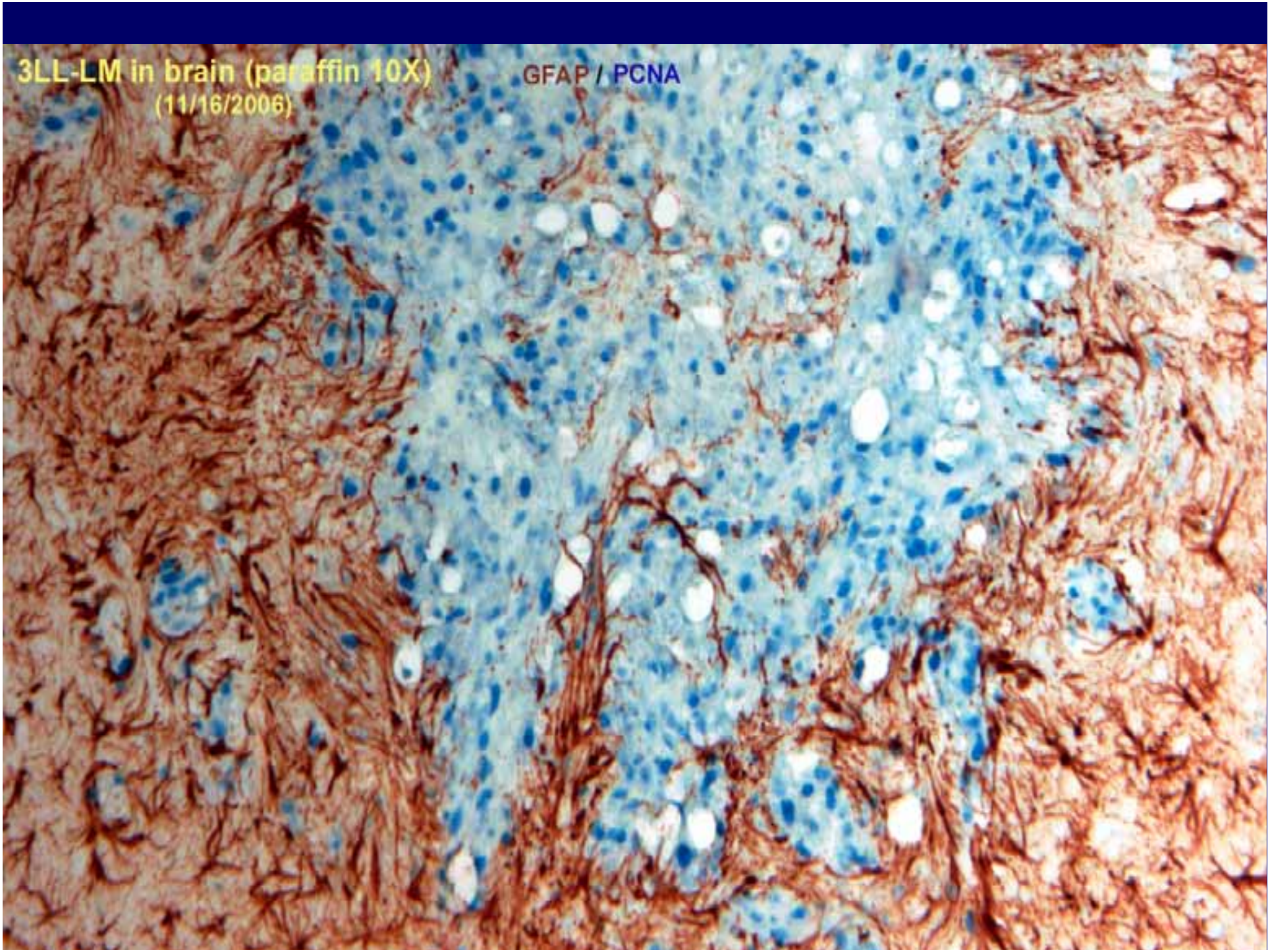
ACTIVATED ASTROCYTES IN BRAIN METASTASES

Breast Cancer

Lung Cancer

Melanoma




3LL-LM in brain (paraffin 10X)
(11/16/2006)
GFAP / PCNA

ACTIVATED ASTROCYTES EXPRESS GFAP

In response to:

Hypoxia

Inflammation

VEGF

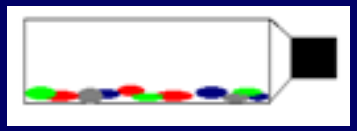
IL-6

IL-8

Isolation of Astrocytes from *H-2K^b-tsA58* Mice

Harvest Brain

Mixed Population of Cells

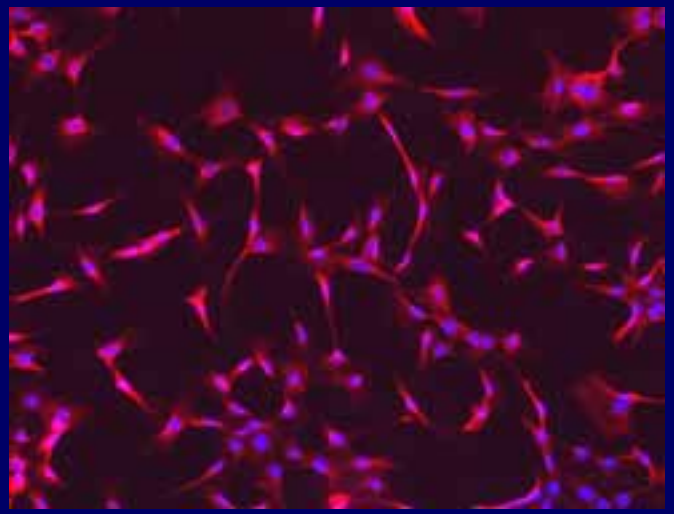


Rotary Shaking

250 RPM Overnight

ImmortoMice
H-2K^b-tsA58
Background: CBA/Ca x C57BL/10

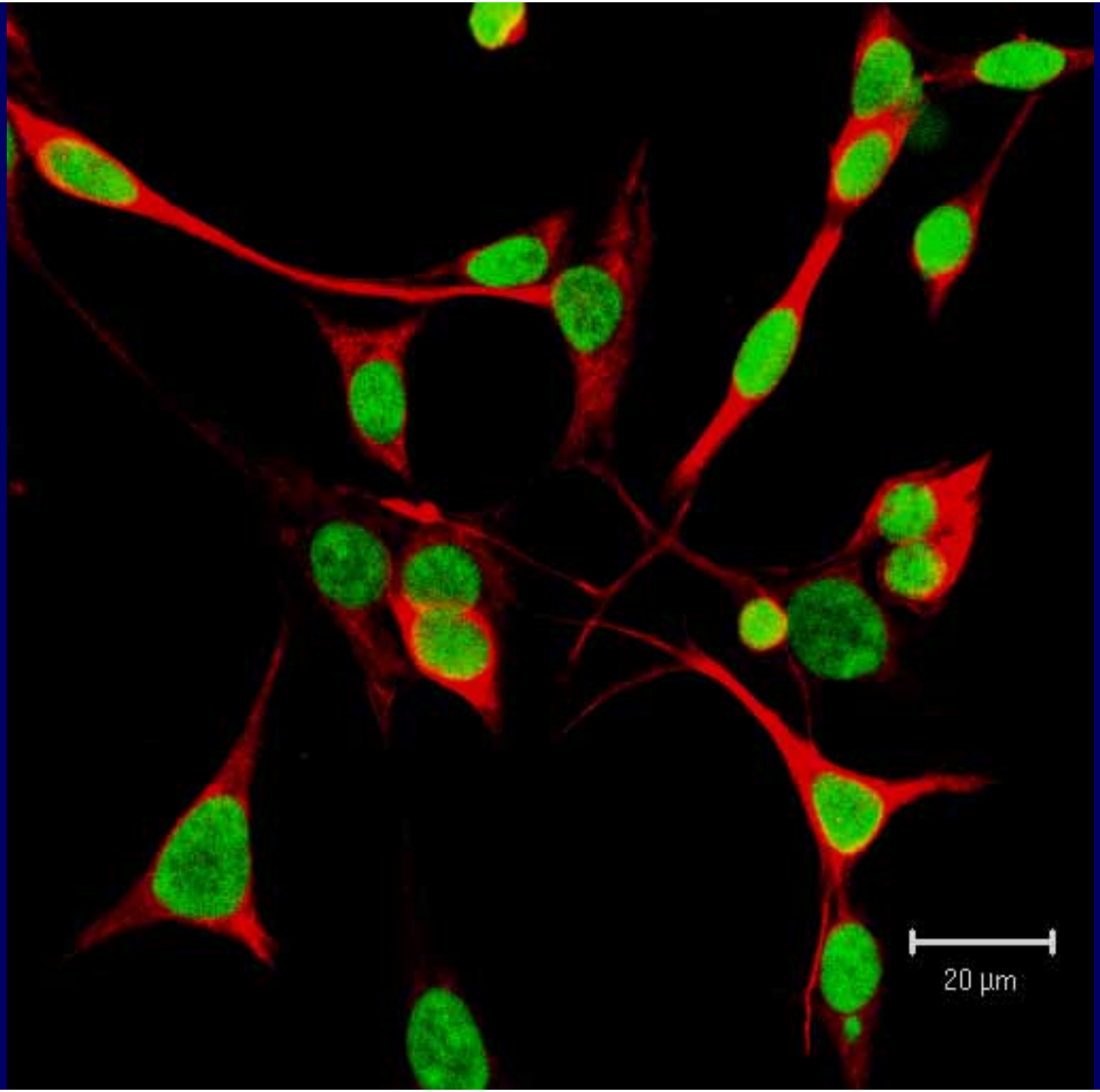
Evaluate GFAP Expression



Langley et al.,

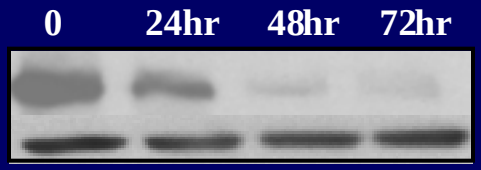
Growing Conditions

- Permissive: 33°
- Non-Permissive: 37°

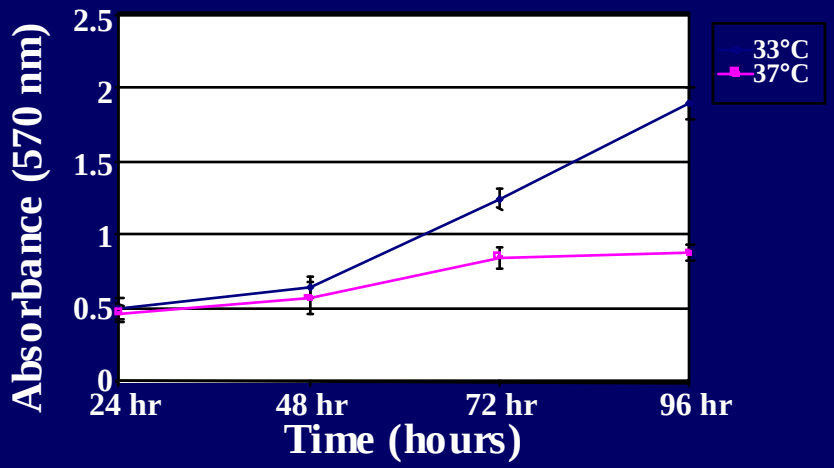

20 µm

Expression of SV40 Large T Antigen and Cell Proliferation of Immortal Mouse Astrocytes

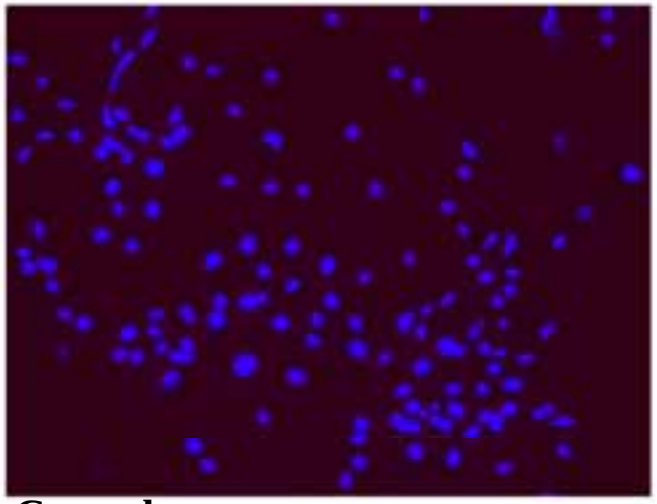
A.



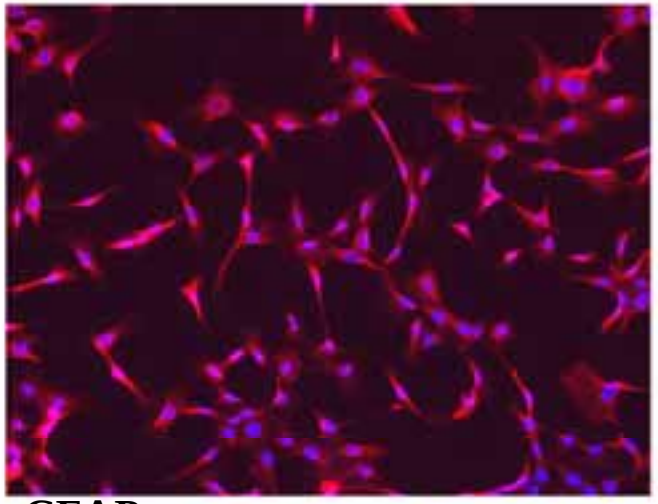
B.



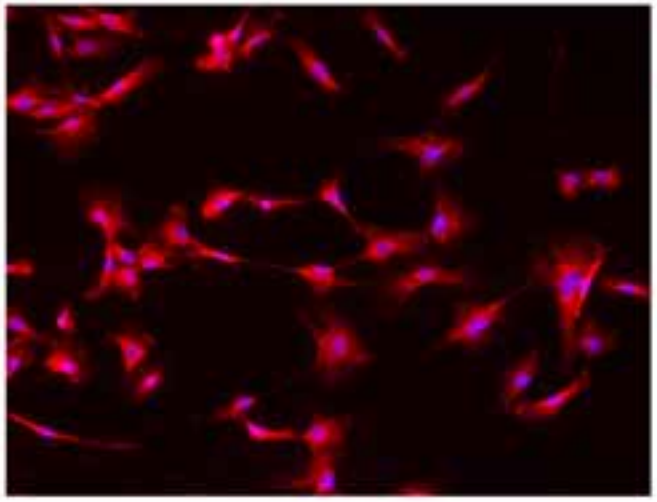
Immortalized Astrocytes from *H-2K^b-tsA58* Mice



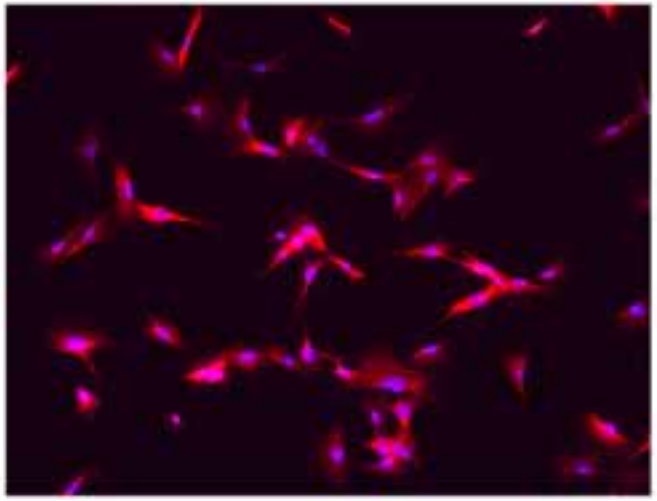
Control



GFAP

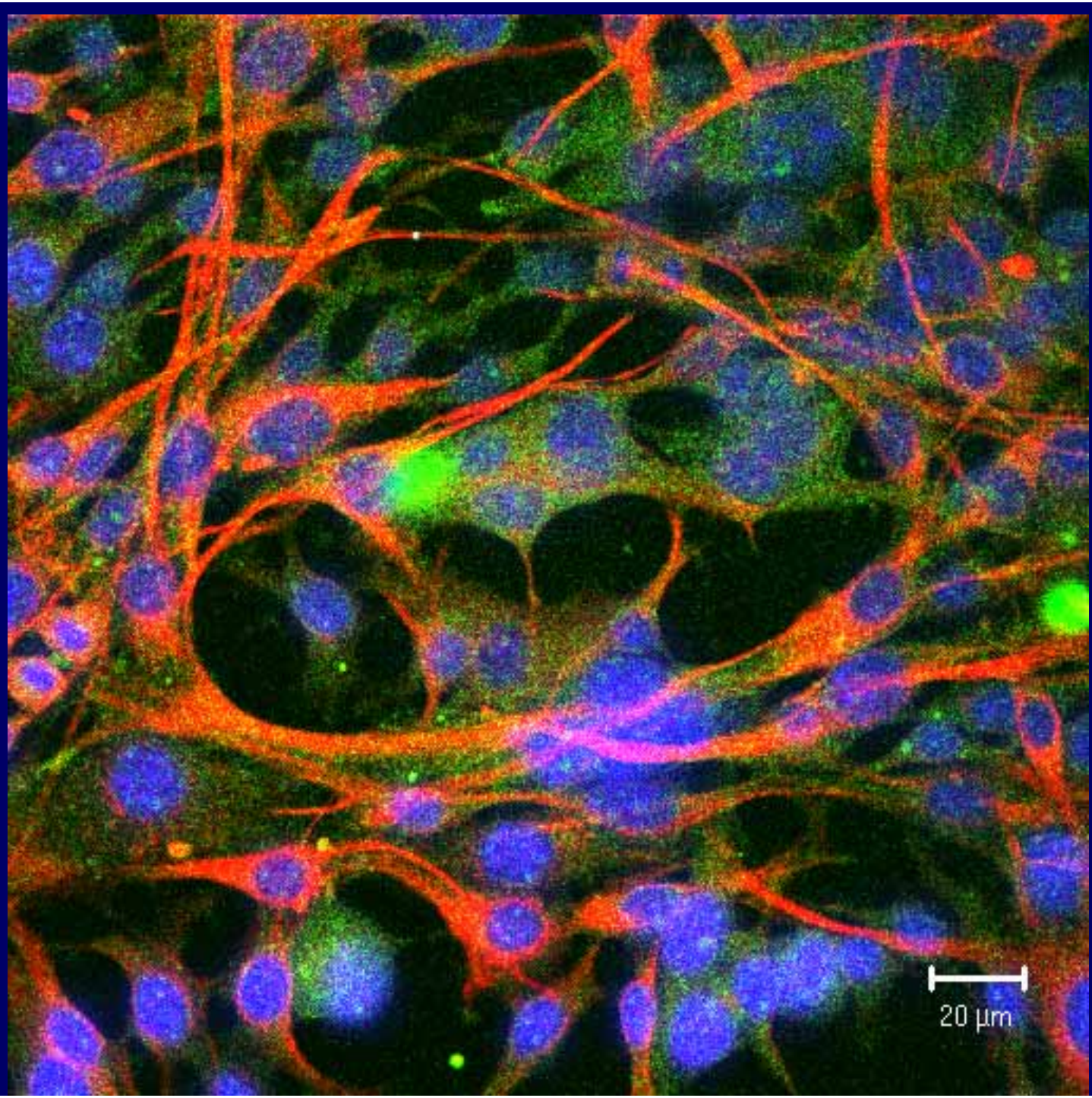


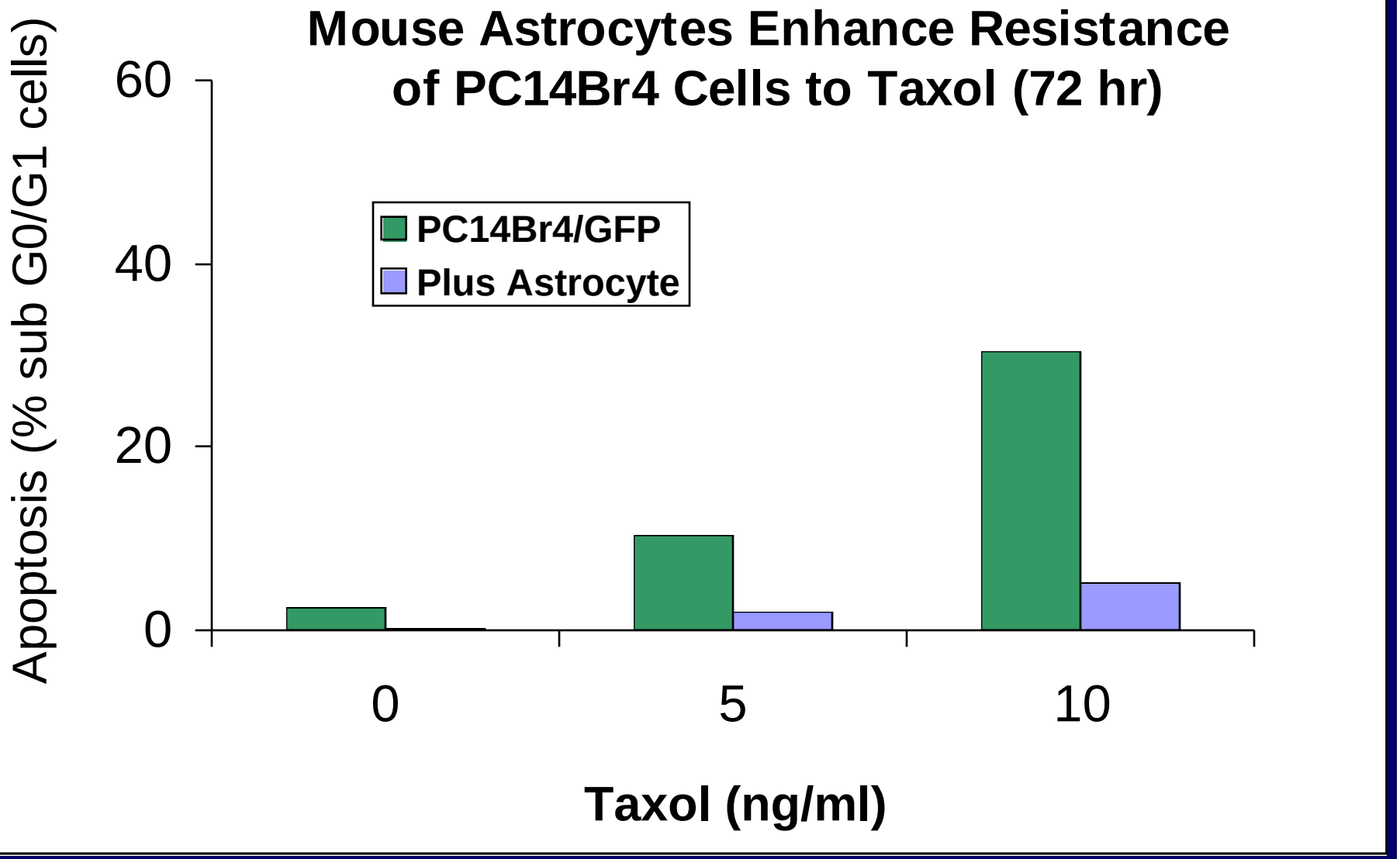
Glutamate Receptor 1

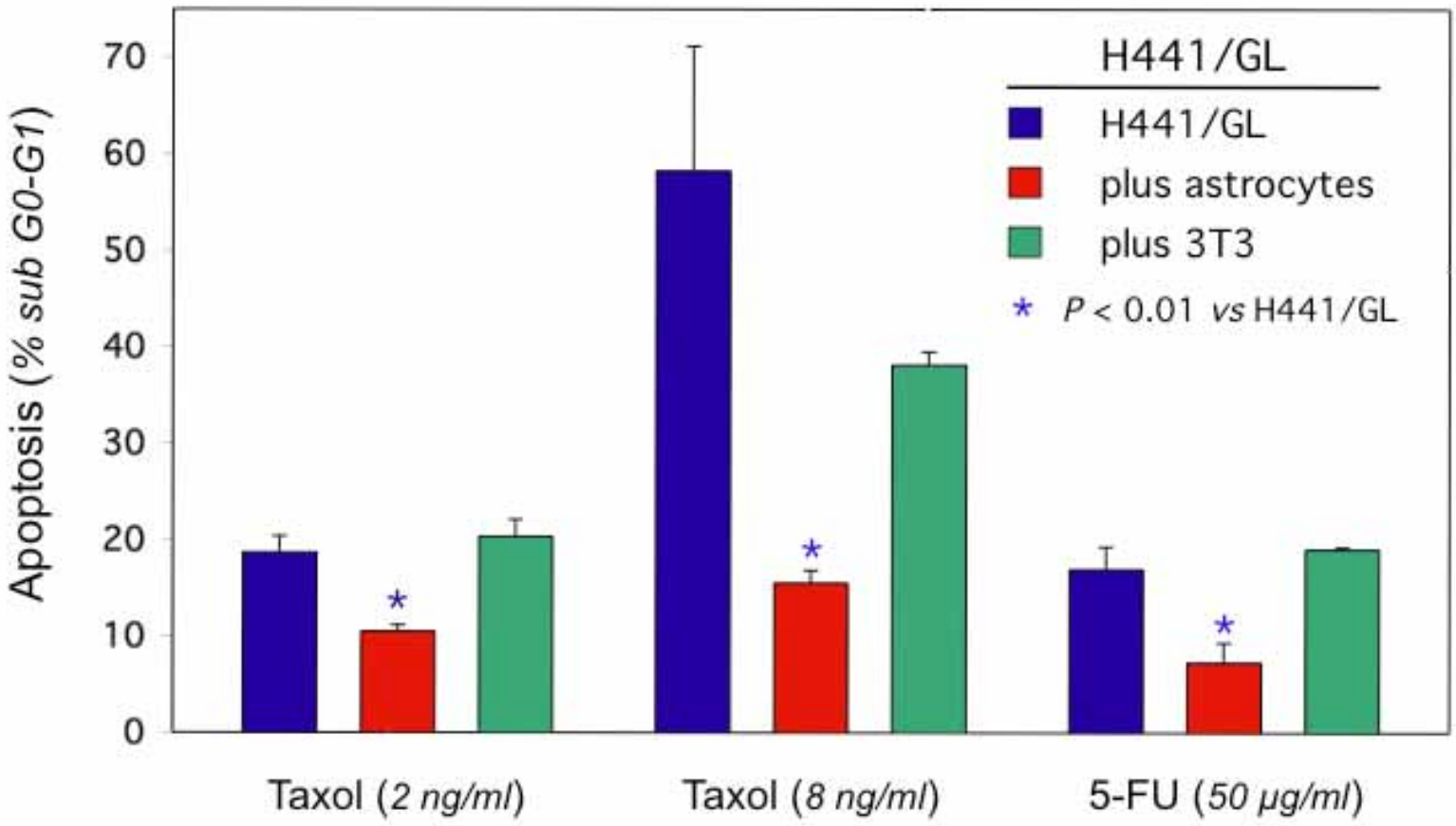


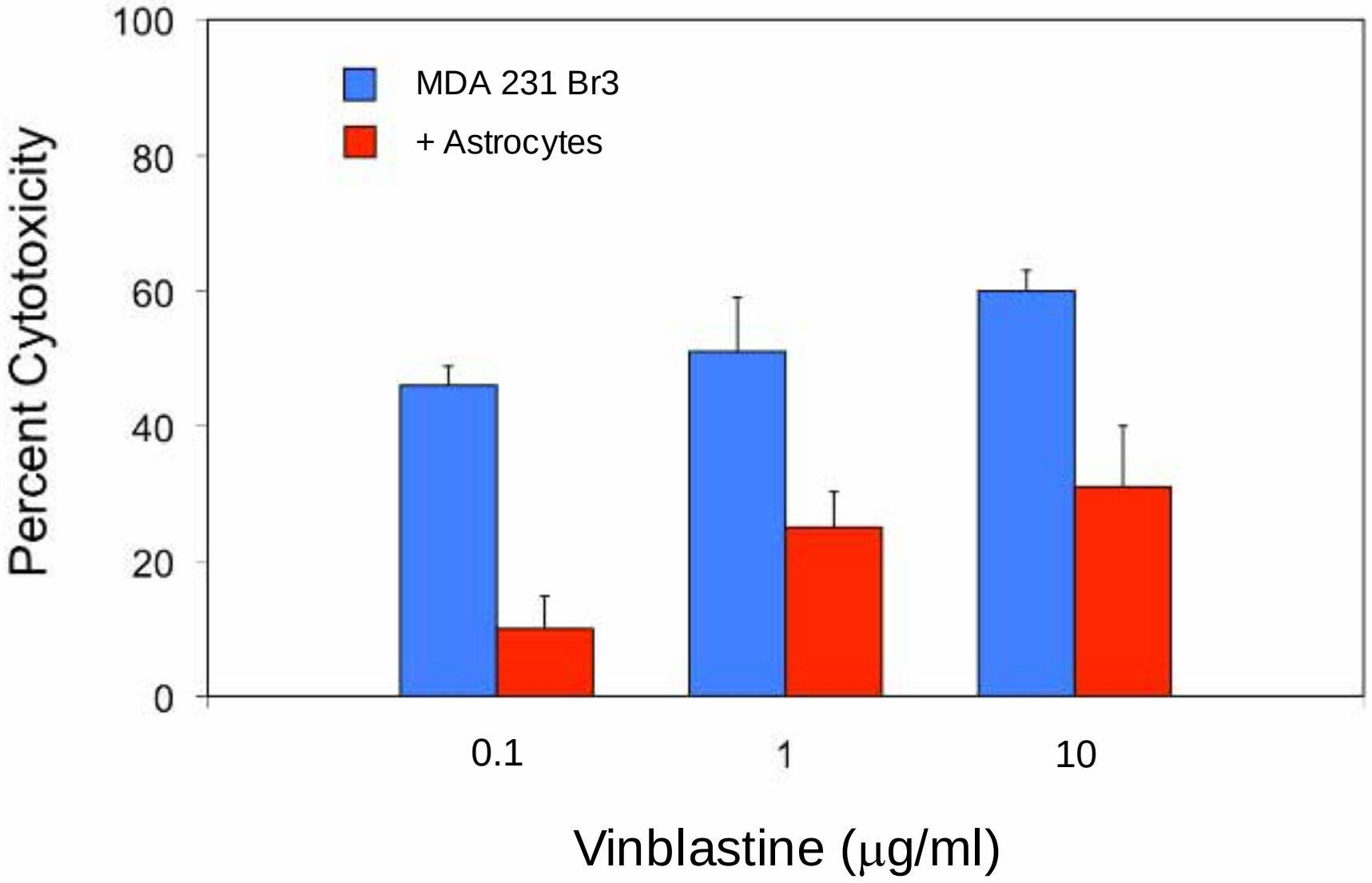
NMDA Receptor

CO-CULTURE EXPERIMENTS

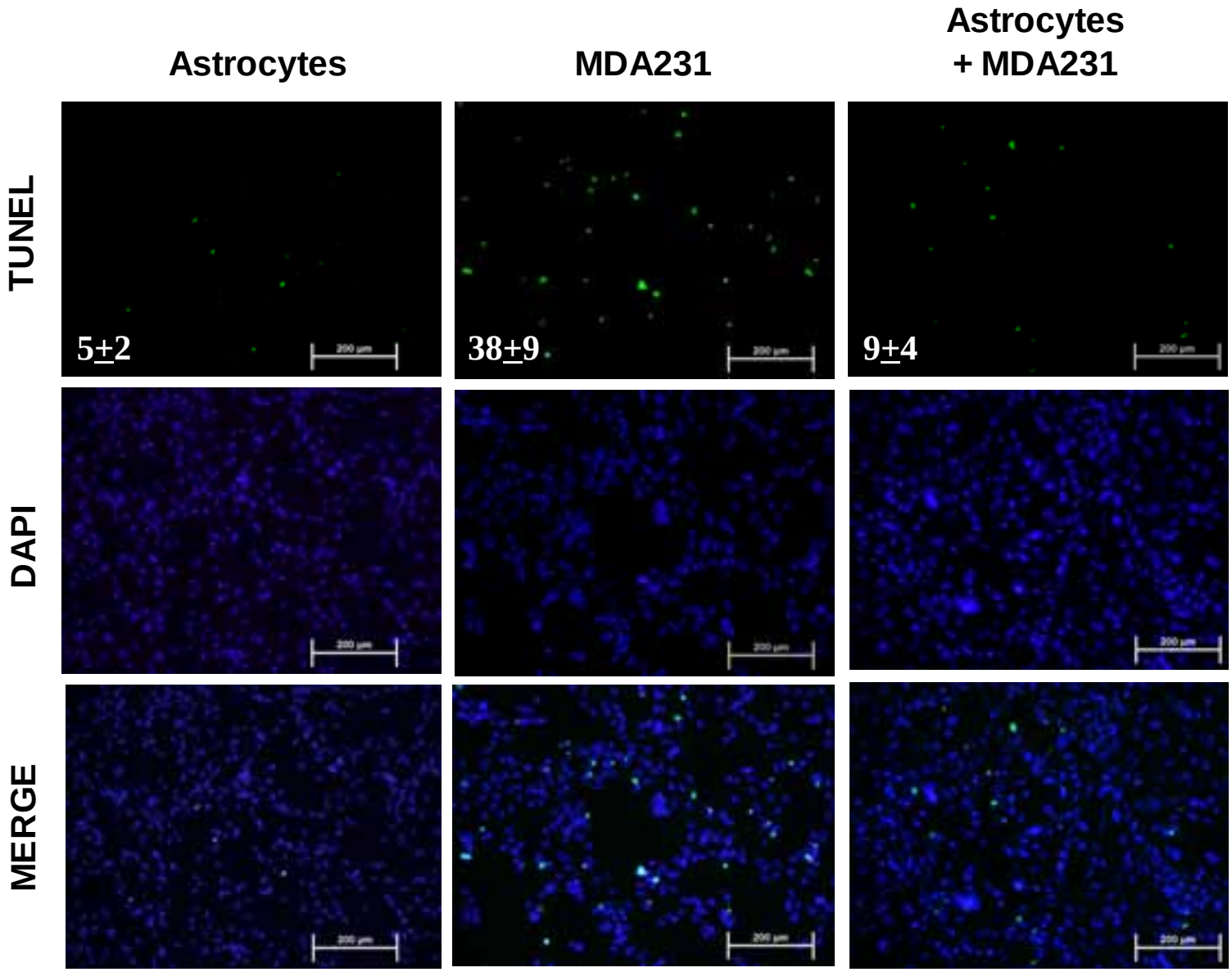

20 µm


Mouse Astrocytes Enhance Resistance of PC14Br4 Cells to Taxol (72 hr)
Apoptosis (% sub G0/G1 cells)
60
40
20
0
PC14Br4/GFP
Plus Astrocyte
0
5
10
Taxol (ng/ml)

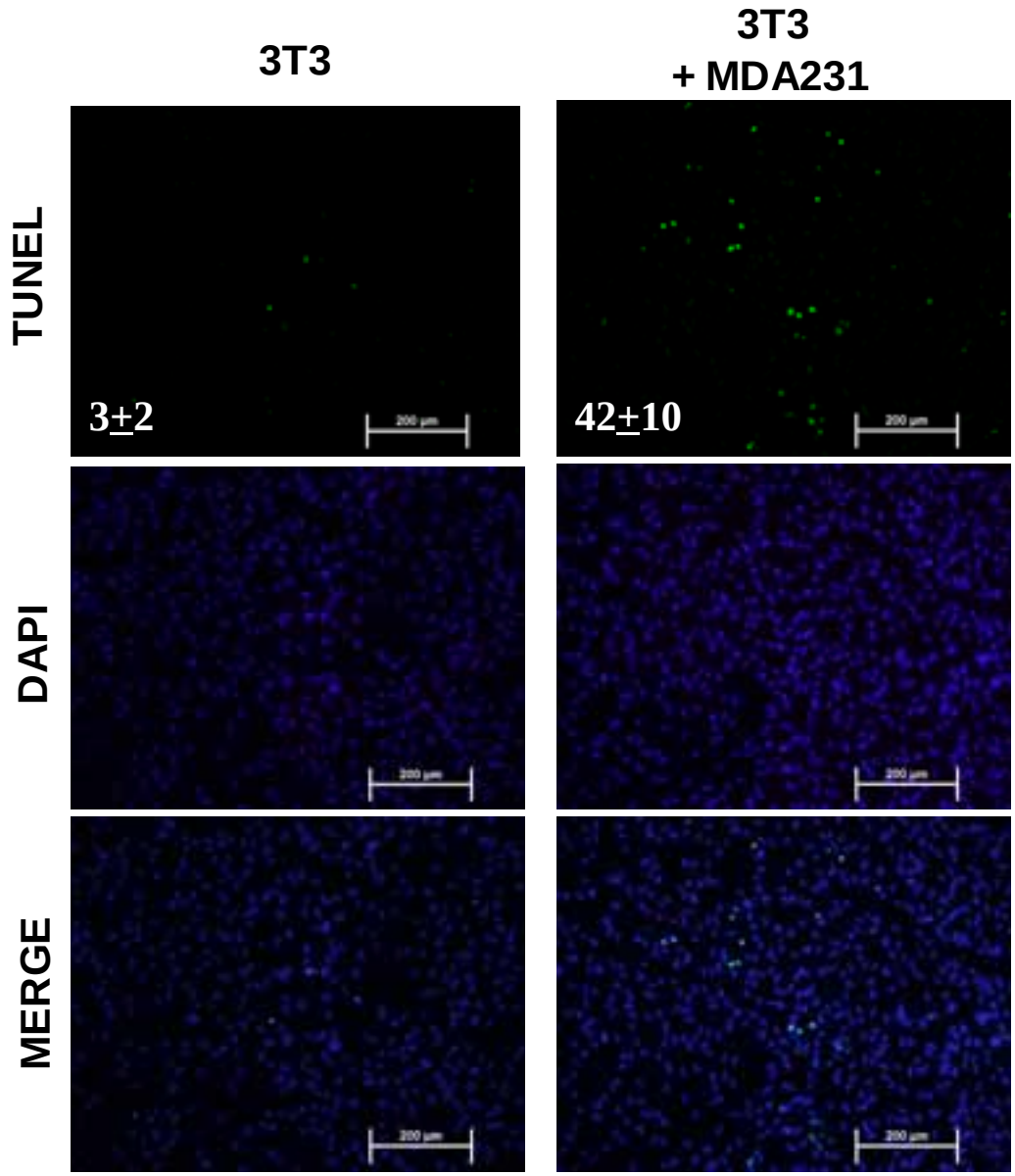

Apoptosis (% sub G0-G1)
70
60
50
40
30
20
10
0
H441/GL
H441/GL
plus astrocytes
plus 3T3
* P < 0.01 vs H441/GL
*
*
*
Taxol (2 ng/ml)
Taxol (8 ng/ml)
5-FU (50 µg/ml)


Percent Cytotoxicity
100
80
60
40
20
0
MDA 231 Br3
+ Astrocytes
0.1
1
10
Vinblastine (μg/ml)

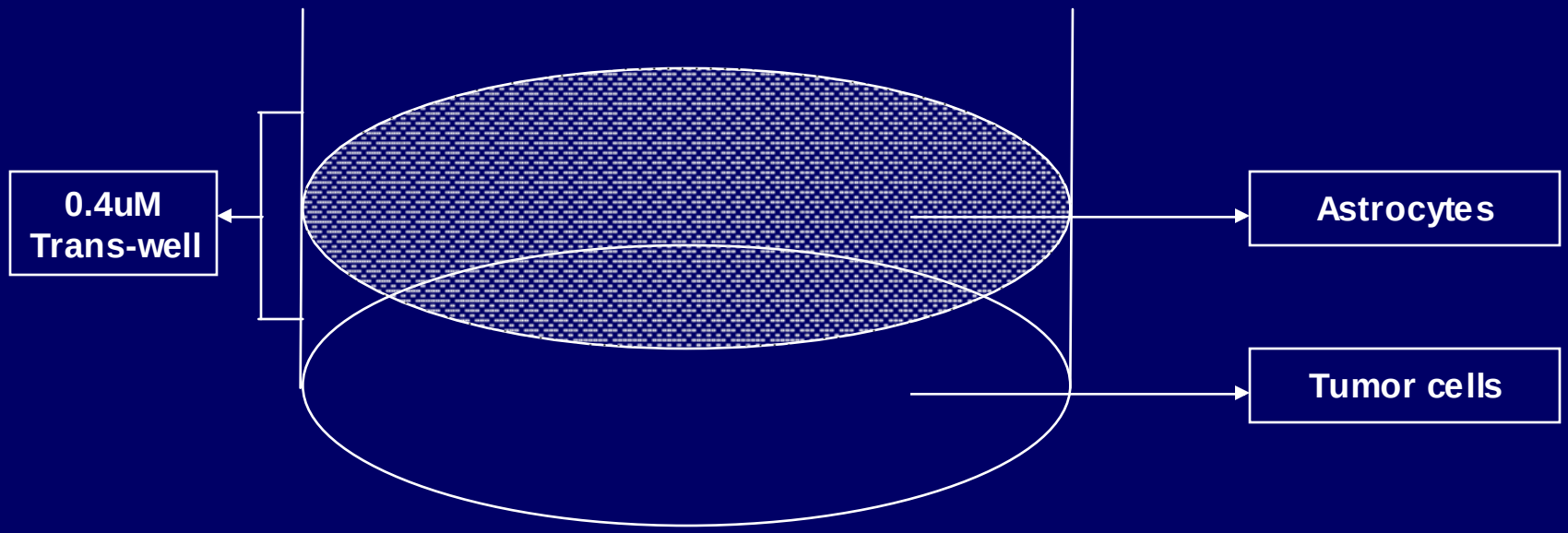
MDA231 /astrocytes Taxol protection assay (TUNEL)

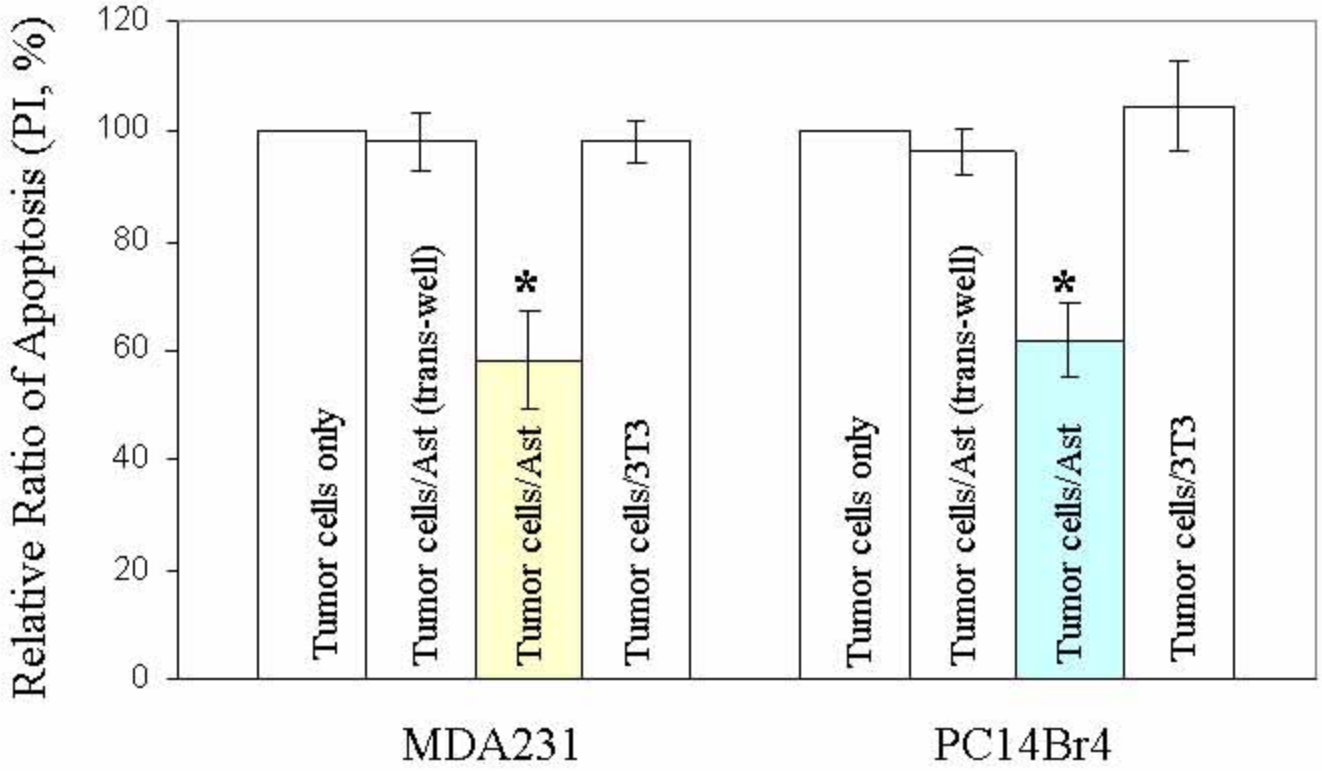


MDA231 / 3T3 Taxol protection assay (TUNEL)

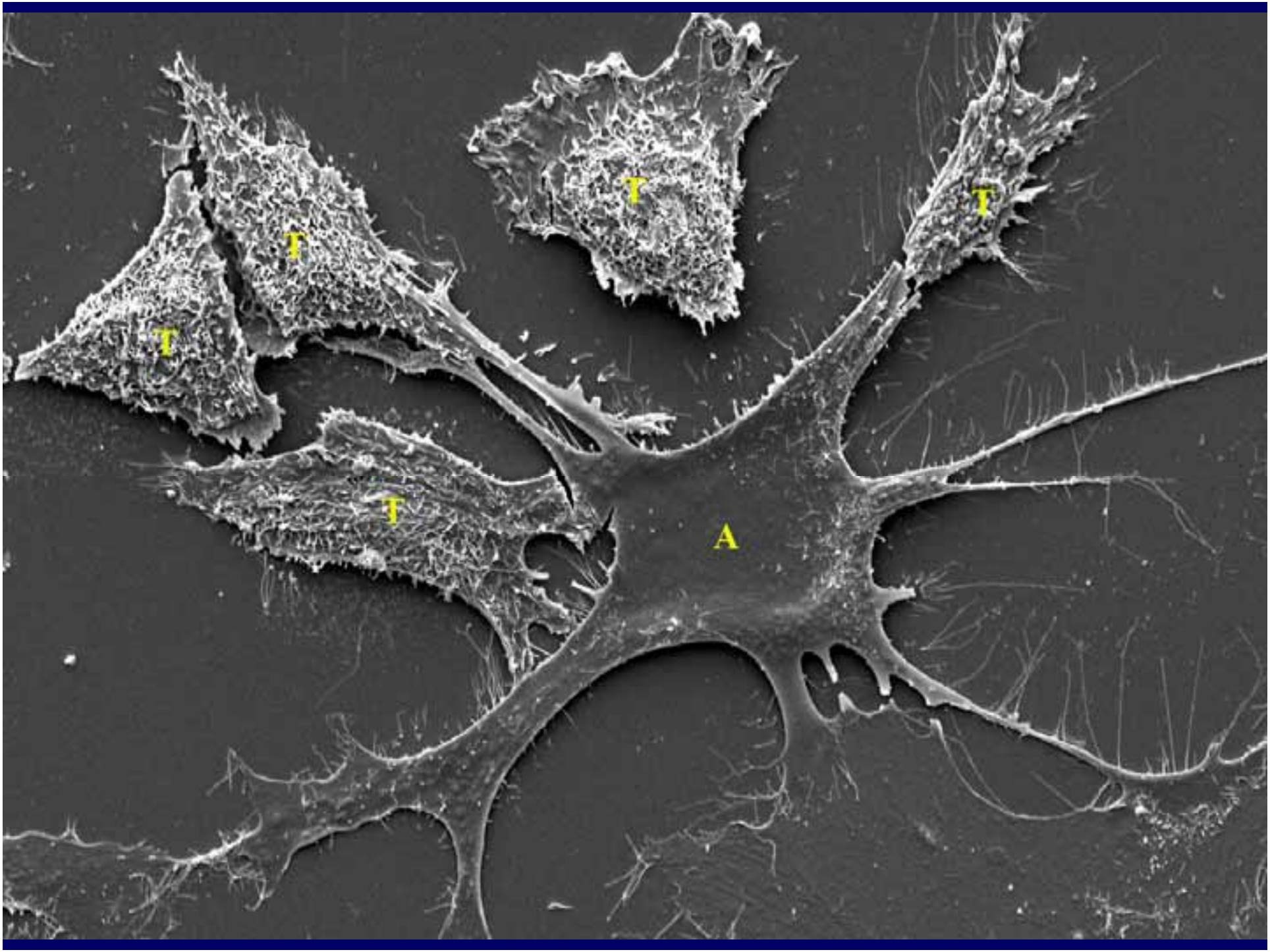


IS THE PROTECTION FROM CHEMOTHERAPEUTIC DRUGS MEDIATED BY SECRETED FACTORS OR IS IT CONTACT DEPENDENT?


0.4uM
Trans-well
Astrocytes
Tumor cells

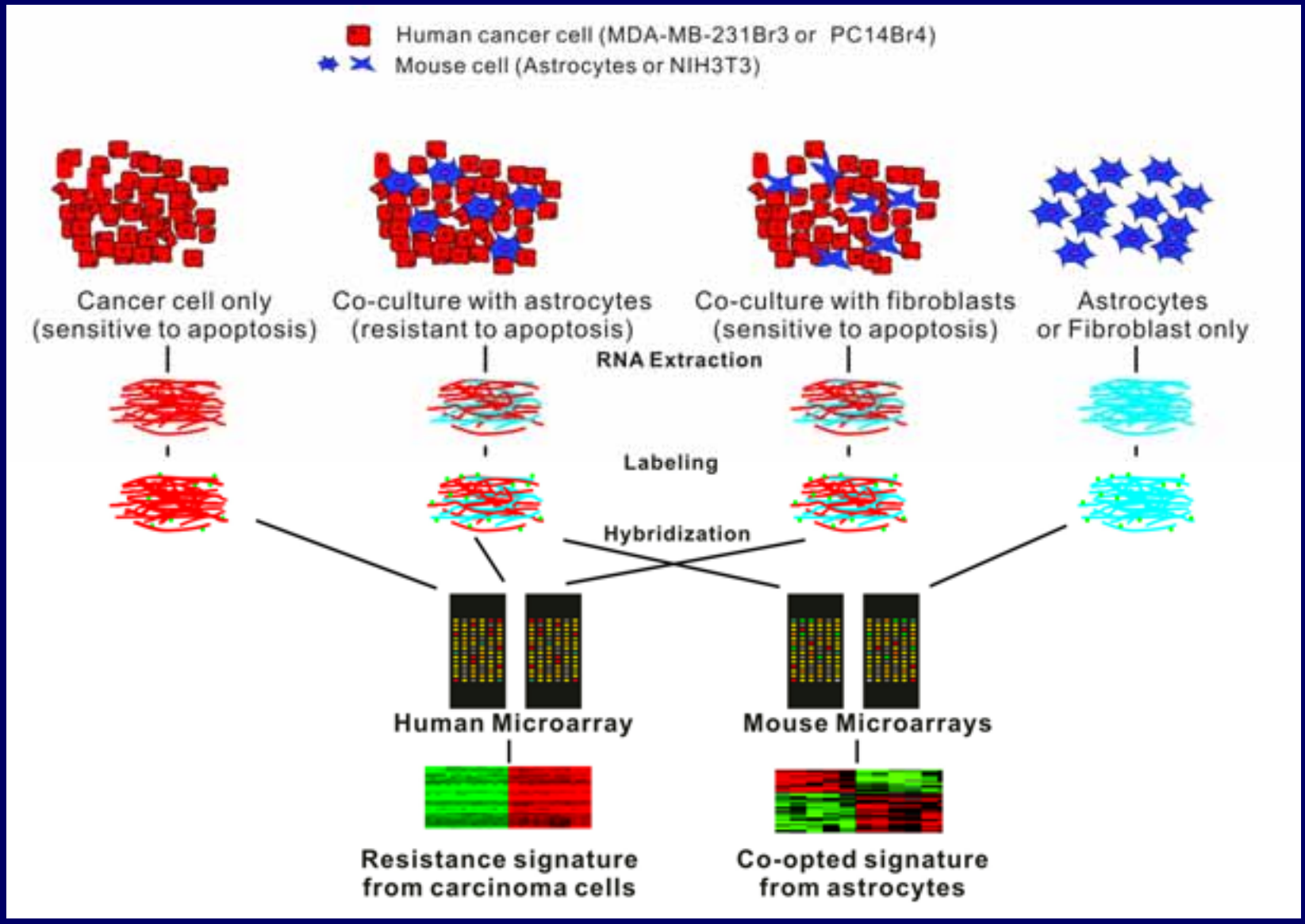

Relative Ratio of Apoptosis (PI, %)
120
100
80
60
40
20
0
Tumor cells only
Tumor cells/Ast (trans-well)
Tumor cells/Ast
Tumor cells/3T3
*
Tumor cells only
Tumor cells/Ast (trans-well)
Tumor cells/Ast
Tumor cells/3T3
*
MDA231
PC14Br4

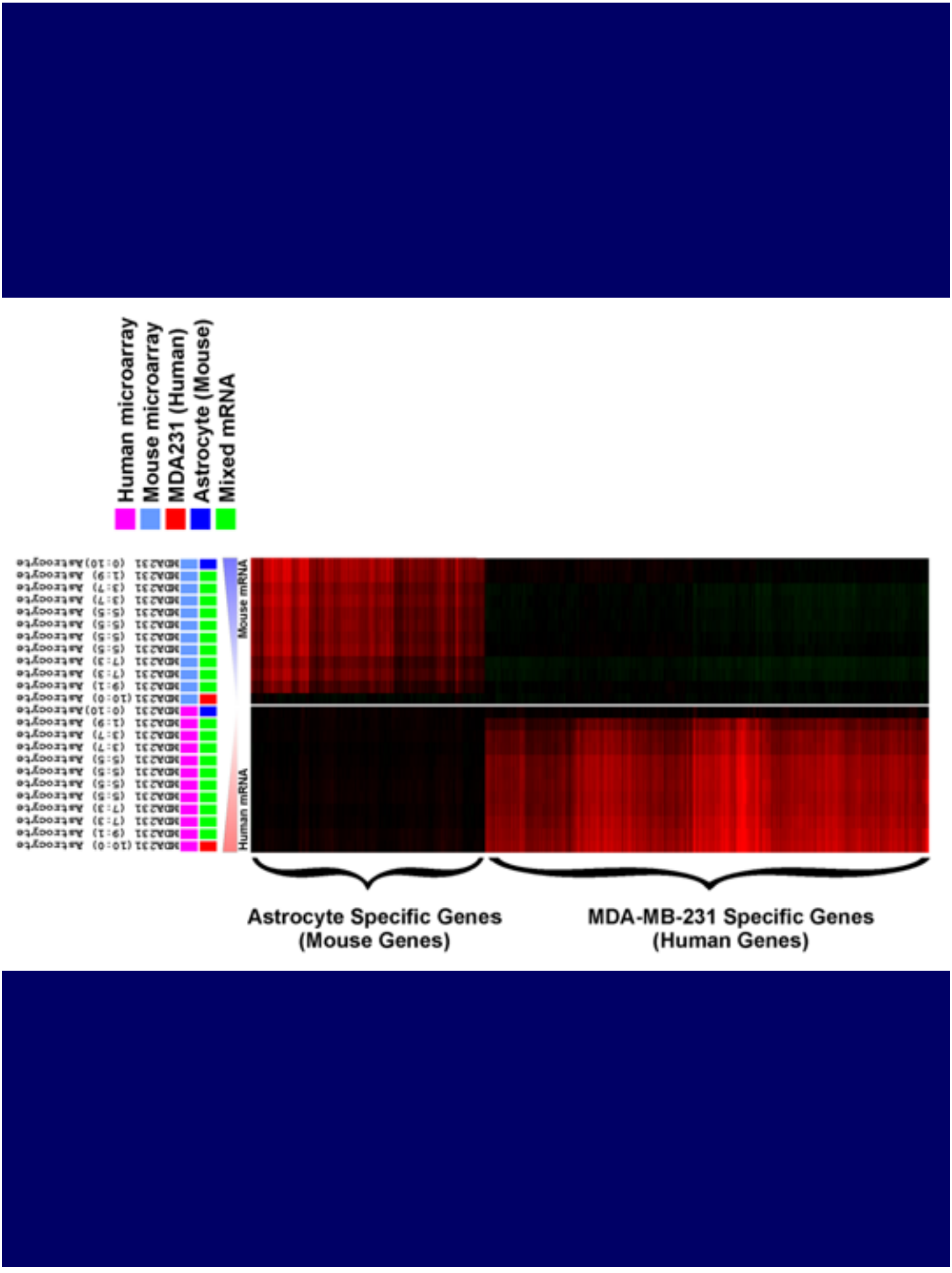
IS THE PROTECTION DEPENDENT ON GAP - JUNCTION CHANNELS (GJC) ?

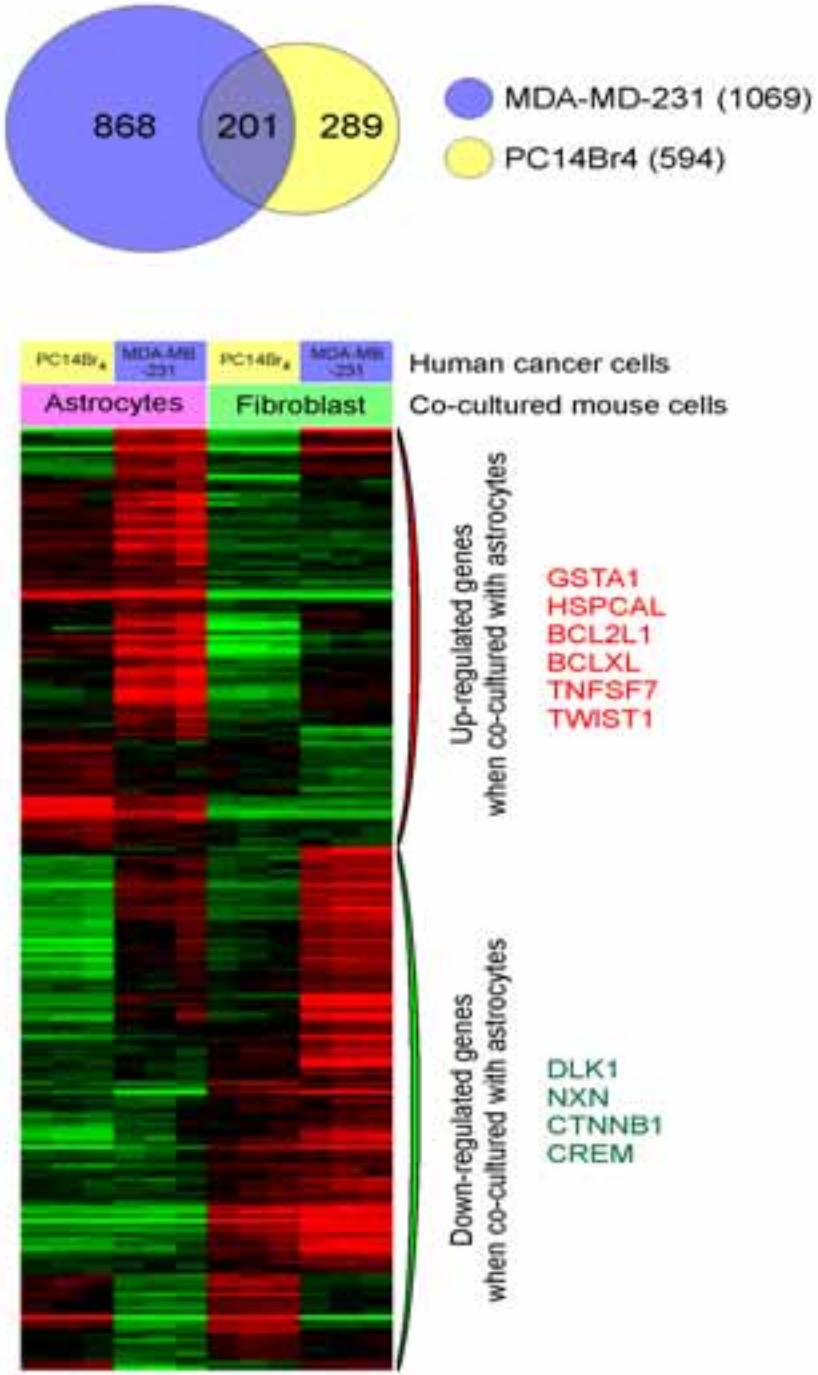

T
T
T
T
T
T
A

IS THE PROTECTION OF TUMOR CELLS FROM CHEMOTHERAPEUTIC DRUGS ASSOCIATED WITH ALTERED EXPRESSION OF SURVIVAL GENES ?

EXPERIMENTAL DESIGN



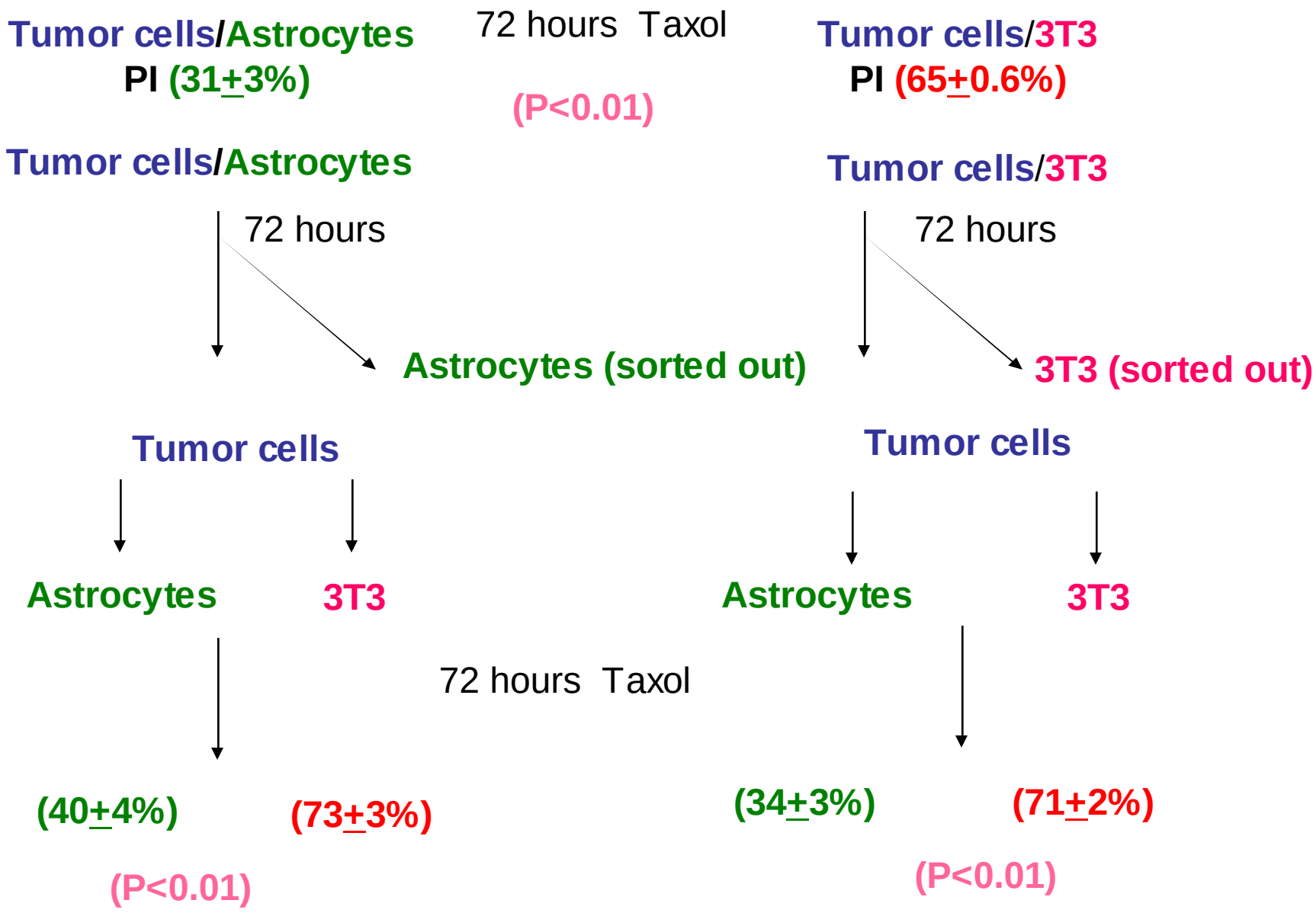

Human microarray
Mouse microarray
MDA231 (Human)
Astrocyte (Mouse)
Mixed mRNA
Mouse mRNA
Human mRNA
Astrocyte Specific Genes
(Mouse Genes)
MDA-MB-231 Specific Genes
(Human Genes)

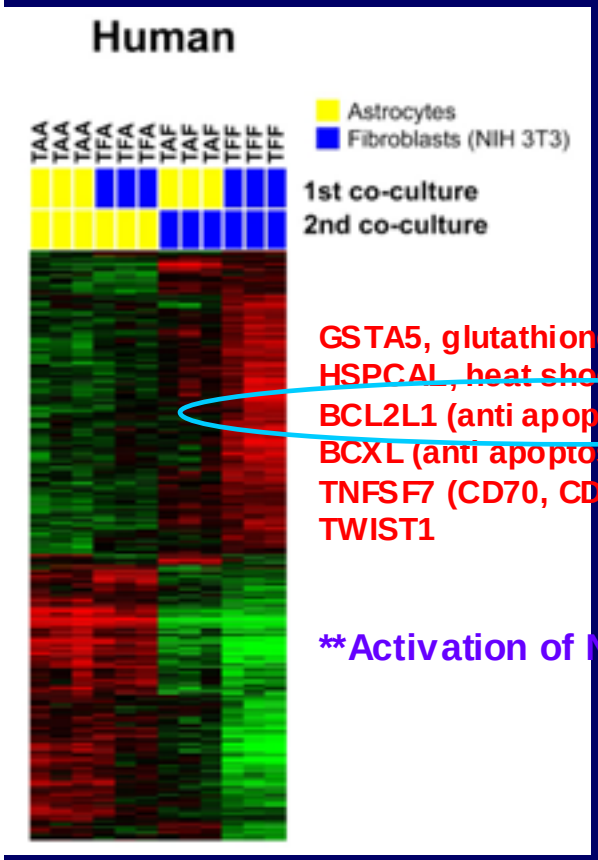

868
201
289
MDA-MD-231 (1069)
PC14Br4 (594)
PC14Br4
MDA-MB-231
PC14Br4
MDA-MB-231
Human cancer cells
Astrocytes
Fibroblast
Co-cultured mouse cells
Up-regulated genes
when co-cultured with astrocytes
GSTA1
HSPCAL
BCL2L1
BCLXL
TNFSF7
TWIST1
Down-regulated genes
when co-cultured with astrocytes
DLK1
NXN
CTNNB1
CREM

ASTROCYTE PROTECTION OF TUMOR CELLS FROM CHEMOTHERAPEUTIC DRUGS IS ASSOCIATED WITH INCREASED EXPRESSION OF SURVIVAL GENES

ASTROCYTE MEDIATED PROTECTION OF PC14 LUNG CANCER CELLS FROM CYTOTOXIC DRUGS

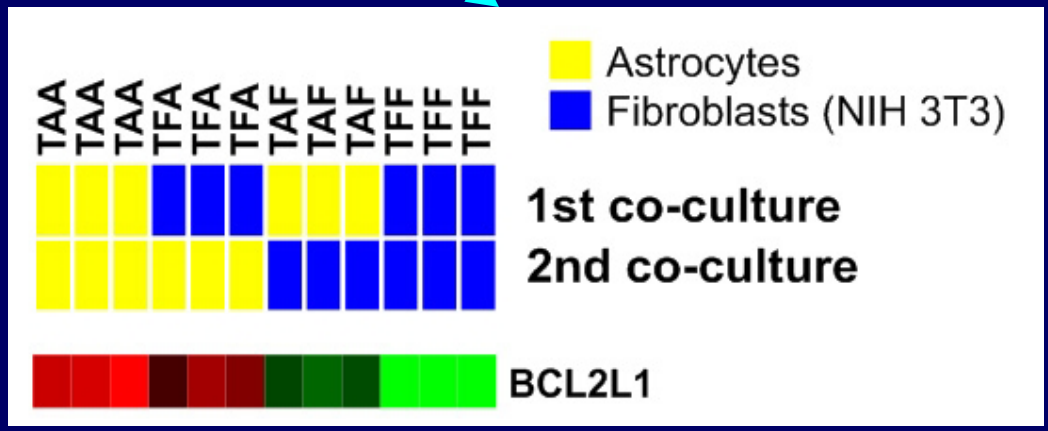
Permanent vs Transient





GSTA5, glutathione S transferase 5
HSPCAL, heat shock 90kDa protein 1-like
BCL2L1 (anti apoptosis gene)
BCXL (anti apoptosis gene)
TNFSF7 (CD70, CD27L)
TWIST1

**Activation of NFKB



ASTROCYTES

SUPPLY GLUCOSE AND OXYGEN TO NEURONS

ASSURE SURVIVAL OF NEURONS AND ENDOTHELIAL CELLS

AND

TUMOR CELLS

THERAPY OF CANCER METASTASIS

OBSTACLES

BIOLOGICAL HETEROGENEITY

RAPID EMERGENCE OF RESISTANT VARIANT CELLS.

PROTECTION BY THE MICROENVIRONMENT

THERAPY OF METASTASIS MUST BE DIRECTED AGAINST THE

METASTATIC CELLS

AND

THE ORGAN MICROENVIRONMENT


FIRE

THANK YOU