Structure and Efficacy of Drugs

Studying the effectivity of different compounds during the drug development in the field of oncology

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Tumors

- Originated from all cell types
- Every 3rd – 4th people
- Hungary is „leading” country
- Genetical inheridity
- Enviromental factors (smoking)
- Benign and malignant
- mutations: peptides with malfunctions

Mutations in DNA → malfunct. pepdides → Tumor cells

Outside reasons

- Smoking
- Alcohol
- Irradiation
- Viruses
- Chemicals

Inside reasons

- Genetical susceptibility/onheretance
- Immun system
- hormones

Smoking

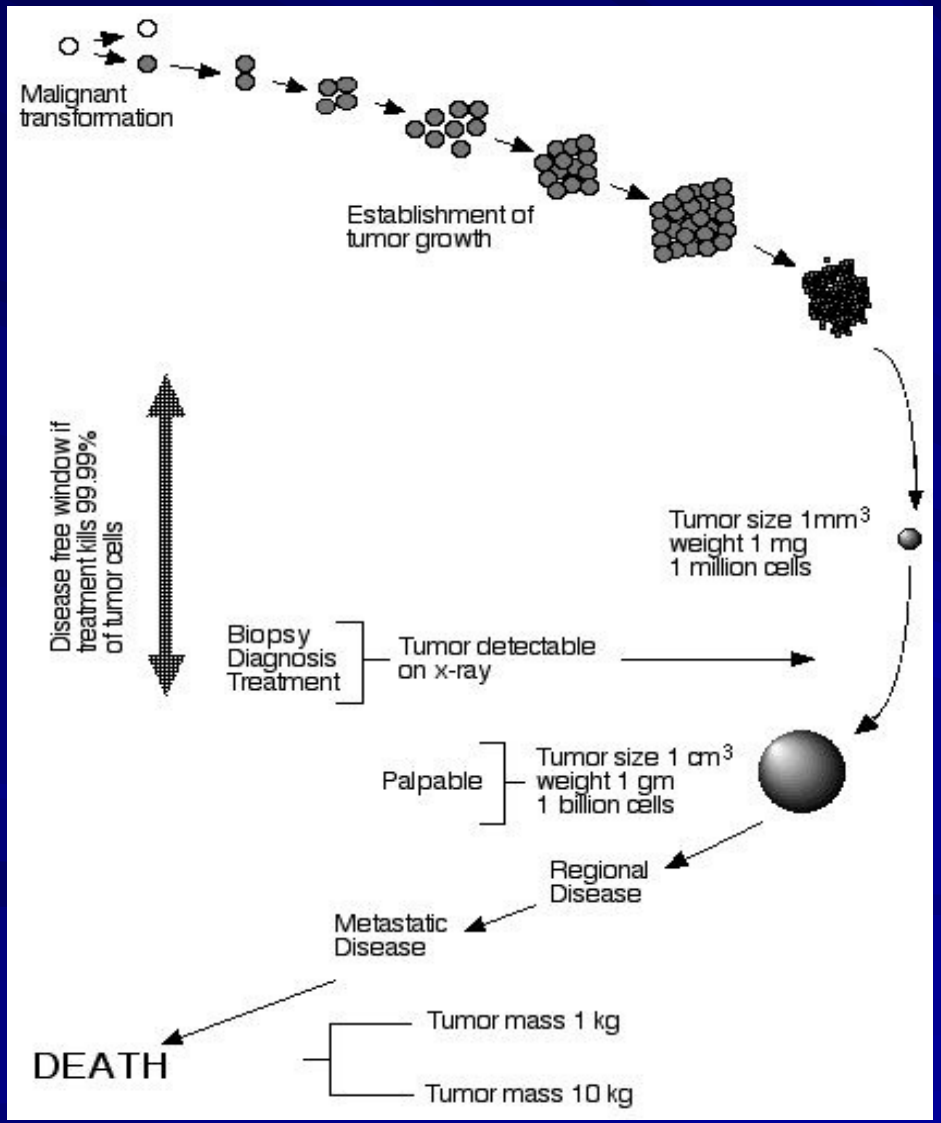
- App. 4000 chemicals in the fume
- More than 40 is carcinogen!!
- Lung, esophagus, trachea, head and neck, bladder, gastric tumors…

Lung cancer: rapid progression, resistance to therapy

Risc factors

- **Alcohol:**
  Soral, head and neck, oesophagus, gastric, liver, breast
- **Irradiation:** cosmic background, UV, radioactivity
- **Viruses:** HPV (16,18); Hepatitis B, C; HIV, HTLV, Eppstein-Barr. Herpes 8 (?)
- **Worms:** (Schistosoma species), bacteria (Helicobacter pilori: stomach)
- **Chemicals:** tar (benzol derivatives), serpentine, stb...

Tumor cell number – tumor size



Classification of the tumors (samples)

- Benign
- Malignant

- Carcinoma (epithelial)
- Sarcoma (mesenchimal)
- Leukaemia, Lymphoma, Melanoma etc

- Grading: differenciation (1-4, well-moderate-week)
- Staging: T (0-4) N (0-3) M (0-1) chategories

Metastasization

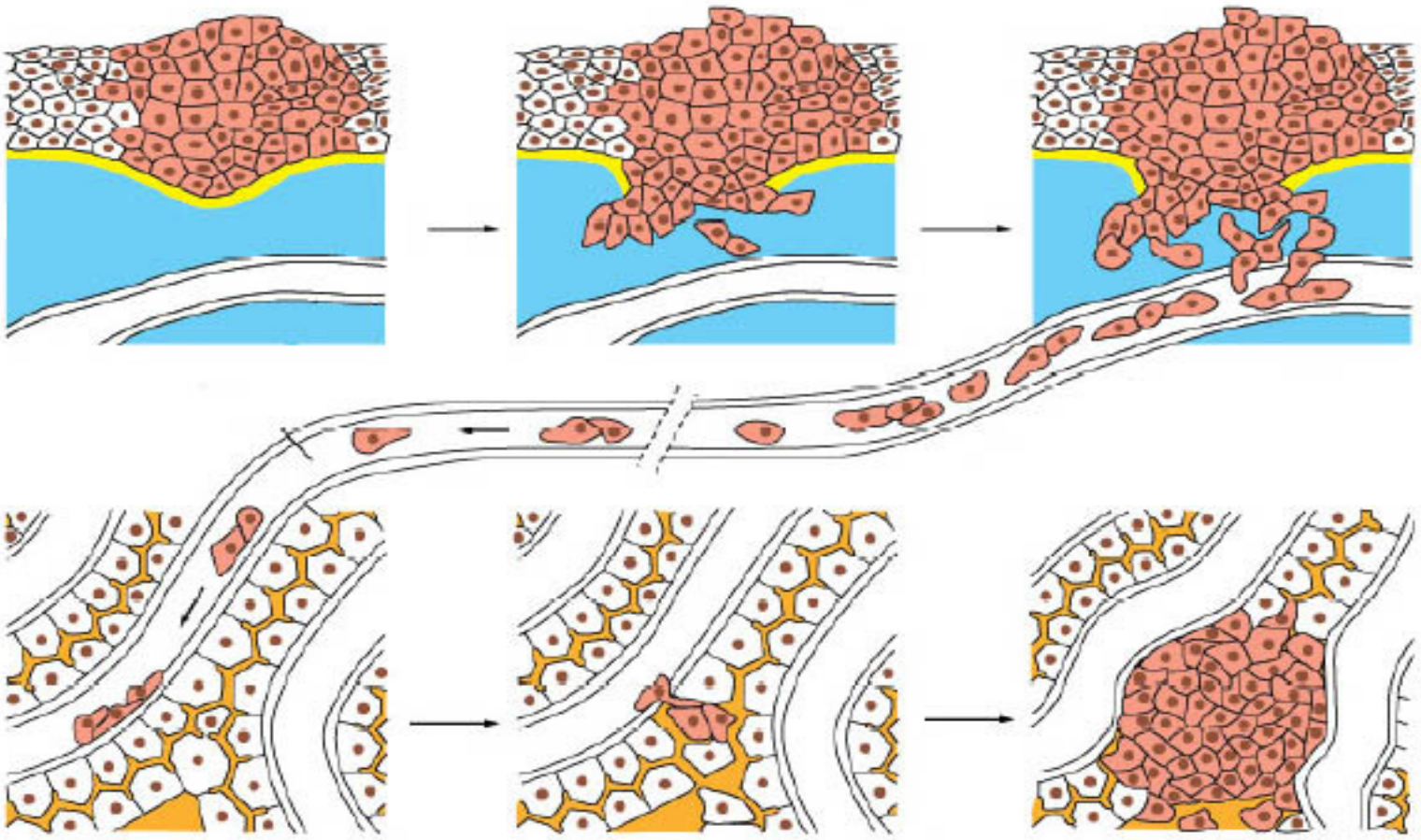
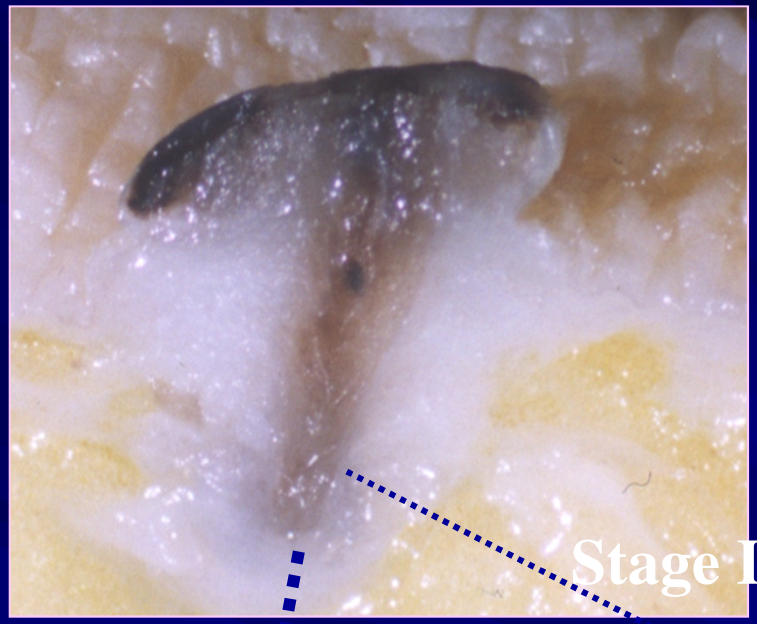
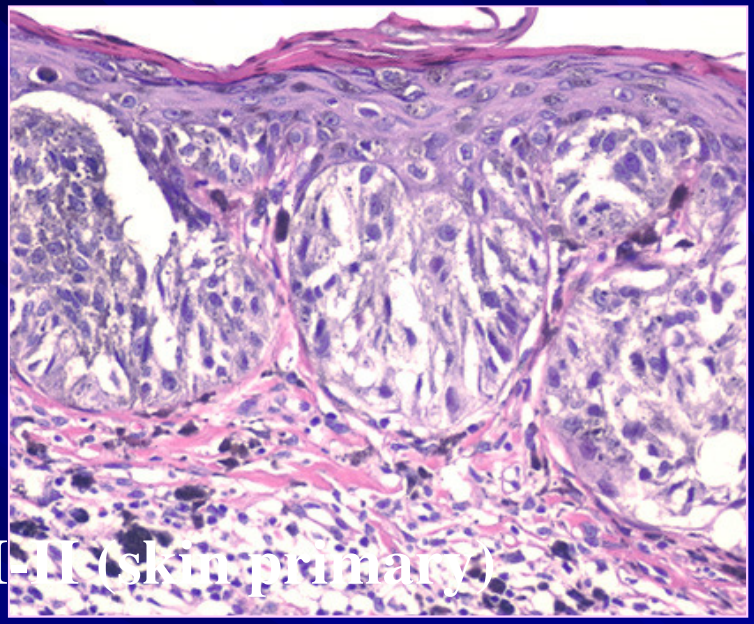
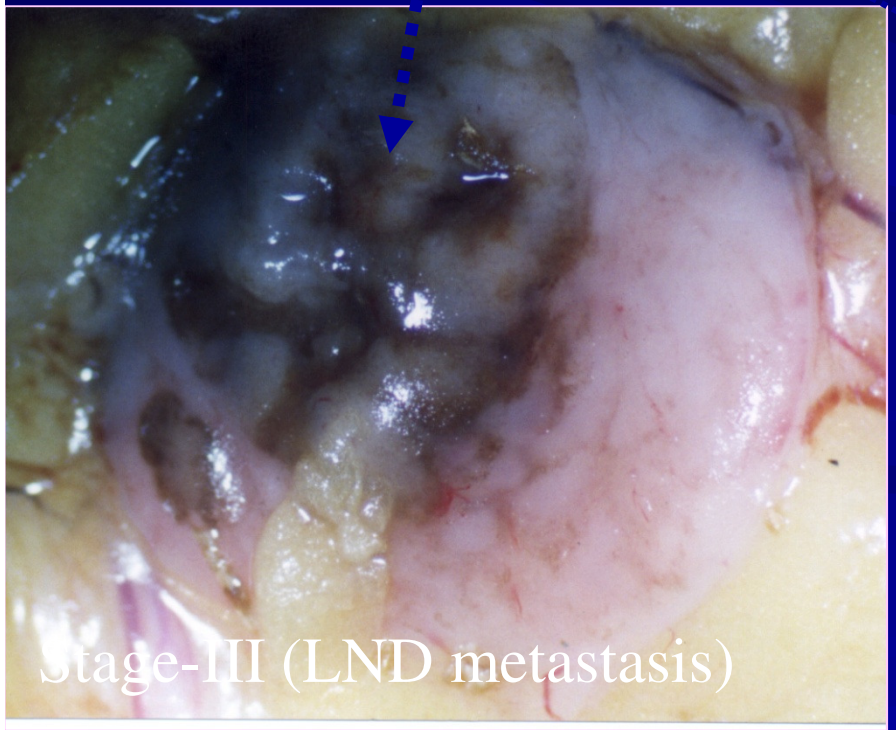


Figure 23–15. Molecular Biology of the Cell, 4th Edition.

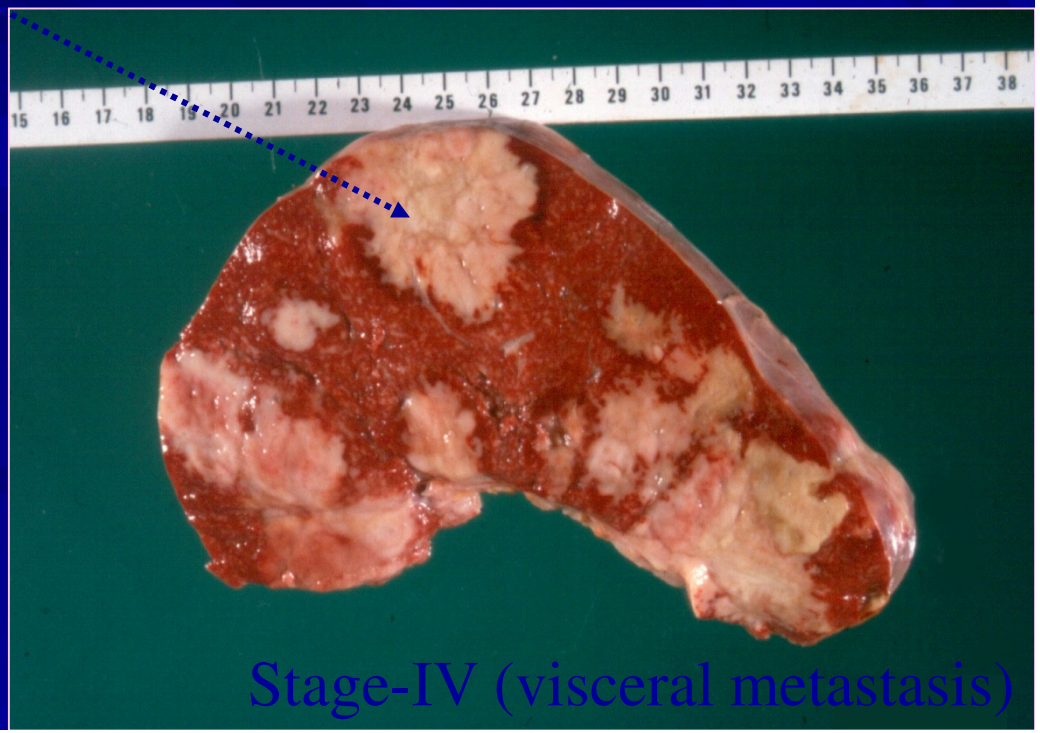




Stage I-II (skin primary)



Stage-III (LND metastasis)



Stage-IV (visceral metastasis)

Steps of the metastatic cascade

1./ Tumor growth, local invasion *, angiogenesis *

2./ Tumor cell escape from the primer mass*

3./ Intravaztion *

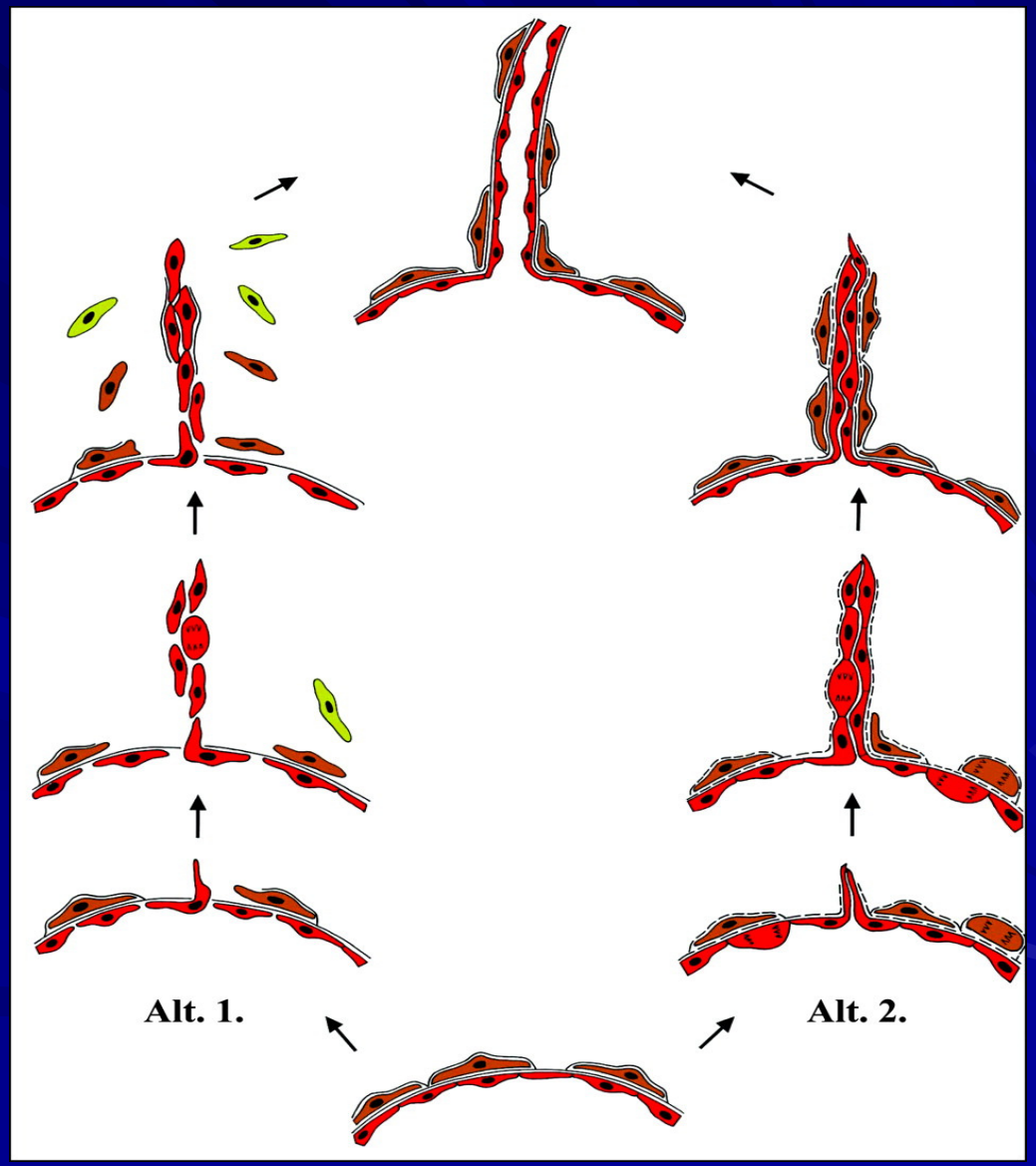
4./ Tumor cell interaction with cells in blood stream *

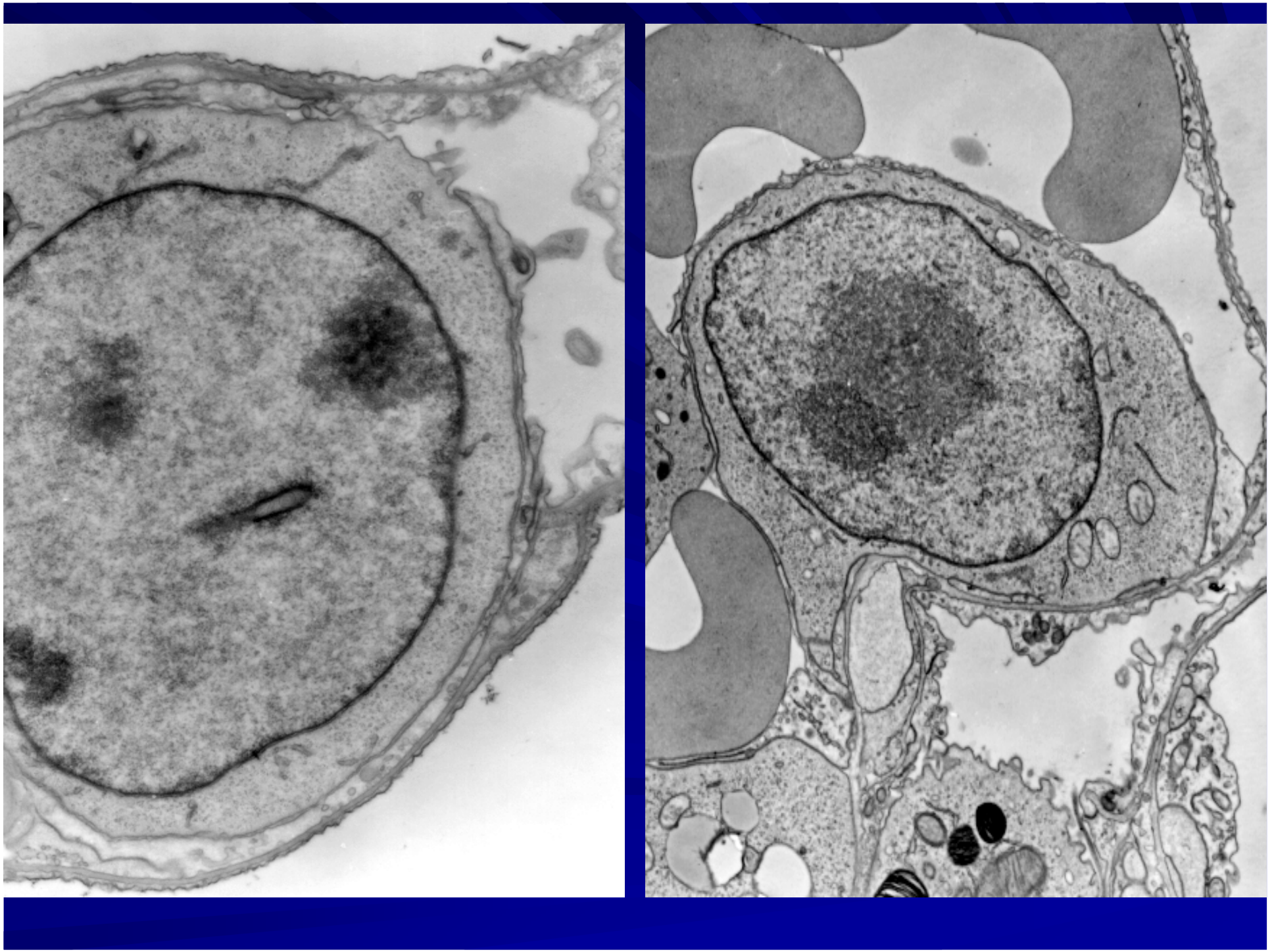
5./ Arrest in the target organ *

6./ Extravazation *

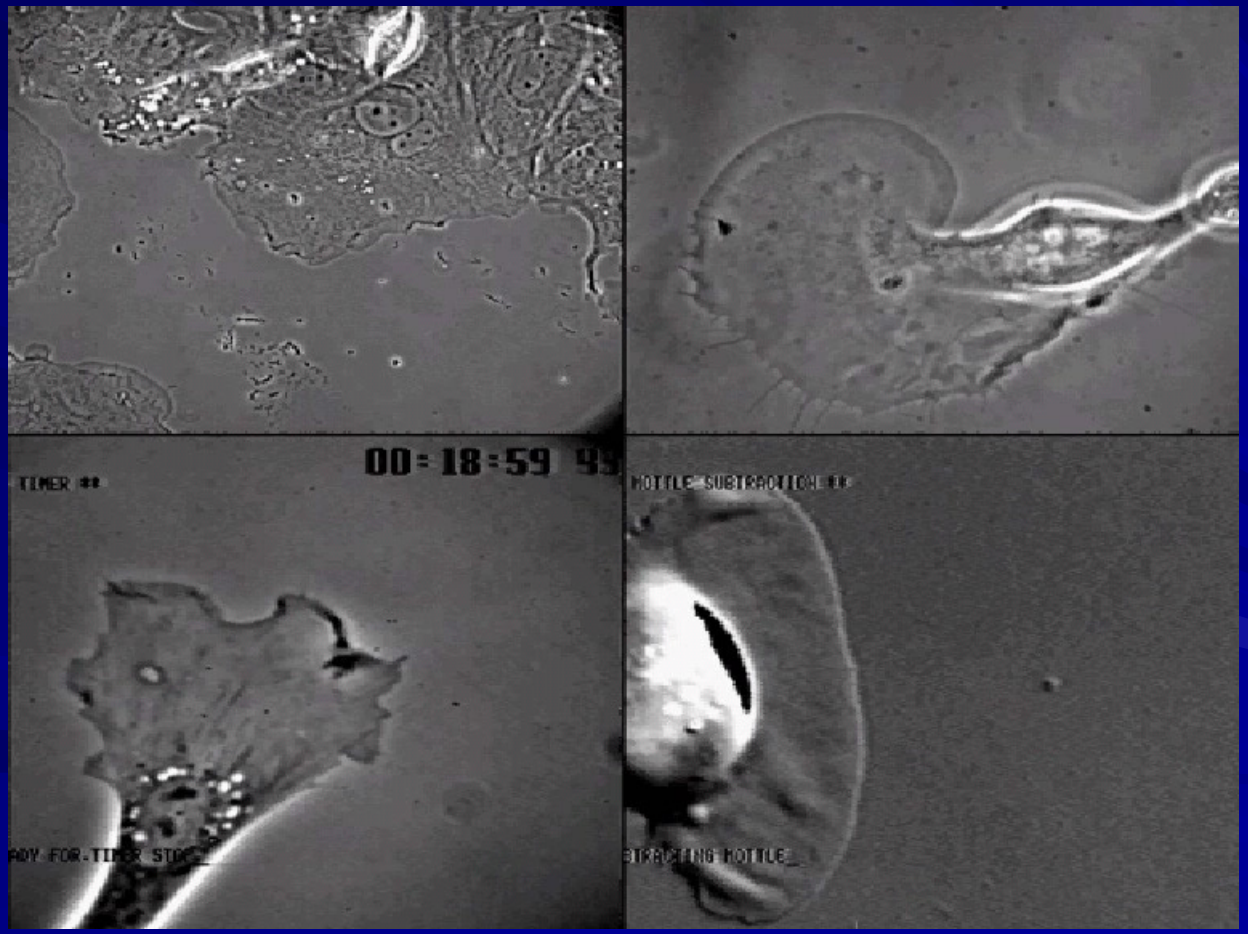
* adhesion - digestion - migration

Tumor-induced neoangiogenesis





Tumor cell migration



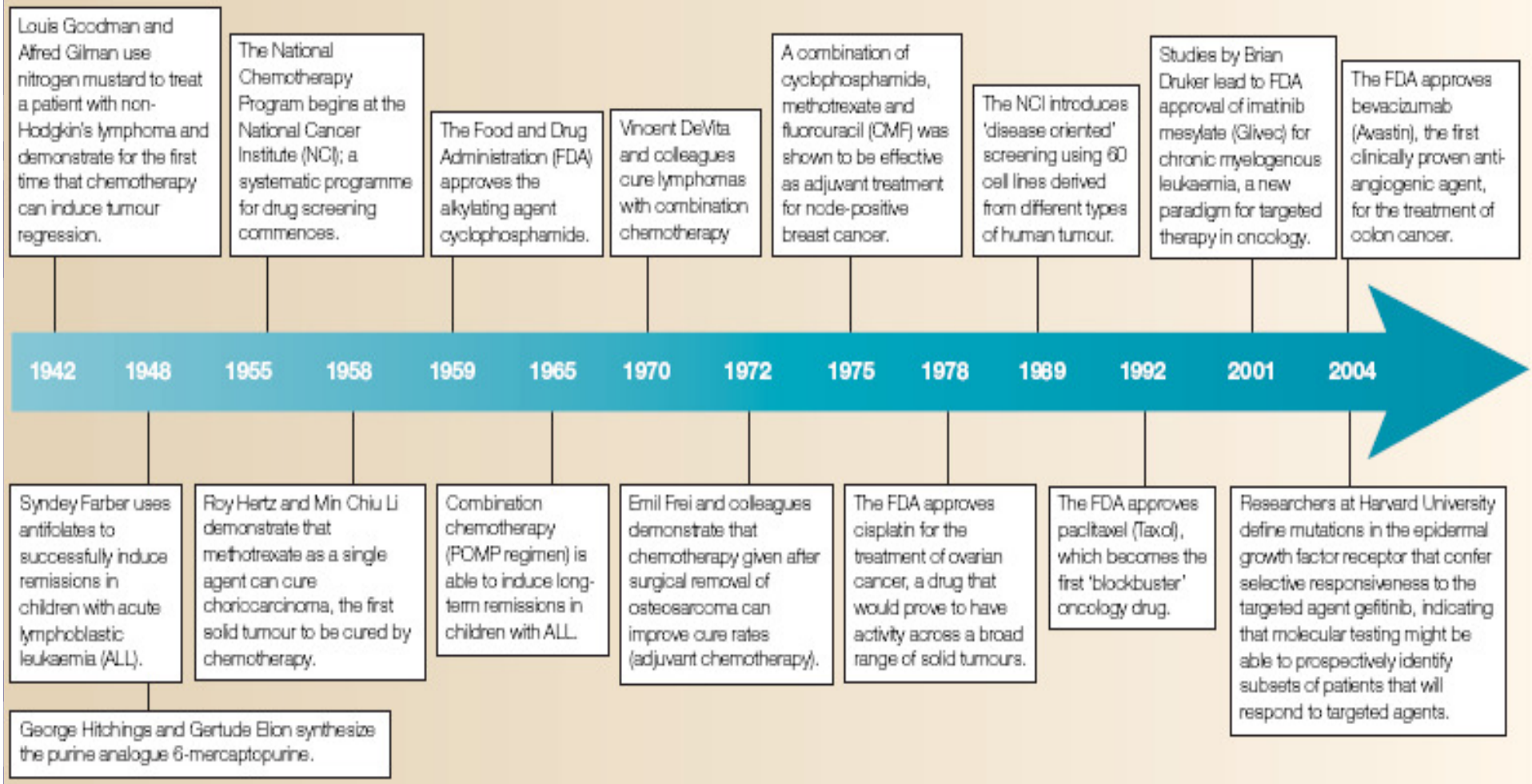
Therapy

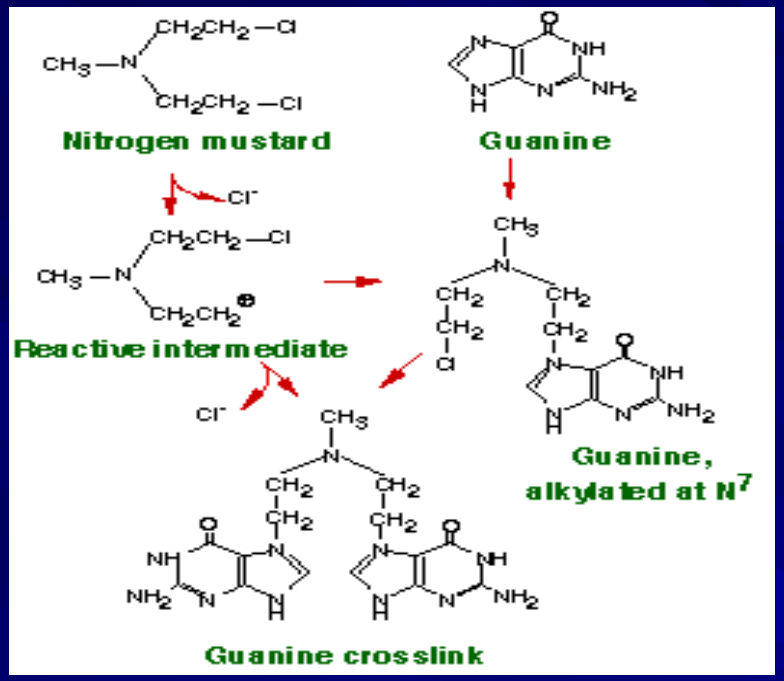
- Surgery (First line in the case of solid tumors, curative if no metastasis)
- Radiotherapy (Ionisation, free radicals, DNA damage)
- Chemotherapy

  Traditional: cell proliferation blockade

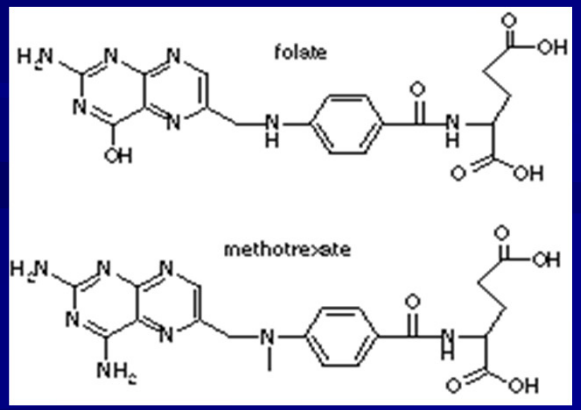
  Innovative: target-specific
  personal based

Timeline | **The history of chemotherapy**





http://www.ovc.uoguelph.ca/BioMed/Courses/Public/Pharmacology/pharmsite/98-409/Cancer/Cancer_images/Nitrog_must.gif



http://www.wellesley.edu/Chemistry/chem227/nucleicfunction/cancer/methotrexate.gif

1) Alkilating agent:

DNA crossbinding, transcription ⬇

1) Antimetabolites:

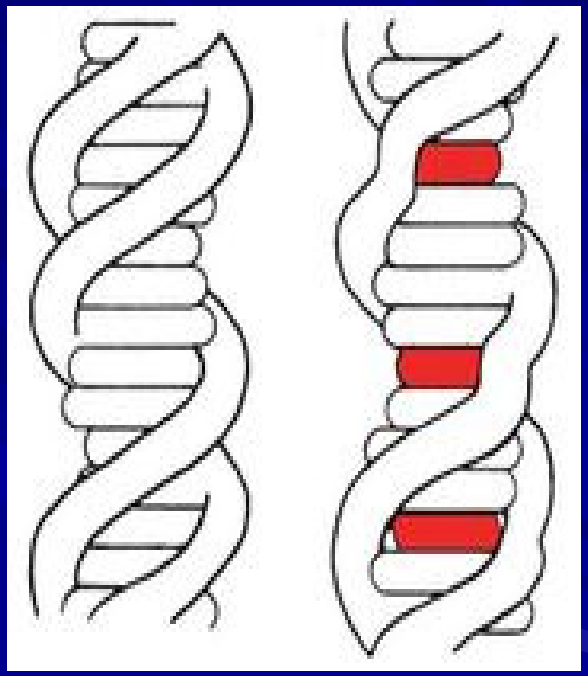
S pahase, DNS synthesis ⬇

Folate analogues (methotrexate)

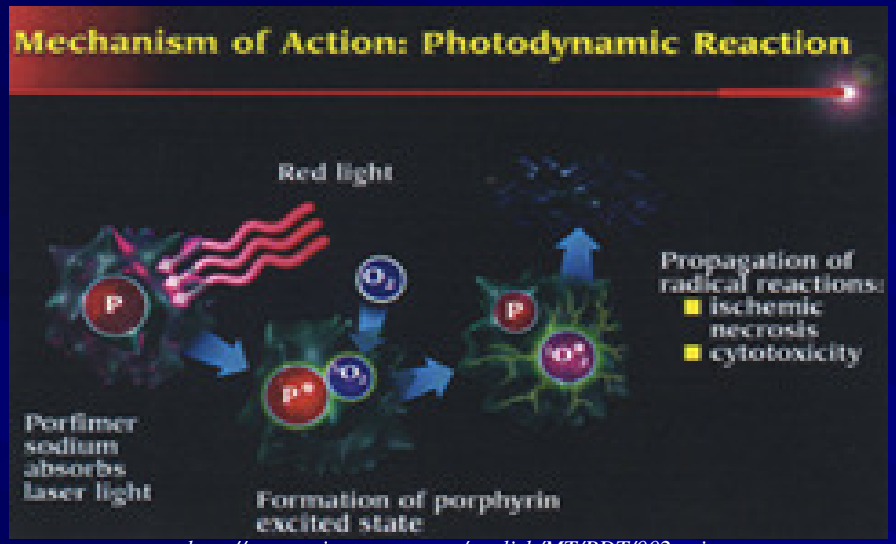
pyrimidine analogues: (fluorouracil)

purine analogues: (mercaptopurine).

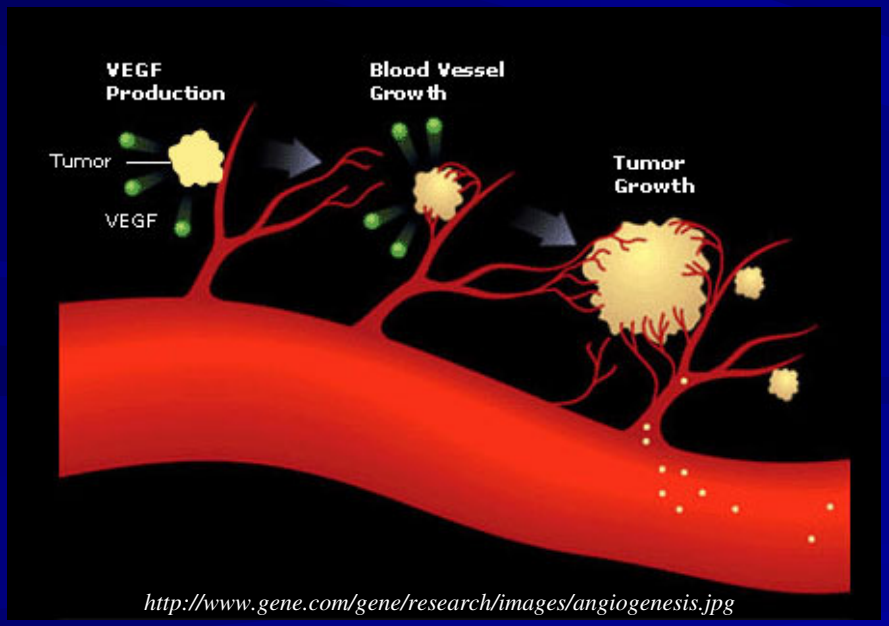
3) Natural compounds:
vinca alkaloides (vincristine),
taxanes (paclitaxel), and
epipodophyllotoxines
(etoposide).
DNA and RNA synthesis ⇩
formation of microtubules ⇩



http://en.wikipedia.org/wiki/DNA_intercalation



http://www.orienttumor.com/english/MT/PDT/002_s.jpg

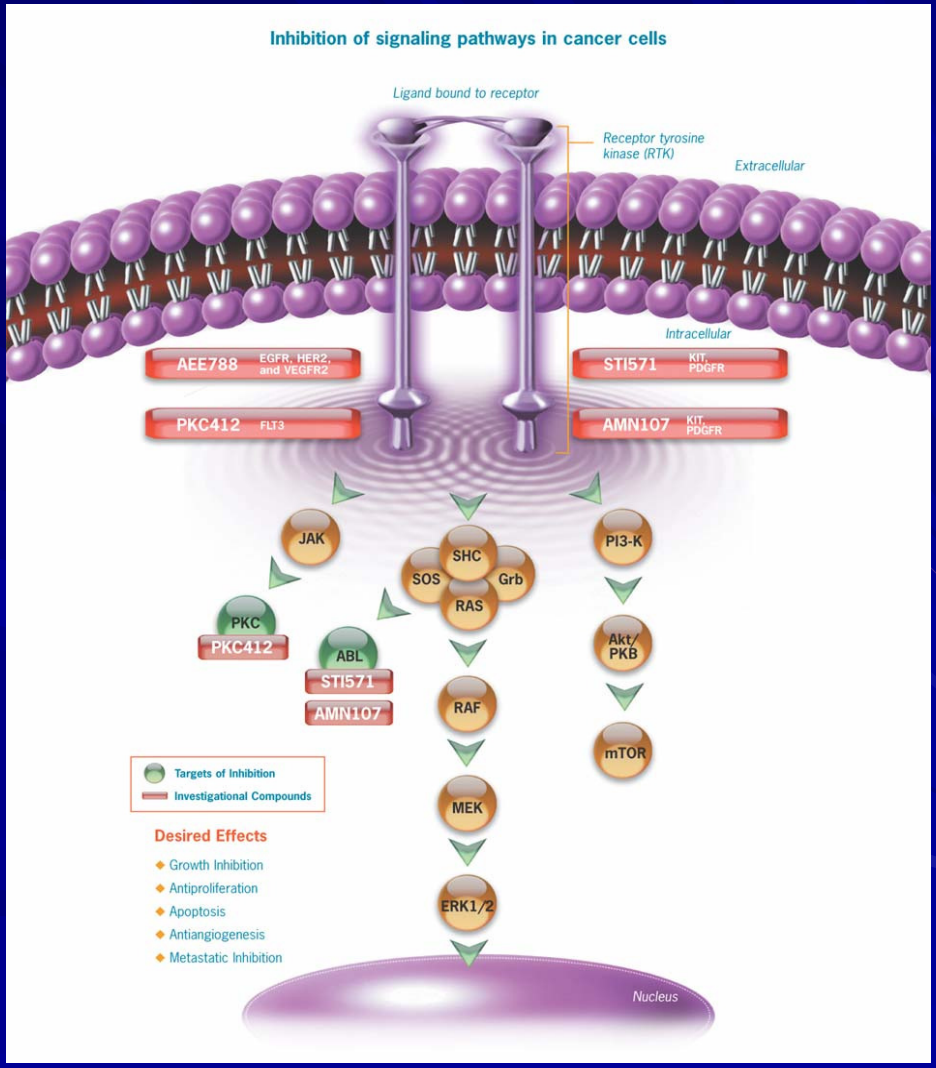


http://www.gene.com/gene/research/images/angiogenesis.jpg

- Photodinamic Therapy: photosensitivity
- Hormon treatments: breast, prostate
- Angiogenesis inhibition: bevacizumab, anti-VEGF antibody.

- Tirozin-kinase Inhibitors:

  EGFR (gefitinib, erlotinib. Cetuximab) Bcr-Abl (Imatinib)



http://www.amn107.com/images/aee788Large.jpg

Strategy in the development

- Early detection
- Diagnosis
- Therapy

Goals:

- Identification of new targets
- New molecules for prediction
- Imaging in early phase of tumor
- Drug delivery
- Monitoring of the therapy
- New methods for inproving of quality of life (QOL)

From development to therapy

Different phases of drug development

- **Preclinical phase**
  in vitro and *in vivo* (animal modells) techniques

- **Clinical Phases**

Phase I.
  Intolerance, insalubrity: healthy volunteer

Phase II.
  Doses, side effects: few patients

Phase III.
  Best dose and formulation: lasrge number of patients
  Registration: EMEA (European Medical Evaluation Agency)

Phase IV.
  Collecting the observations from the practice

Difficulties

- **„Pills”: annual 700 Billion USD (45% USA, 30% EU)**
- **10.000 molecules - 1 drug**
- **12 years from first results to the market**
- **1 Billion USD total cost**
- **1/10 blockbuster, 1-2 give profit**

Steps of drug development

- Molecule design/synthesis - (drug „targeting” find the target molecules or biological step)

- *in silico, in vitro/ex vivo* screening (High throughput, HTS): lead molecule

- Lead optimization: millions of molecules

- Biological screenings

- Animal experiments

- Formulation, synthesis, volume increasion

Pharmacology

- Primer pharmacodynamics: effect on target

-Secunder: effect on off-targets

- Safety pharmacology: to find unwanted effects

- Dose – efficacy scale

- central nervous system, circulation, respiration,secretion

Toxicology

- Dose level enumeration

- LD50: median lethal dose

- Acute, subacute/chronic toxicity

- genotoxicity: mutagenesis (carcinogenecity)

- Immuntoxicity

- local tolerability

3R rules in animal expeiments

Replacement, Refinement and Reduction of animals

Alternative techniques (in vitro cell-based, ex vivo)

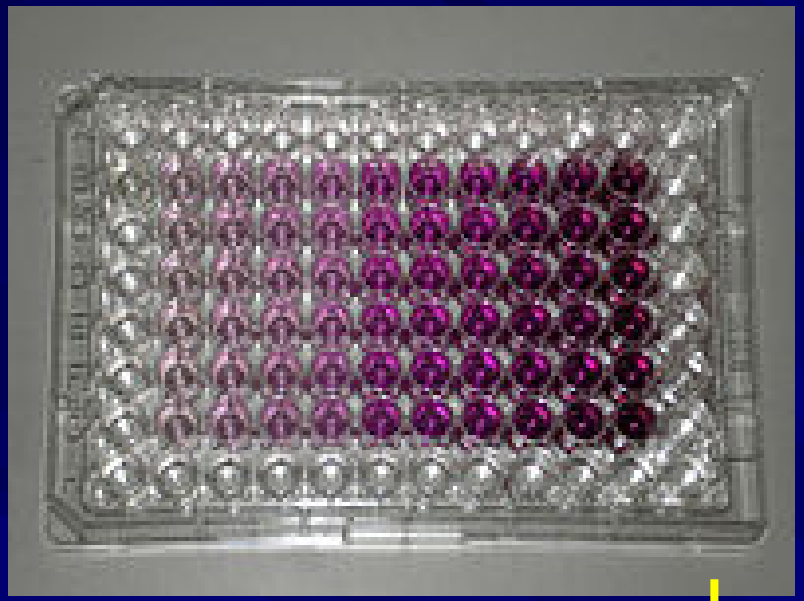
Optimalization of the target/goals, decreased burden

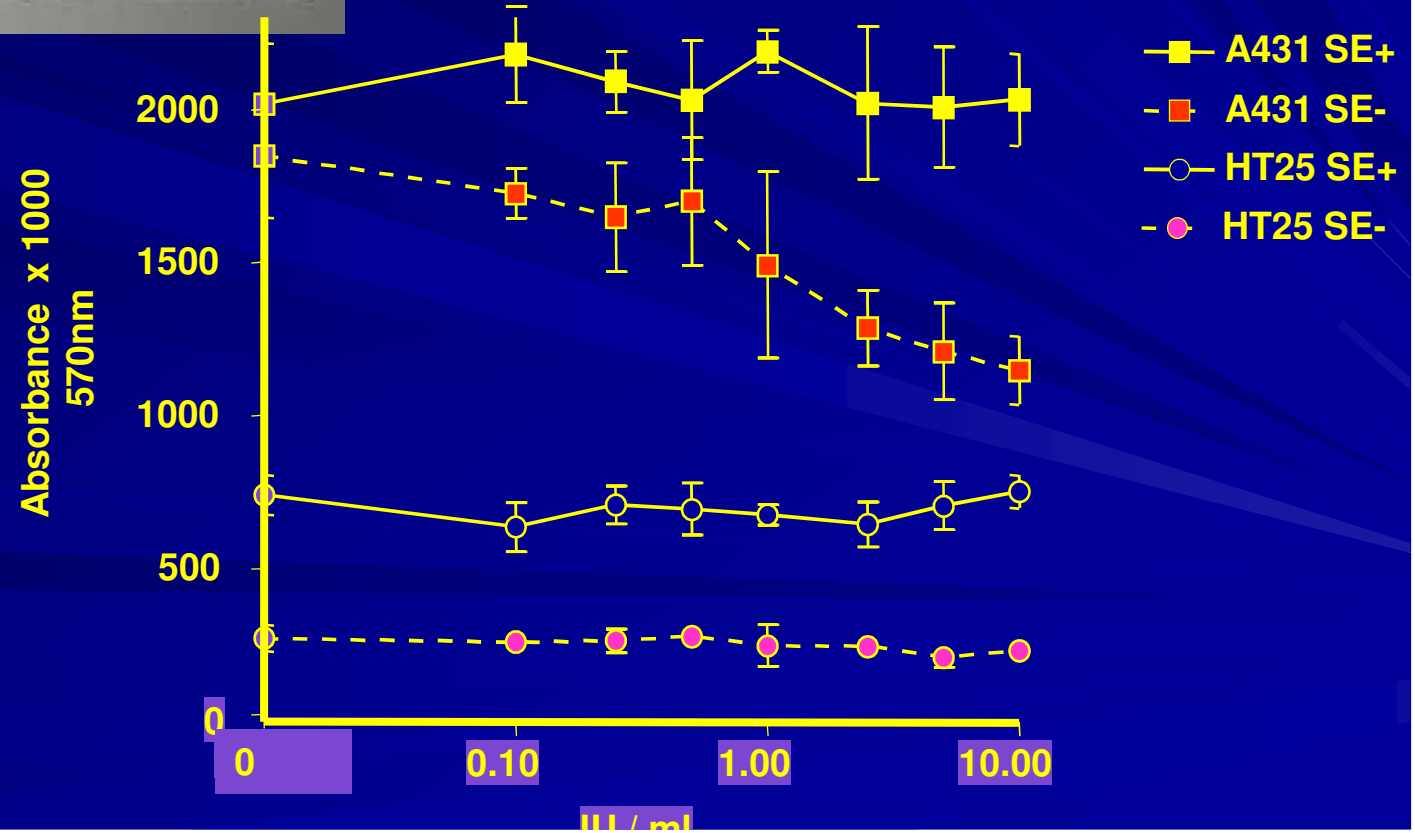
Minimal animal number / groups

Drugs against tumors

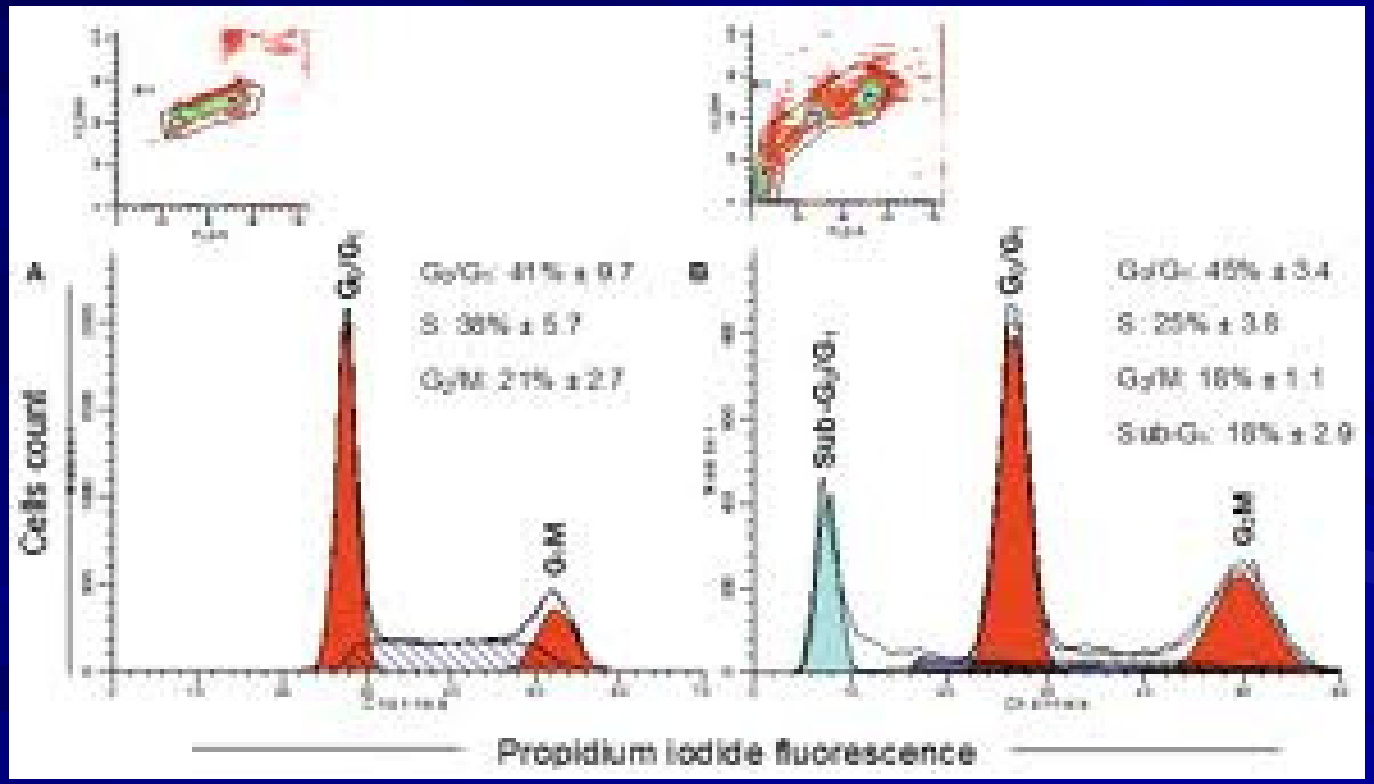
- Cell Proliferation
- Apoptózis (cell death)
- Cell movement / cell adhesion
- Angiogenesis

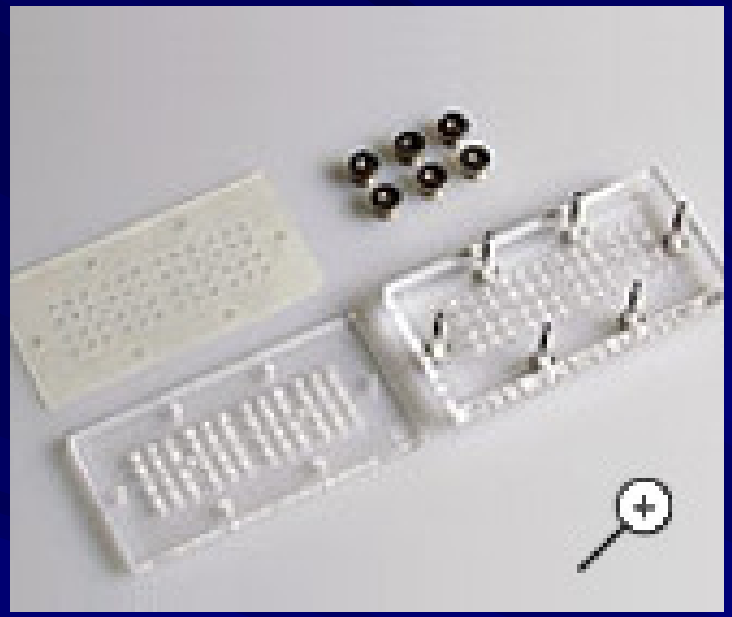
MTT proliferation assay



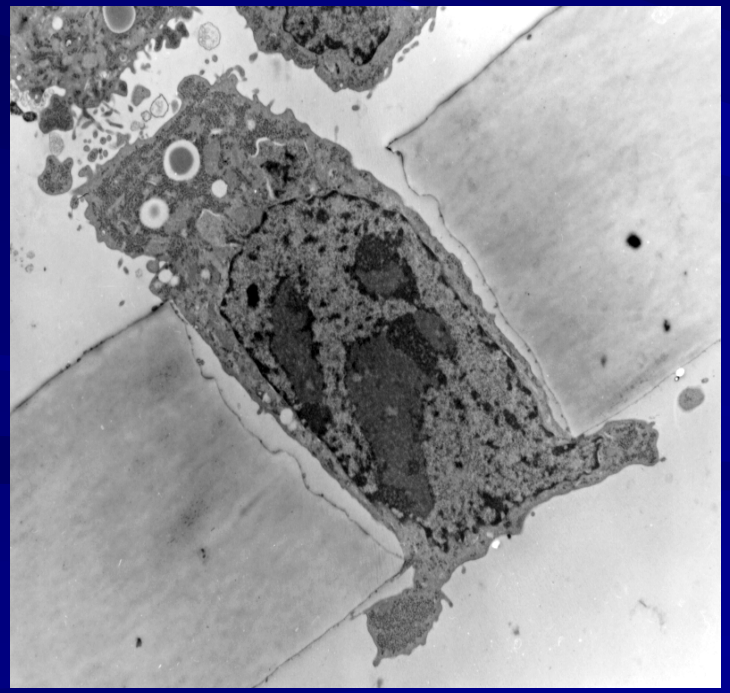


Apoptosis (flow cytometry)





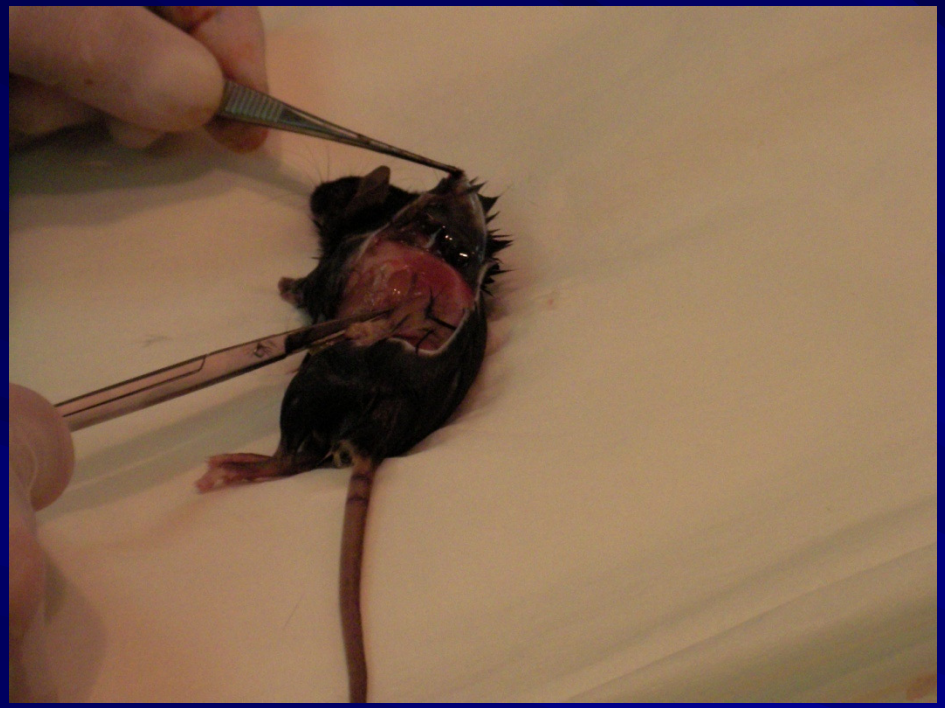
Cell migration
(Boyden chamber)



Animal models

- Allograft (mouse in mice); xenograft (human tumor cells in mice)

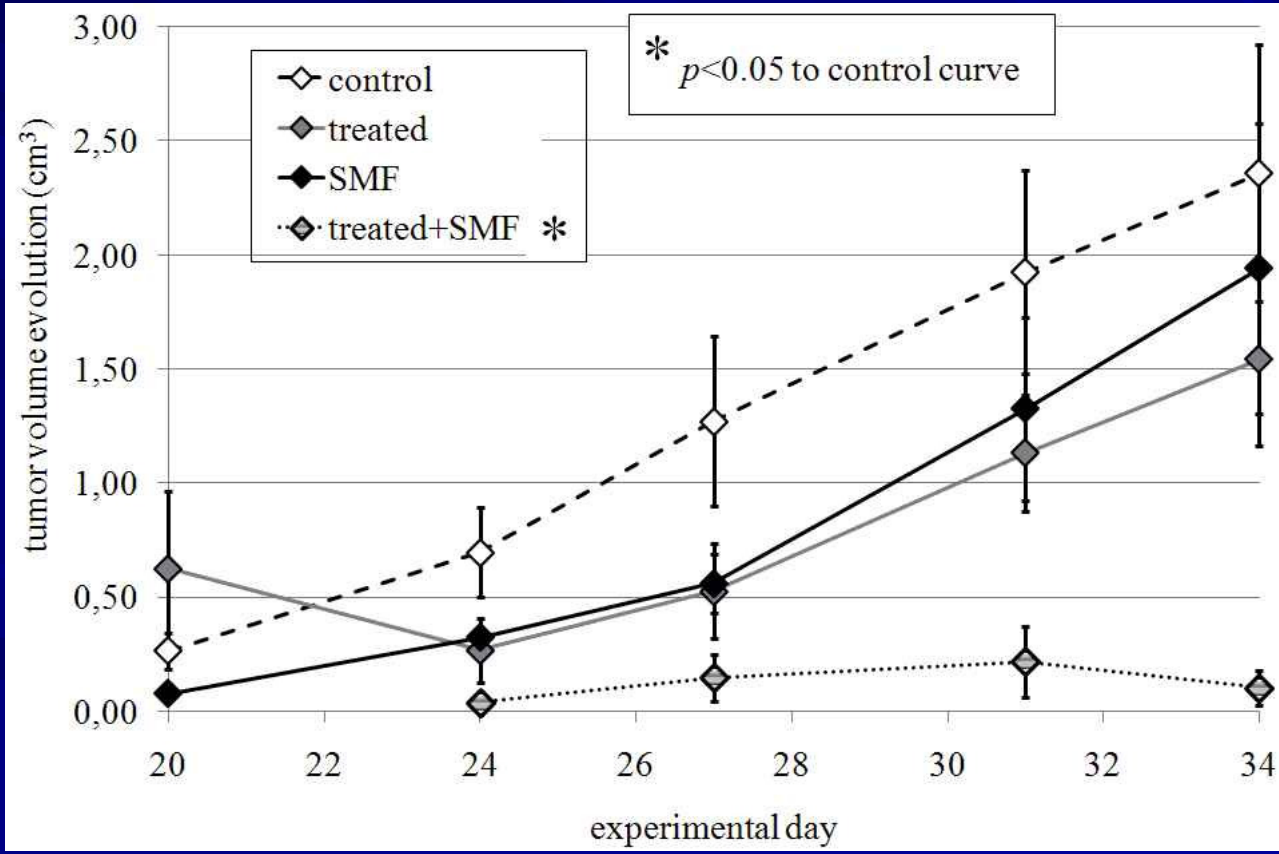
-*Subcutan transplantation*: tumor cells / tumor fragments: growth measurement; inhibitors dose-time correlations
- *orthotopic transpl.:* original tissue enviroment (prostate, breast, colon, lung brain…)
- *metastasis modells*: intra cardiac-, intra venous transplantations…



Subcutan inoculation

Tumor volumes

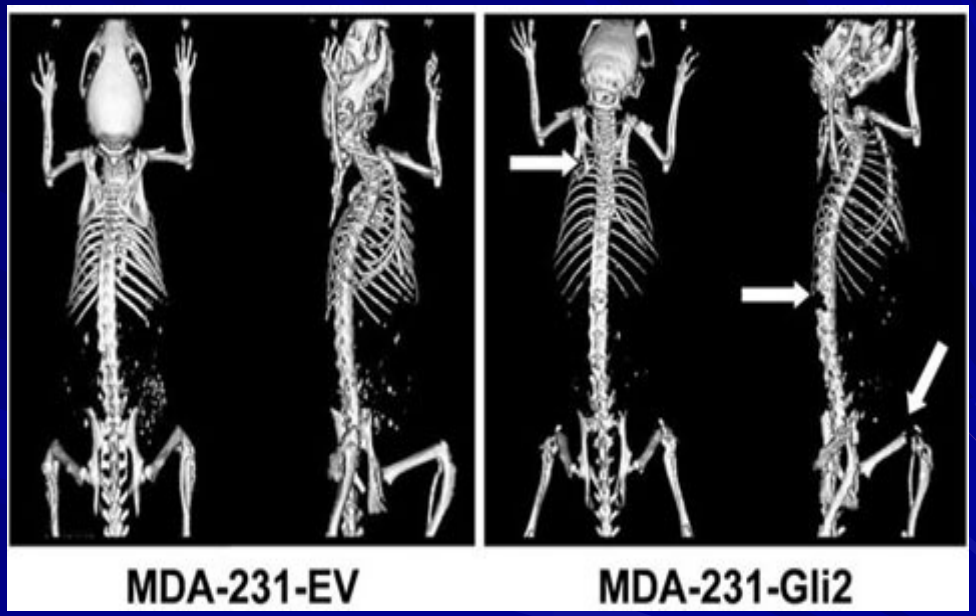
Volume=lenght*width^2*π/6



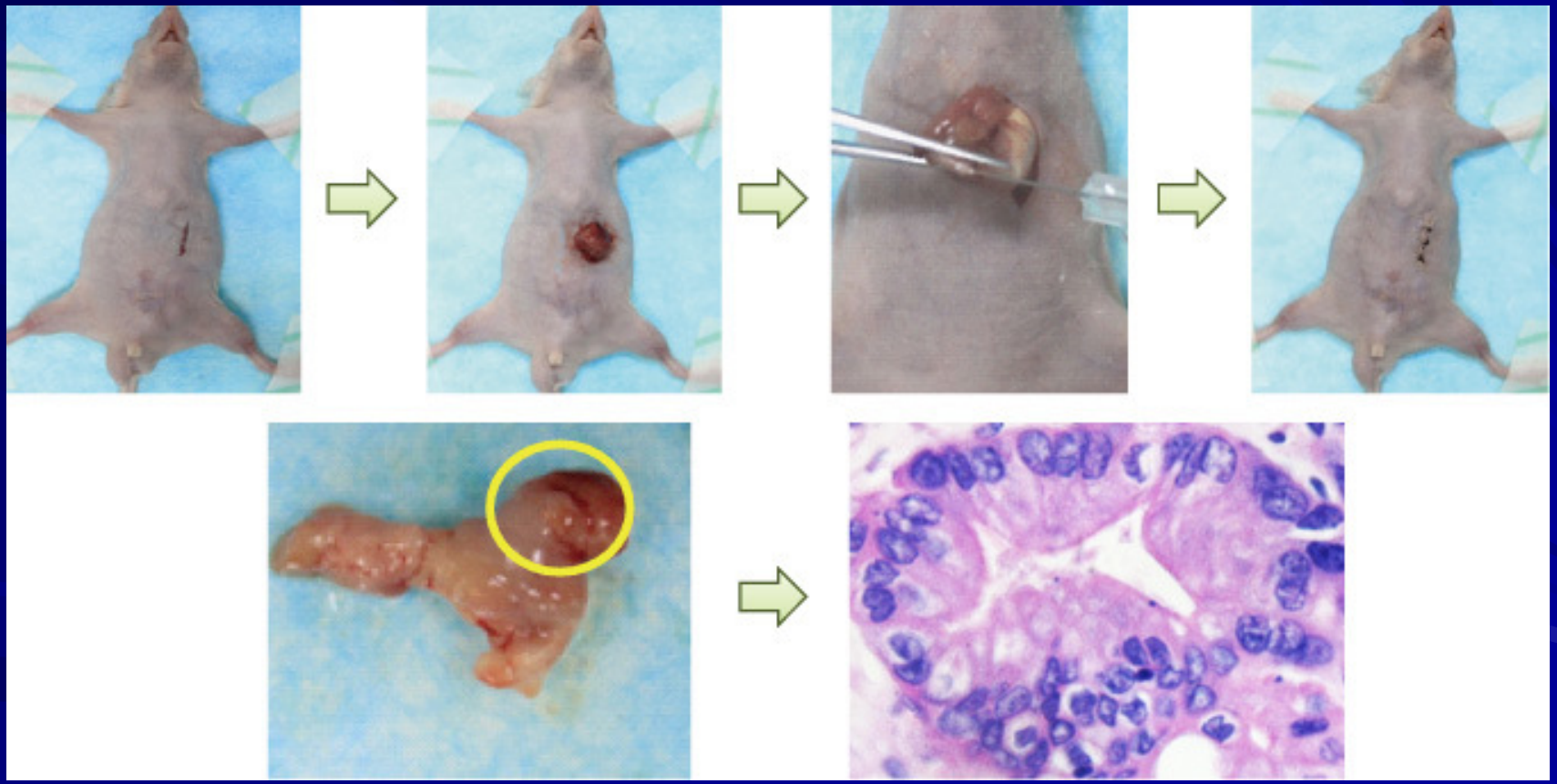
Orthotopic transplantation

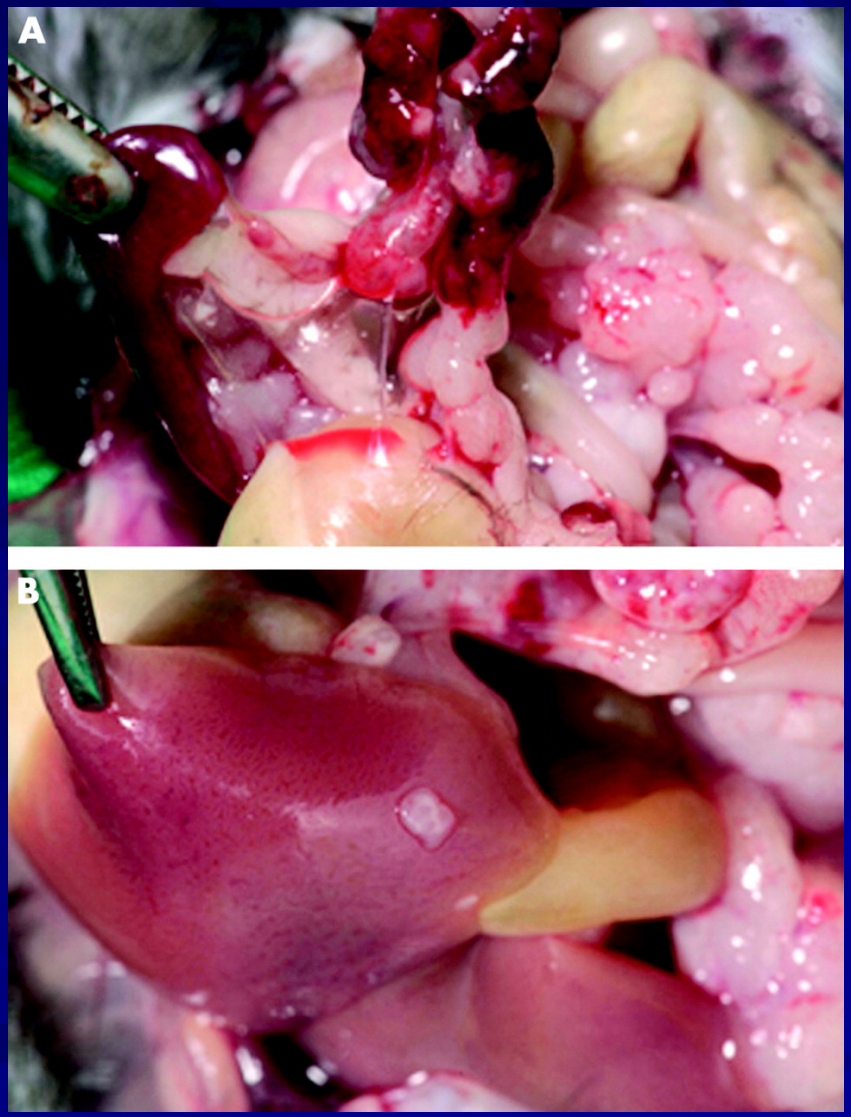
Zhao MD et al. Biomaterials 33(2) 2012. 634-43.

Intra cardiac

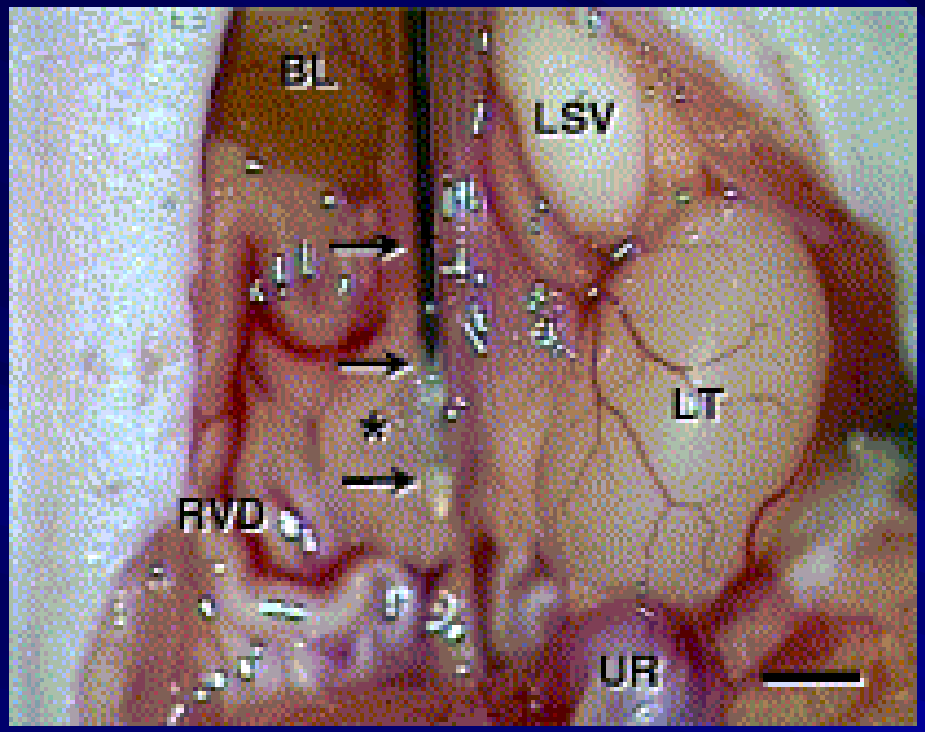


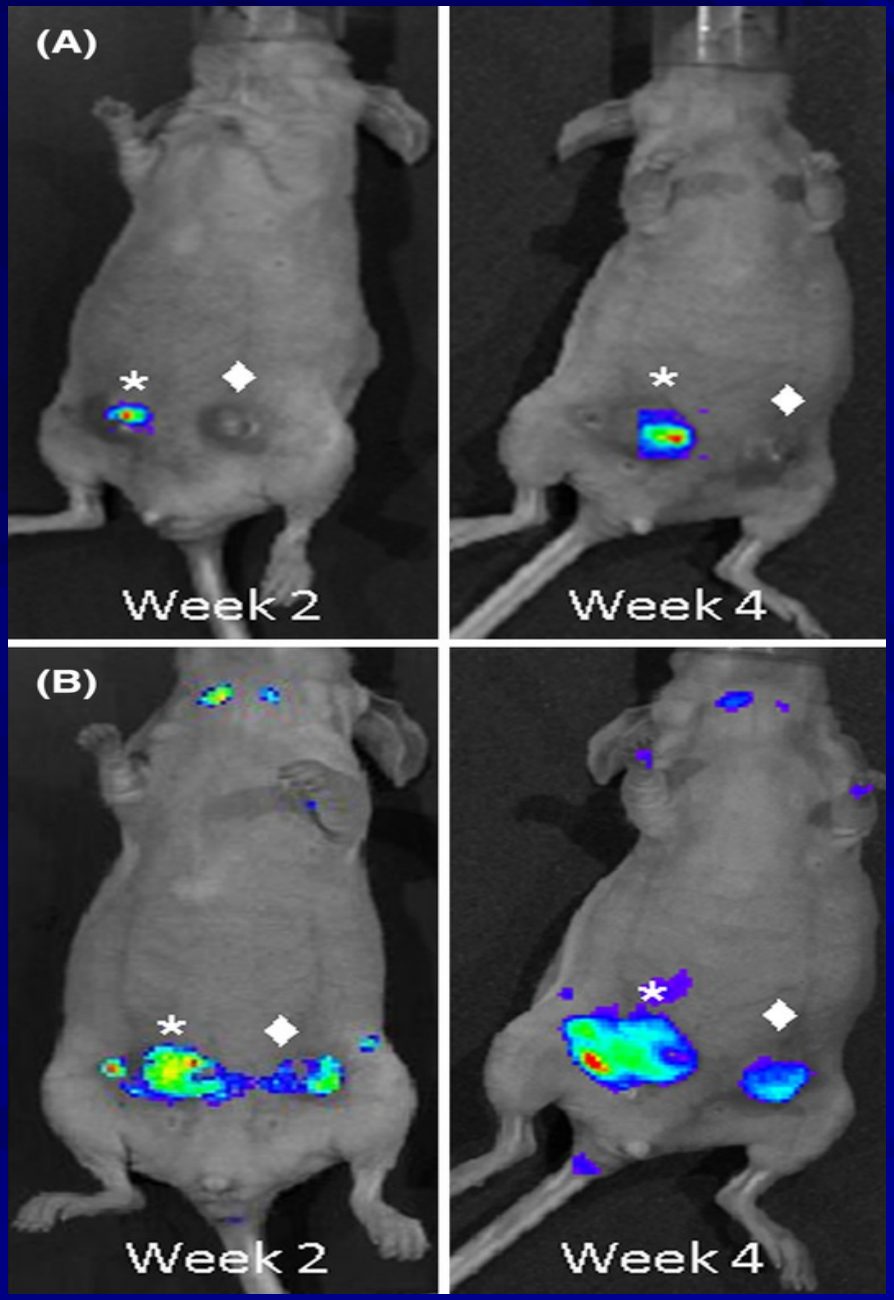
intra bowel




A
B

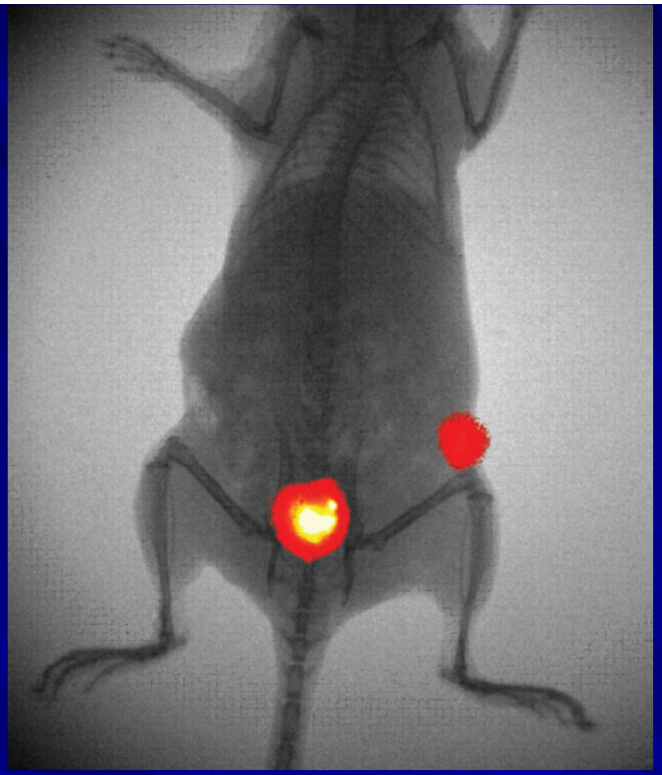
Prostate

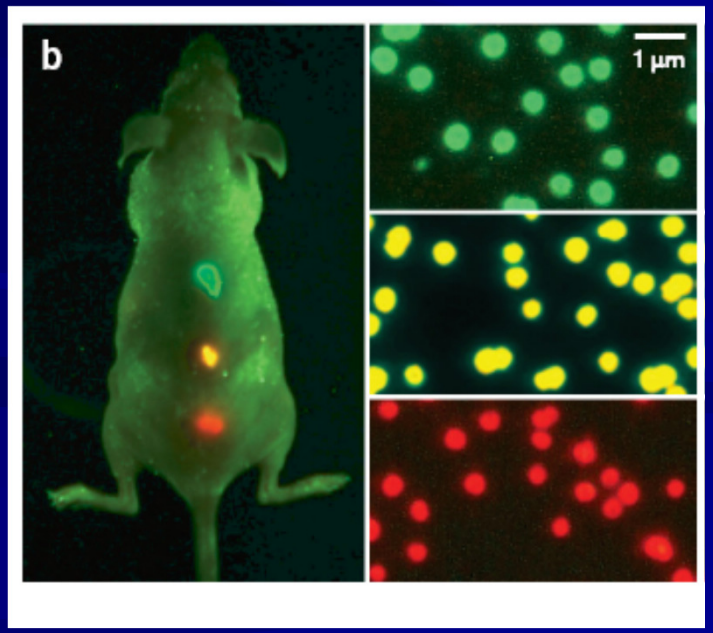


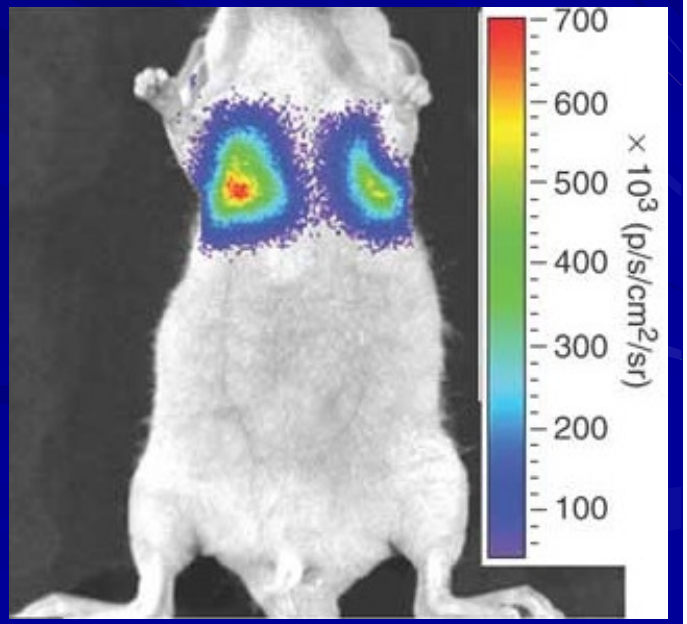


Mammary fat pad

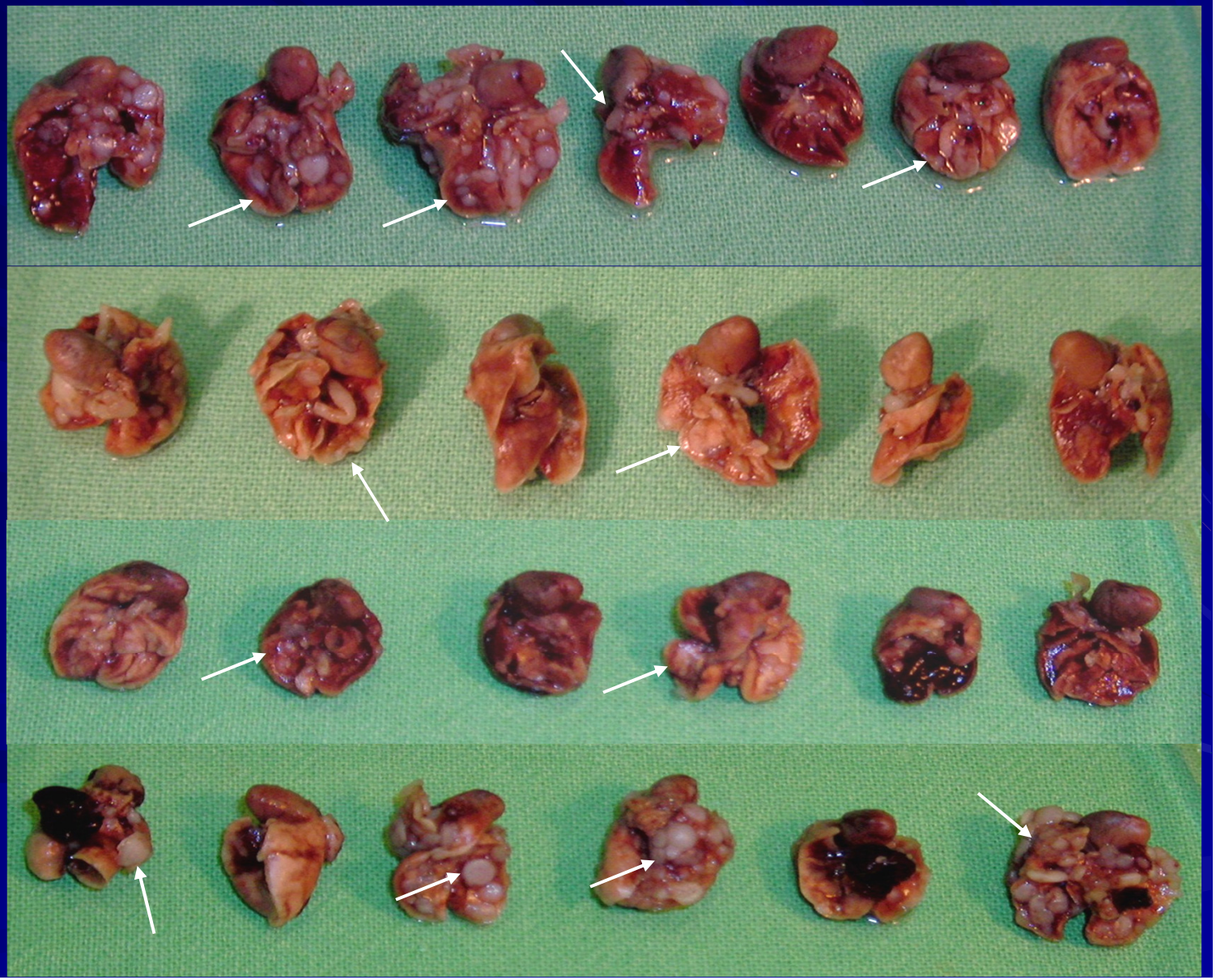
Imaging



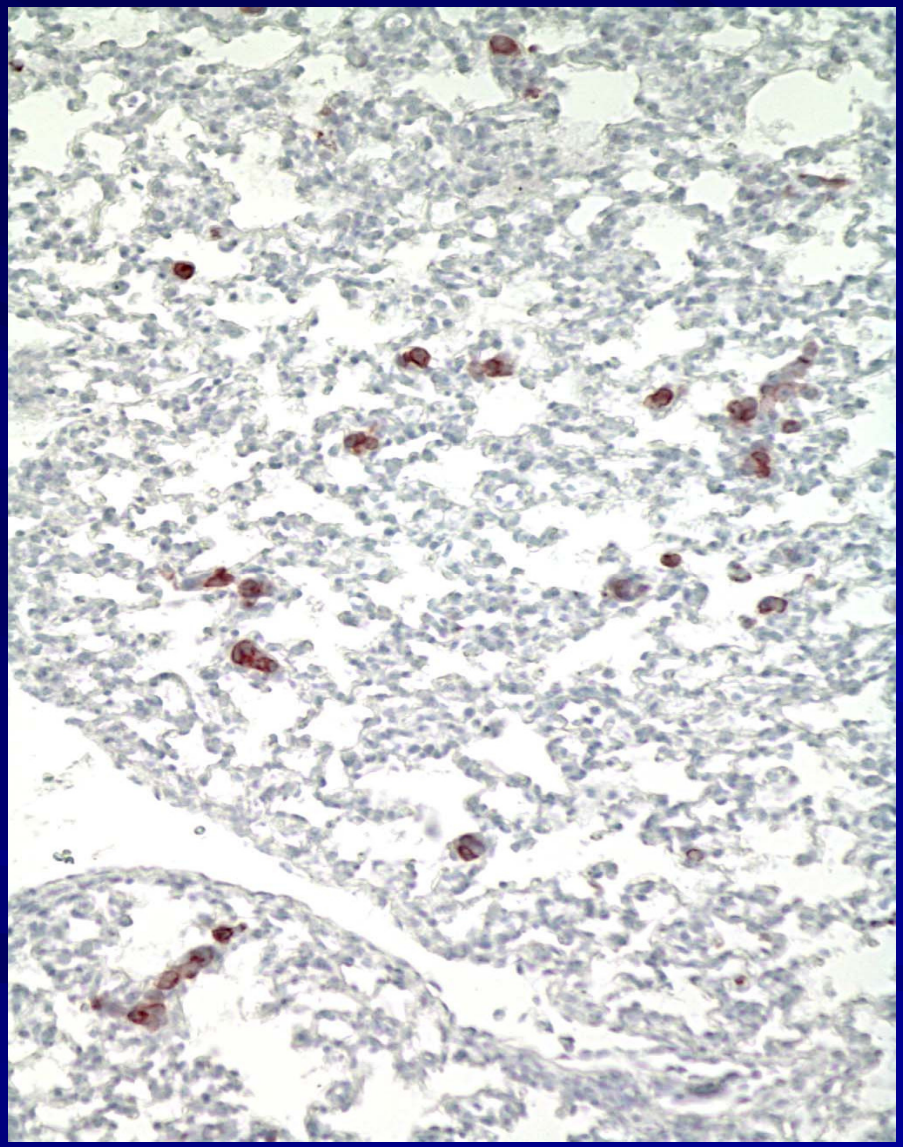


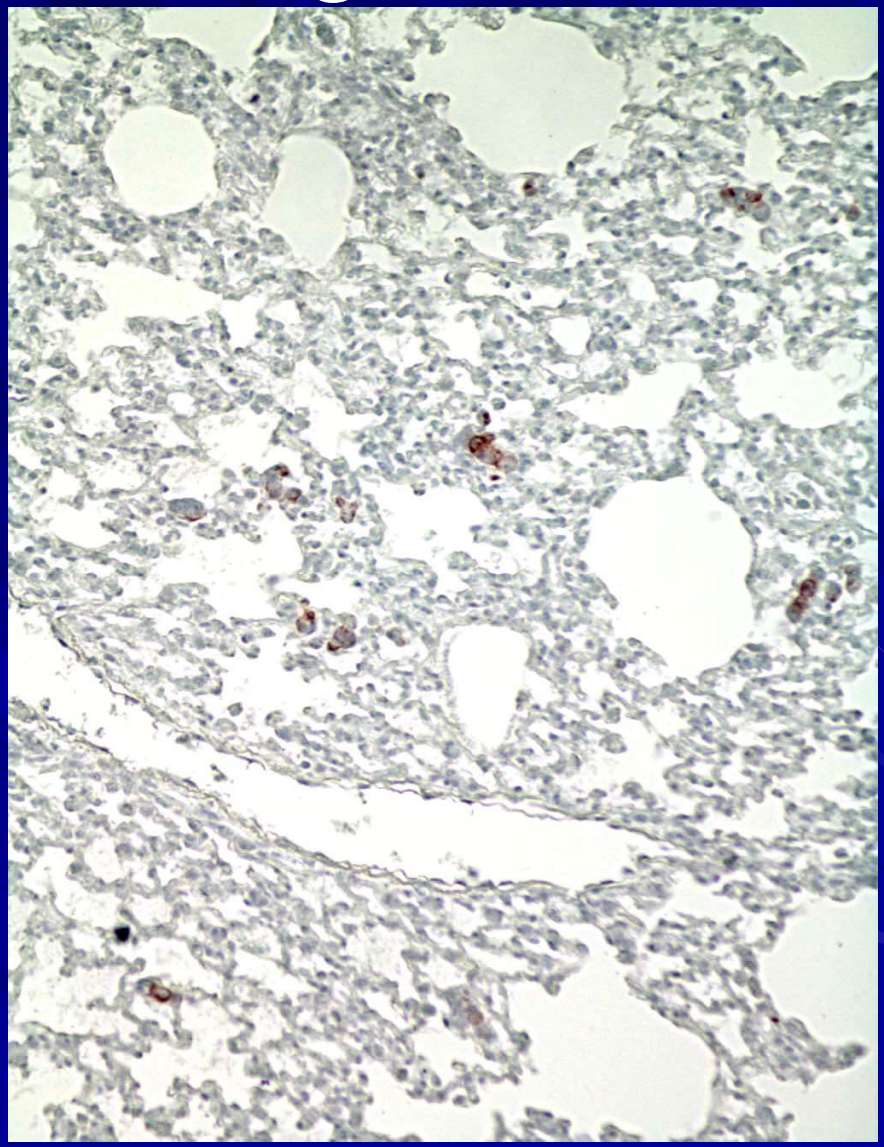


Intra venous – lung colonies



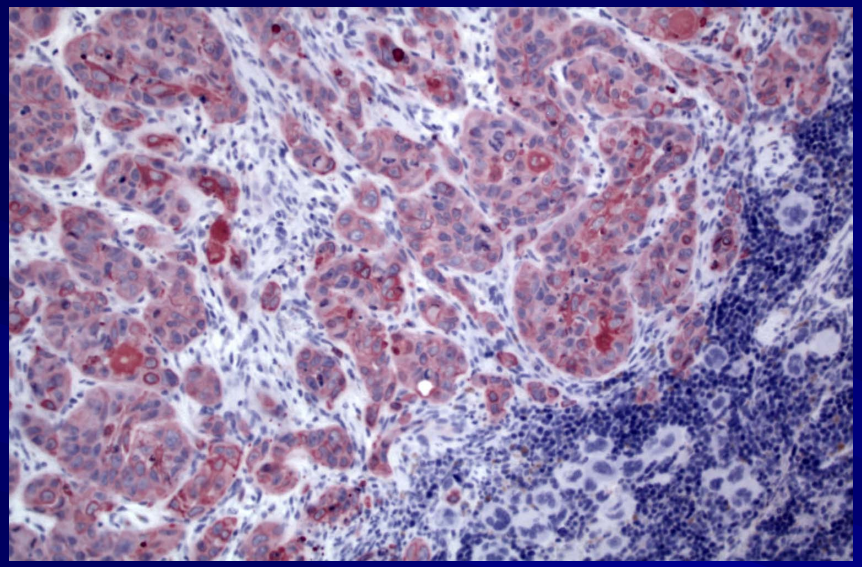
Tumor cells in the lung tissue

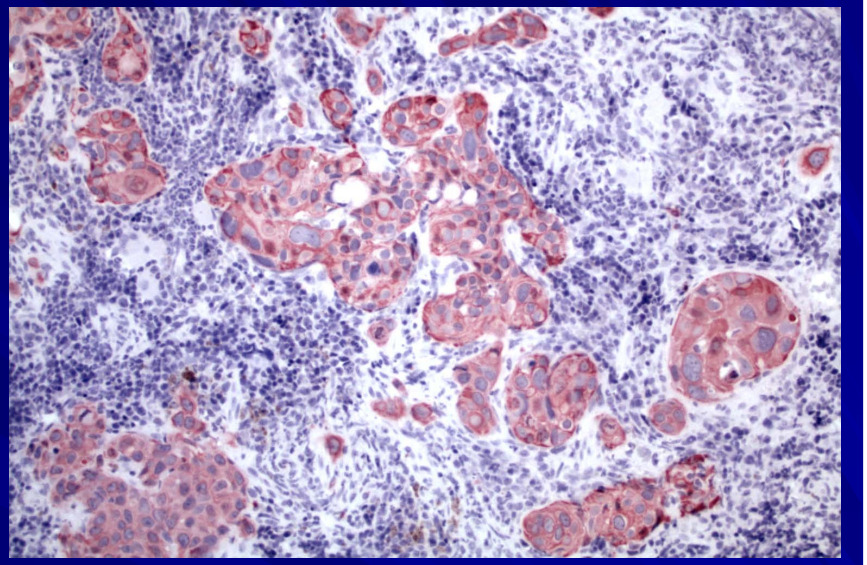


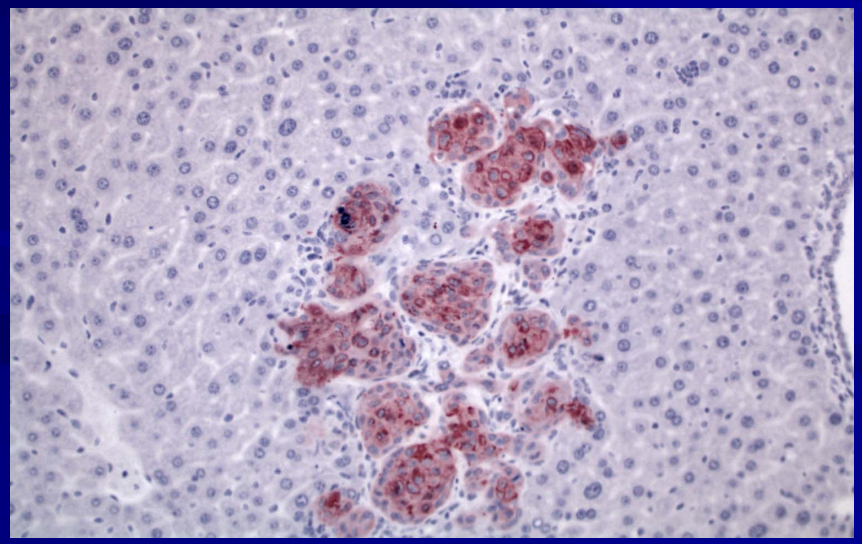


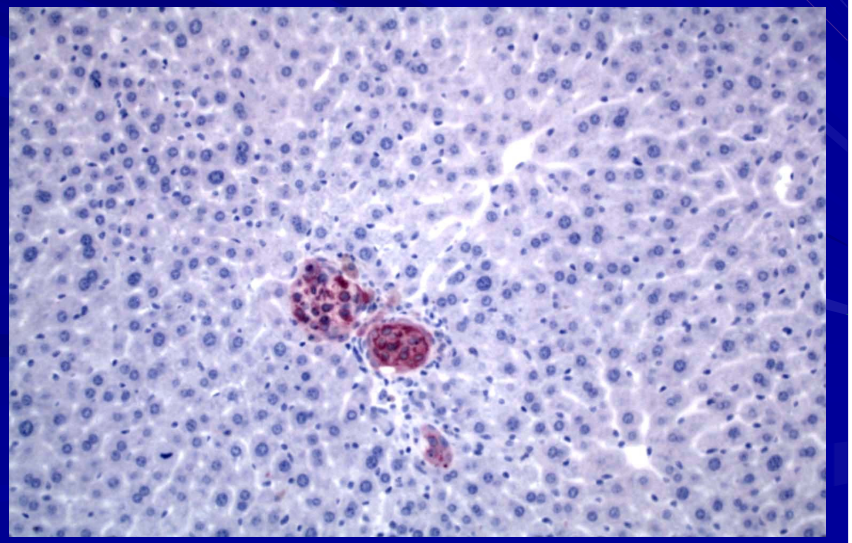
Spleen – liver model

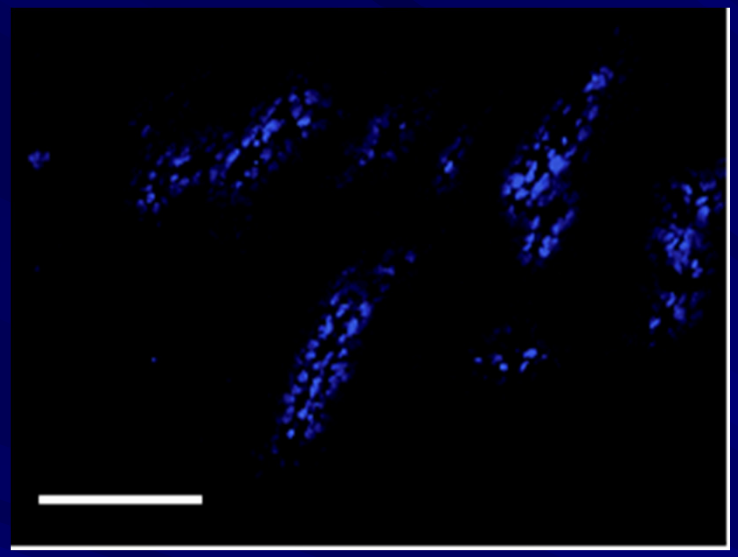
Spleen primer – liver colonies



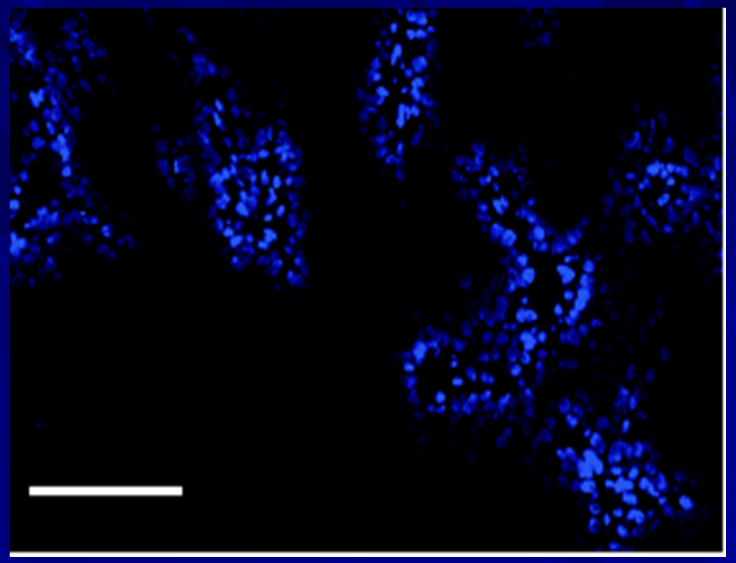




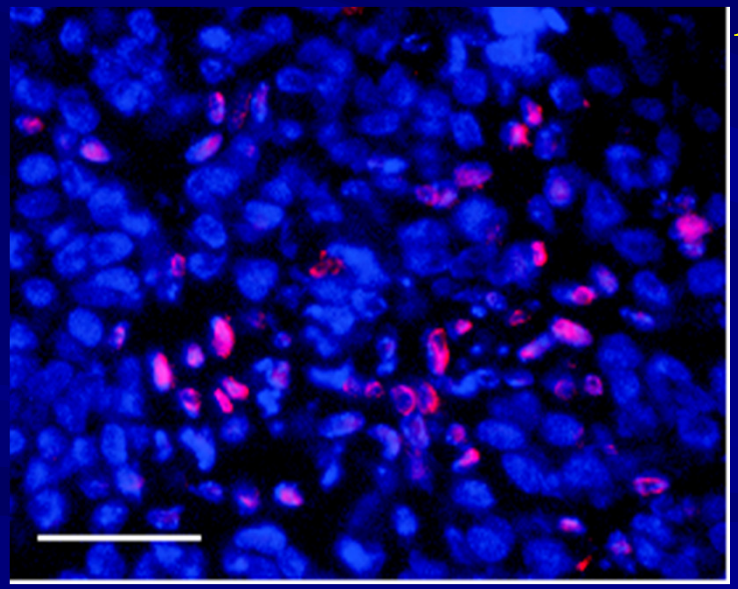




Hoechst

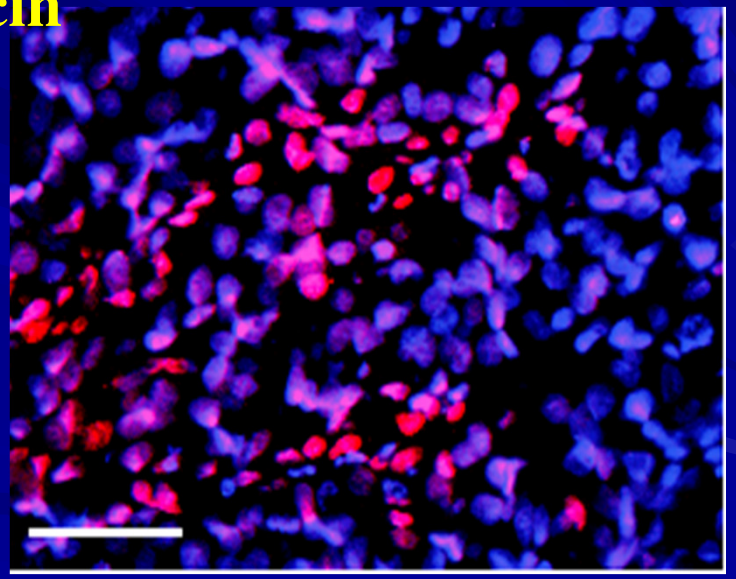


Control



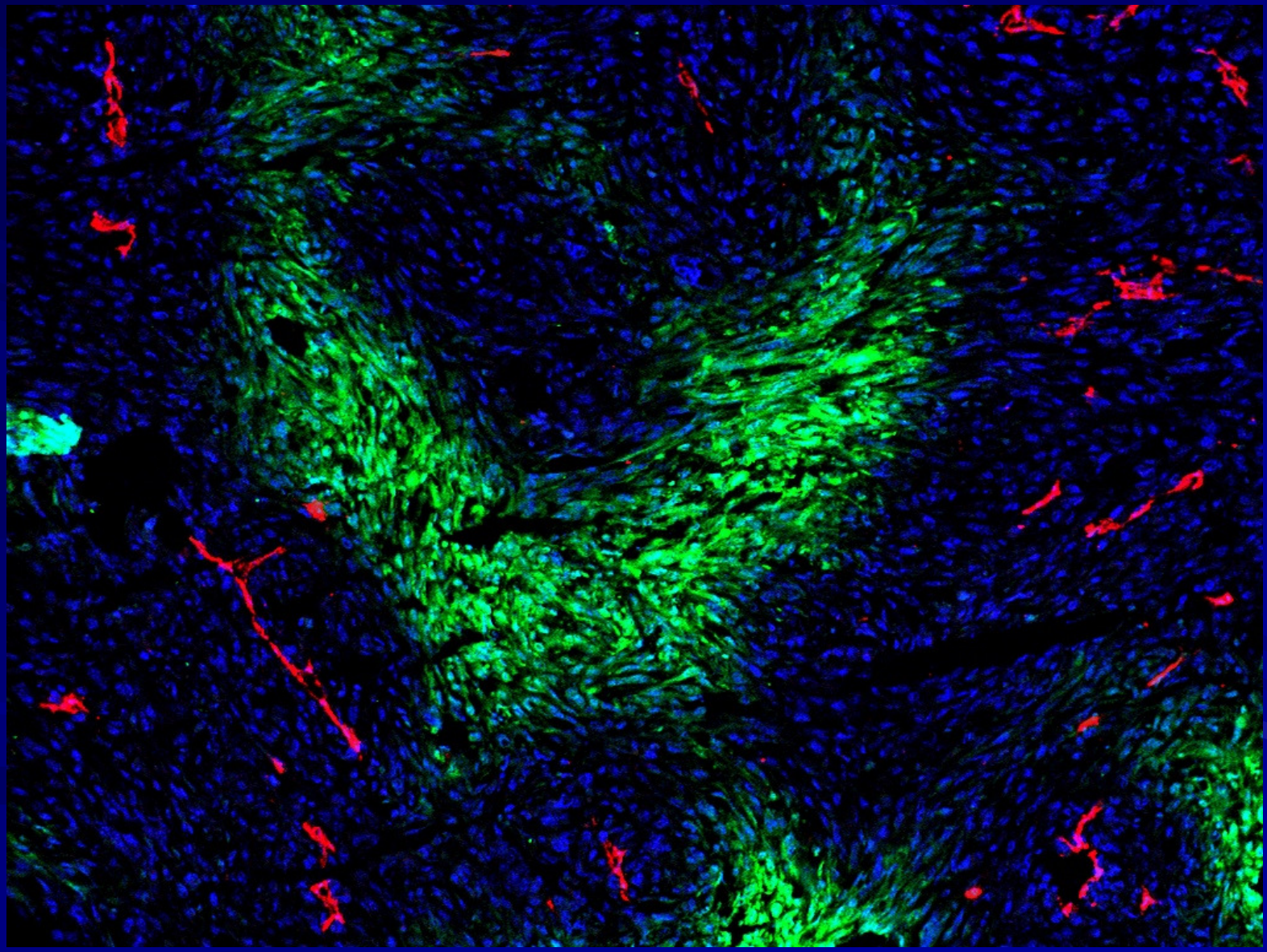
Doxorubicin

rHuEPO



Tovari et al: Cancer Res 2005.

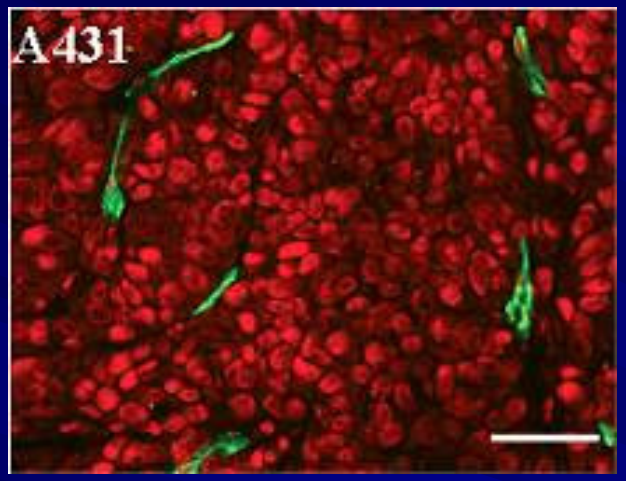
Immunohistochemistry: hypoxic areas



CD31+ vessels in A431 and HT25 tumor xenograft

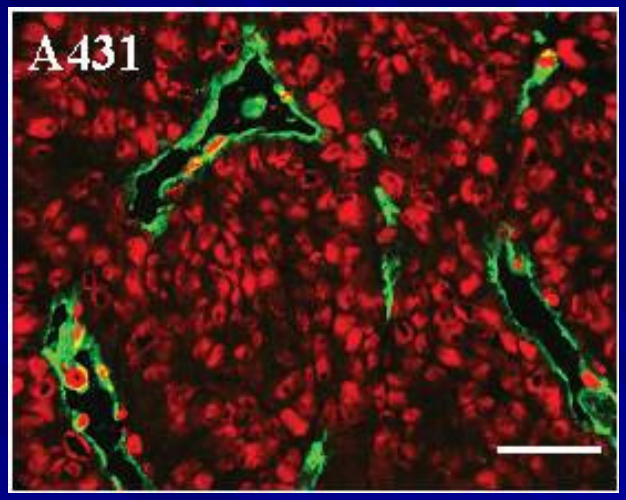
Control

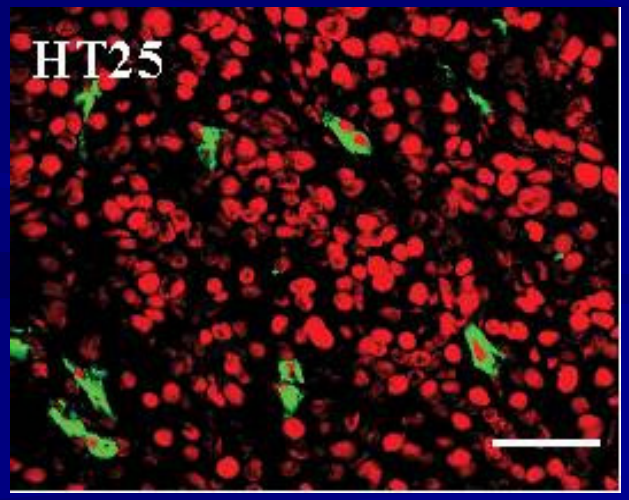
rHuEPO

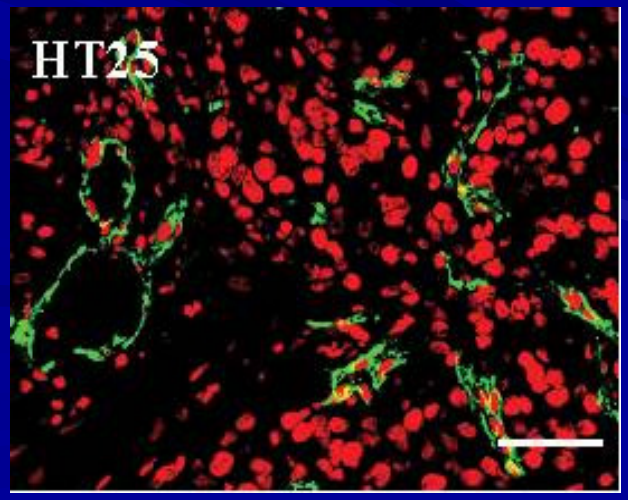


CD31

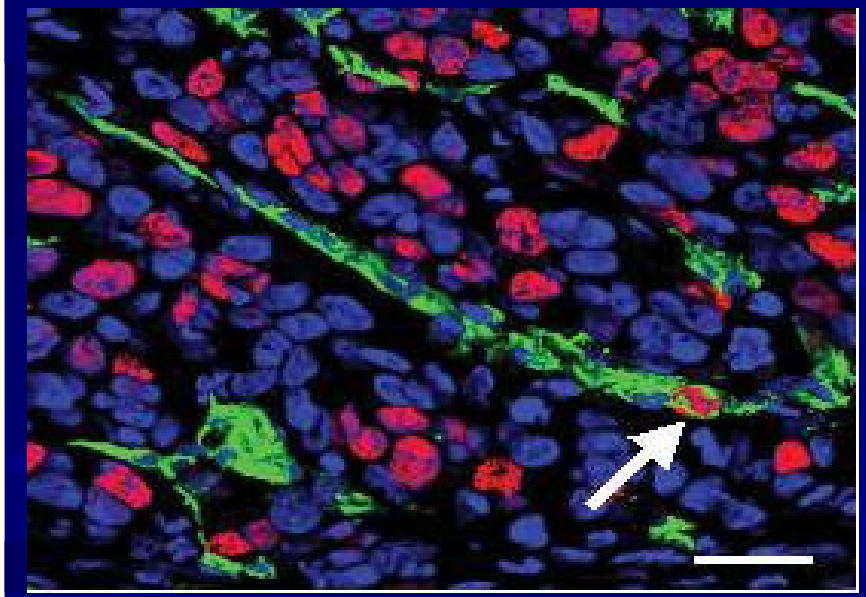
PI







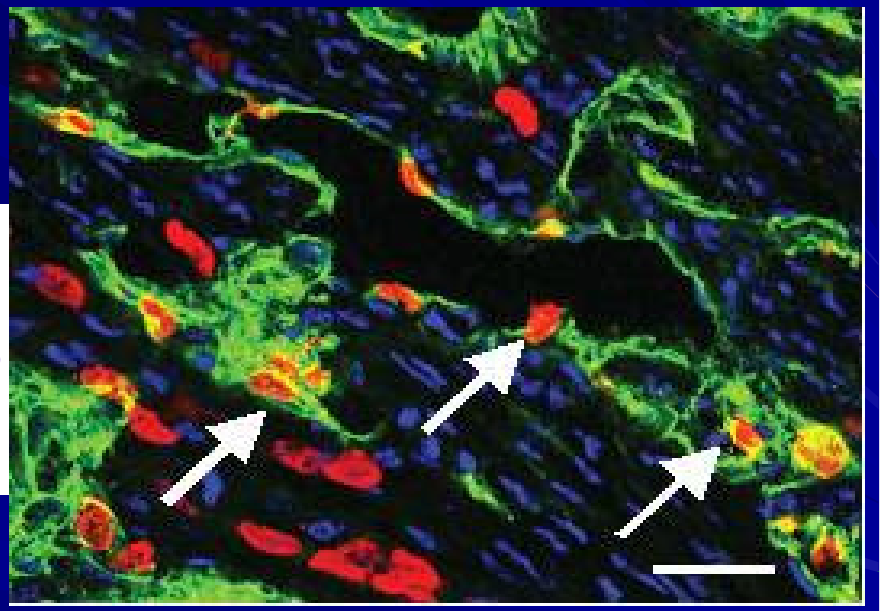
Effects of rHuEPO on tumor and endothelial cell proliferation *in vivo*



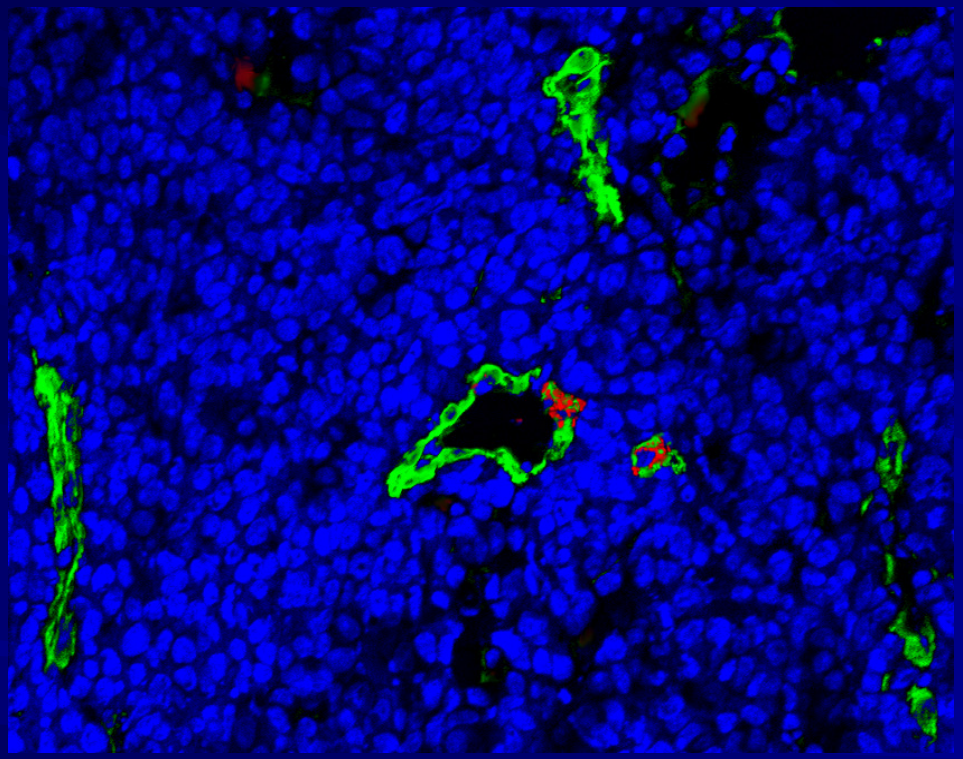
CD31

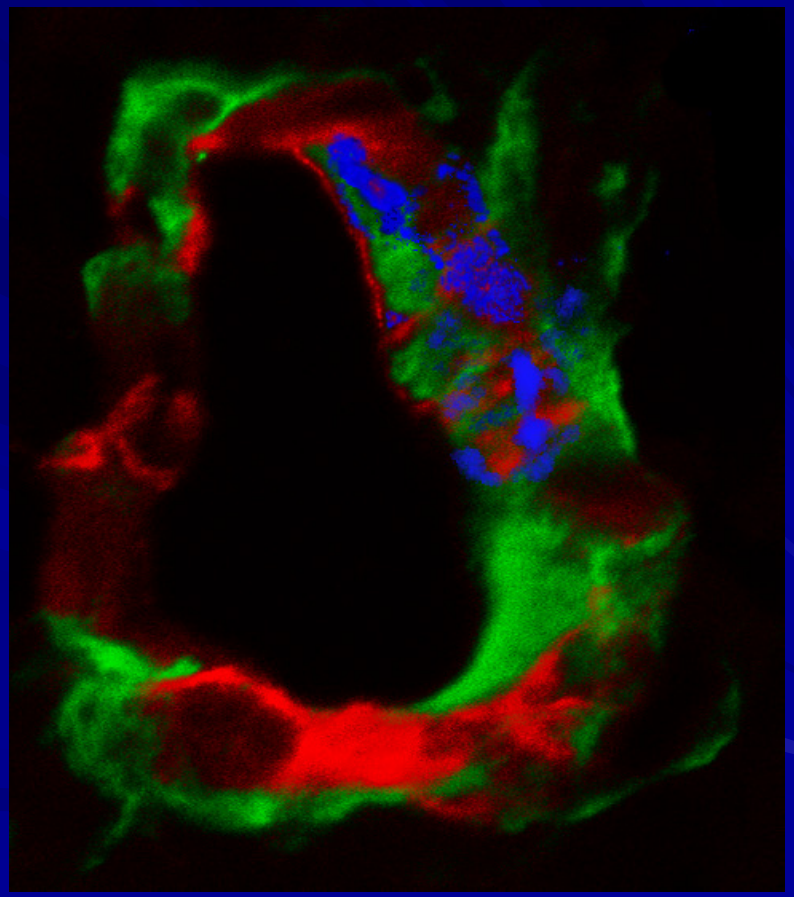
Nucleus

BrdU



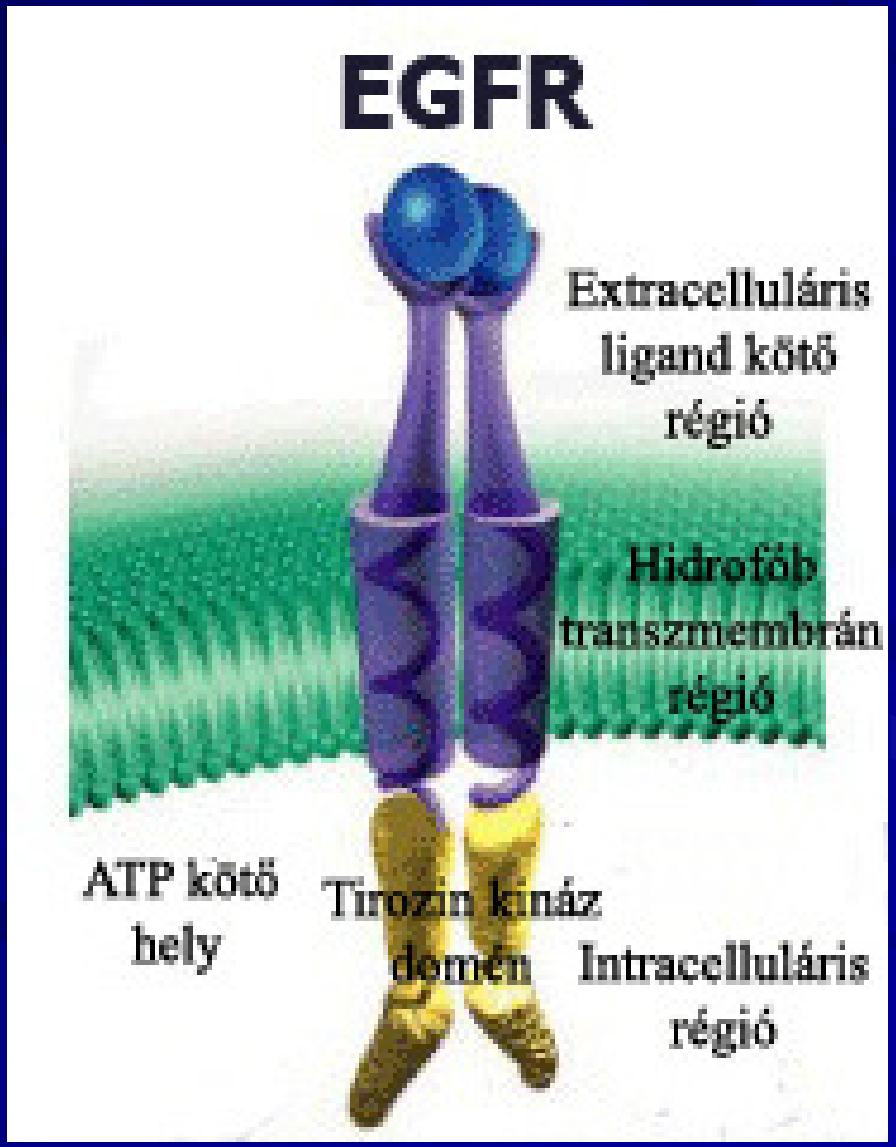
Endotheliális precursor cells in NSCLC (confocal microscopy)



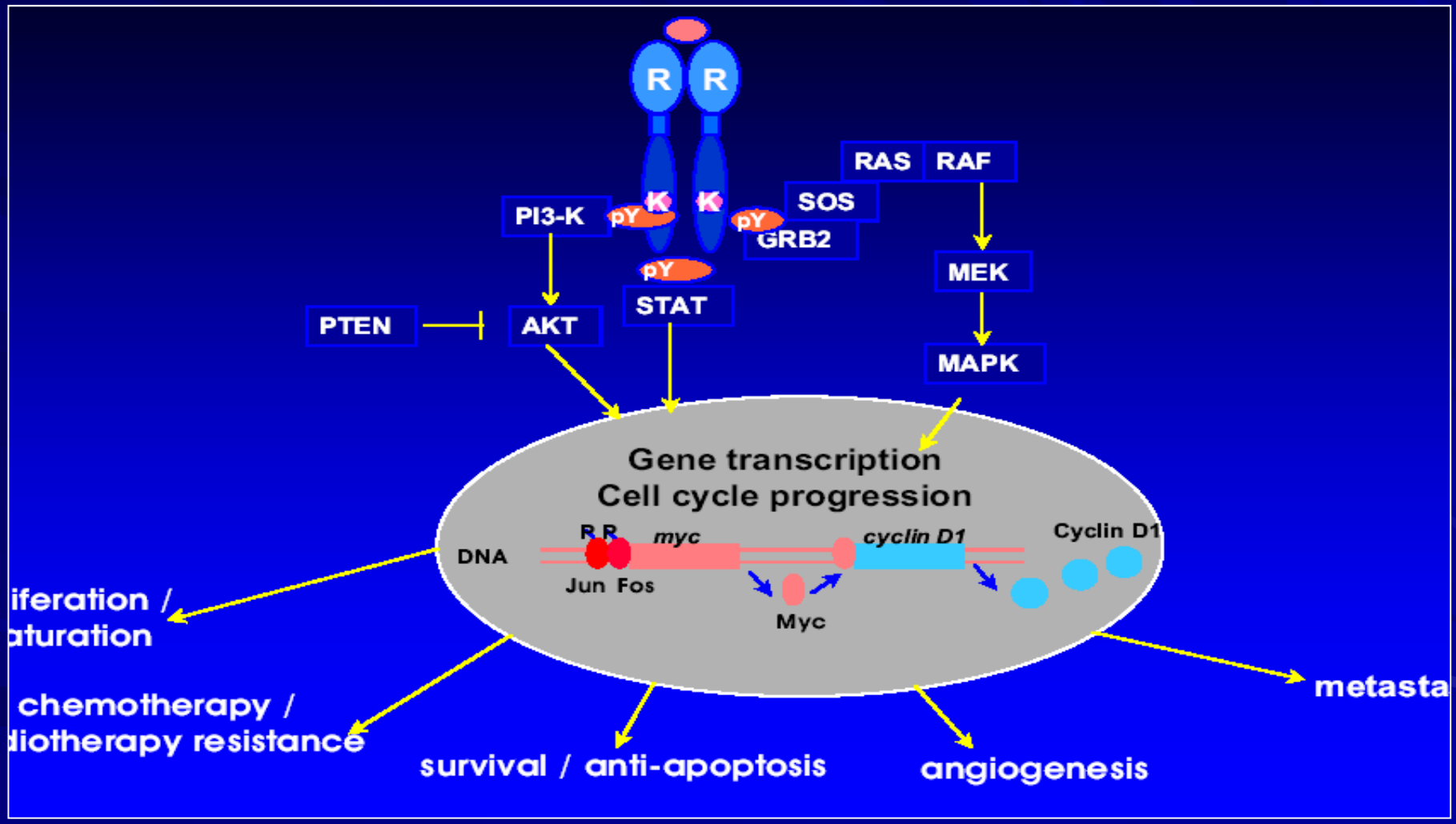


CD133, sejtmag, CD31

Target-specific therapy (EGFR)



EGFR signaling



Anti-EGFR therapies

- Monoclonal antibodies:
  – CETUXIMAB (Erbitux - *Merck*)
  HNSCC combined with radiotherapy

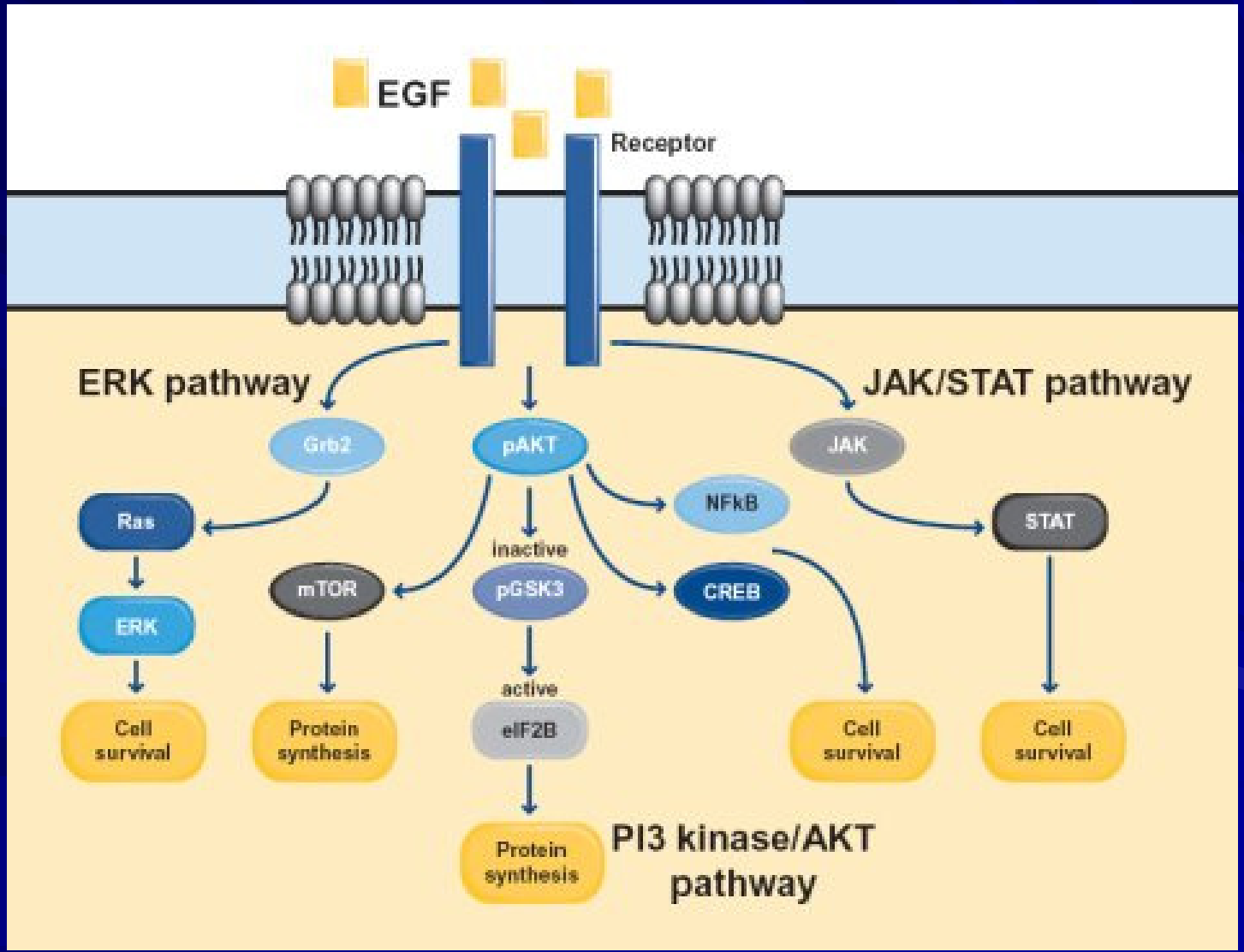
- Tirozin- kinase inhibitors:
  – ERLOTINIB (Tarceva – *Roche*)
  NSCLC, pancreatic tumors

  – GEFITINIB (Iressa – *Astra Zeneca*):

Alterations of EGFR in tumors

- Overexpresssion
- Gene amplifiction
  (~ 13% *Freier és mtsai, Cancer Res. 2006*)
- TK domain mutations: exon 19, 21
- Extracellular deletion– vIII variant
  (~ 42% - *Sok JC és mtsai, Clin. Cancer Res. 2006*)
- + RAS mutations ⟶ anti-EGFR therapy ineffective

Role of RAS in EGFR signaling



Development of Anti-EGFR small TK-inhibitors

Isolated enzyme (TK activity IC50): 33nM

Cell culture (proliferation inhibit. IC50): 1-2uM

In vivo usage: 2 mg/kg

Challenges in the drug development

- Protection against biological degradation
- Delivery
- Side effects
- Cost benefit analysis

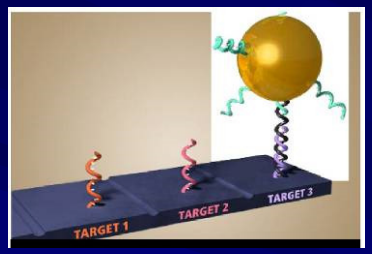
Drug administrations

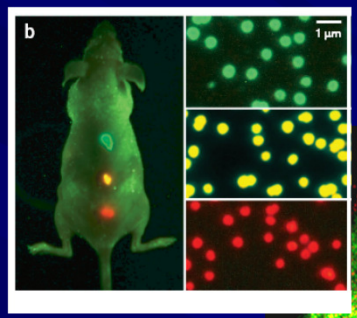
- Oral Delivery

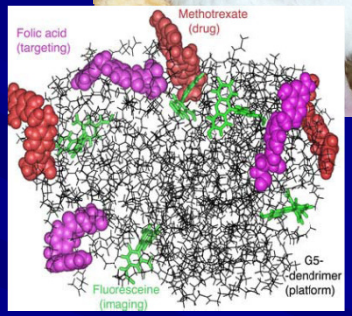
- Inhalation
- Transdermal

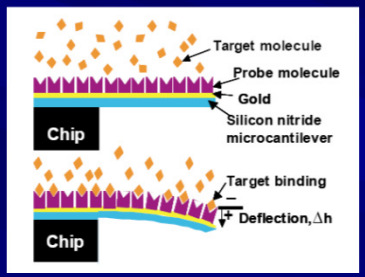
- Implantation
- Injection

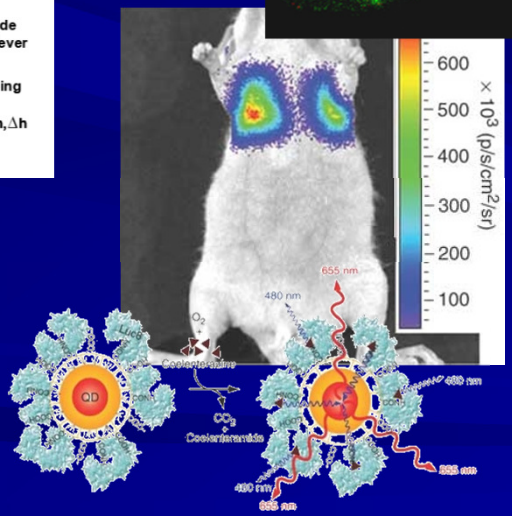
Nanotechnology in Oncology

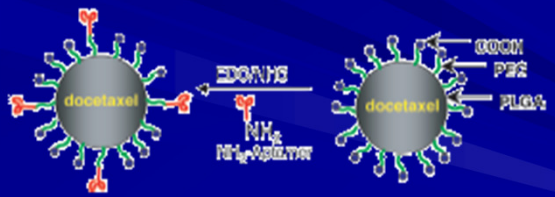








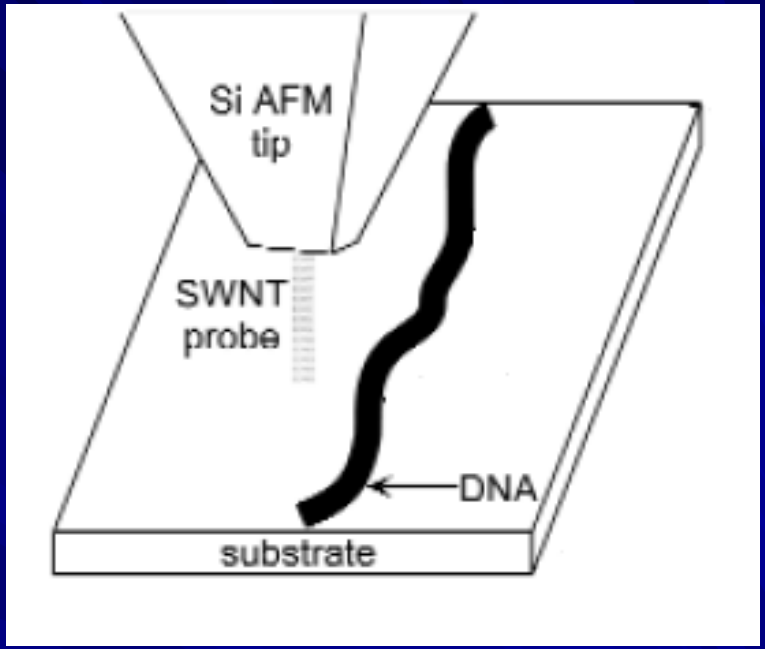




Early detection **Imaging** **Therapy**

Early Cancer Detection

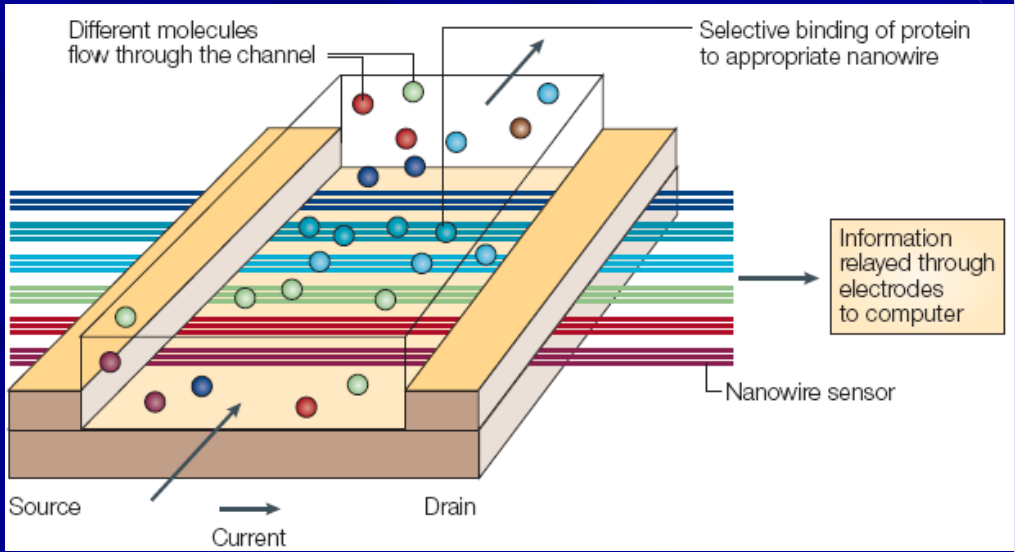
- AFM microscopy



- Nanowires

  Conduction

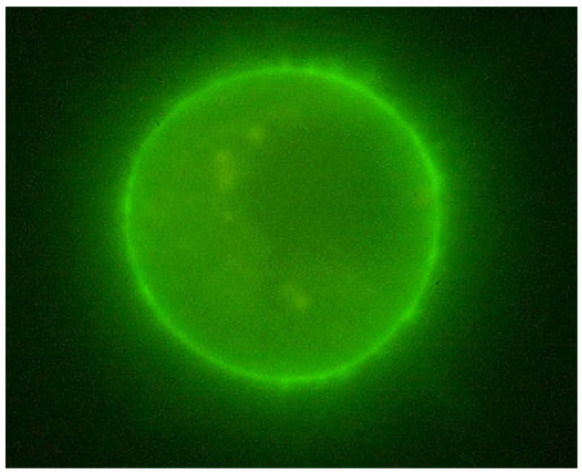
  cc: 10 X 10^{-15}

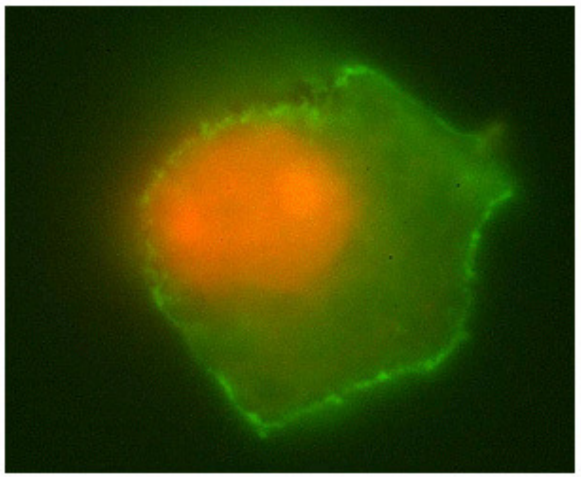


Molecular Cancer Imaging (QDs)

- **Tumor Targeting and Imaging**

  Size: (2 nm-7 nm)
  Color is size dependent

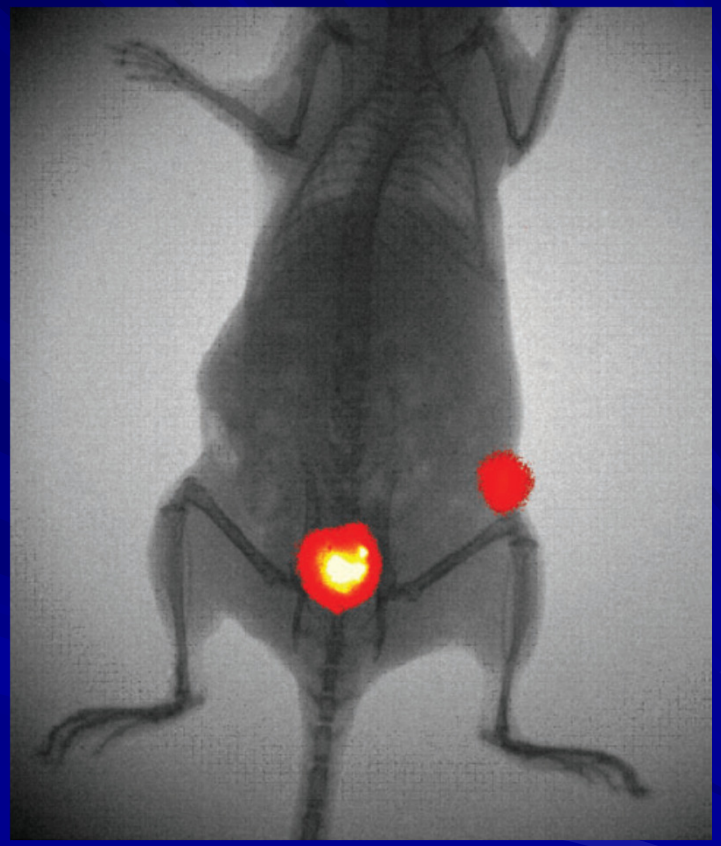


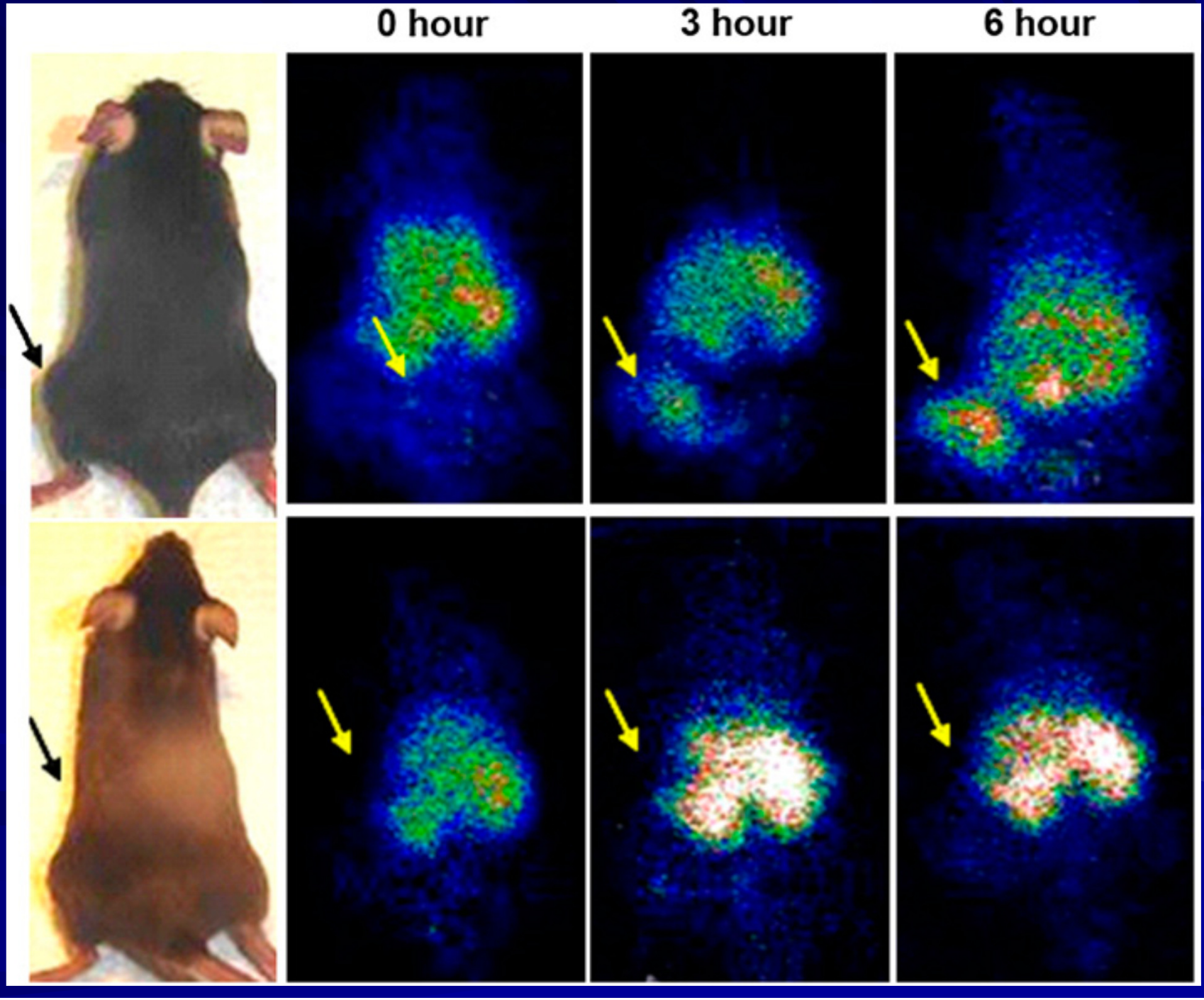


➢X-ray + Optical Imaging

Small tumors

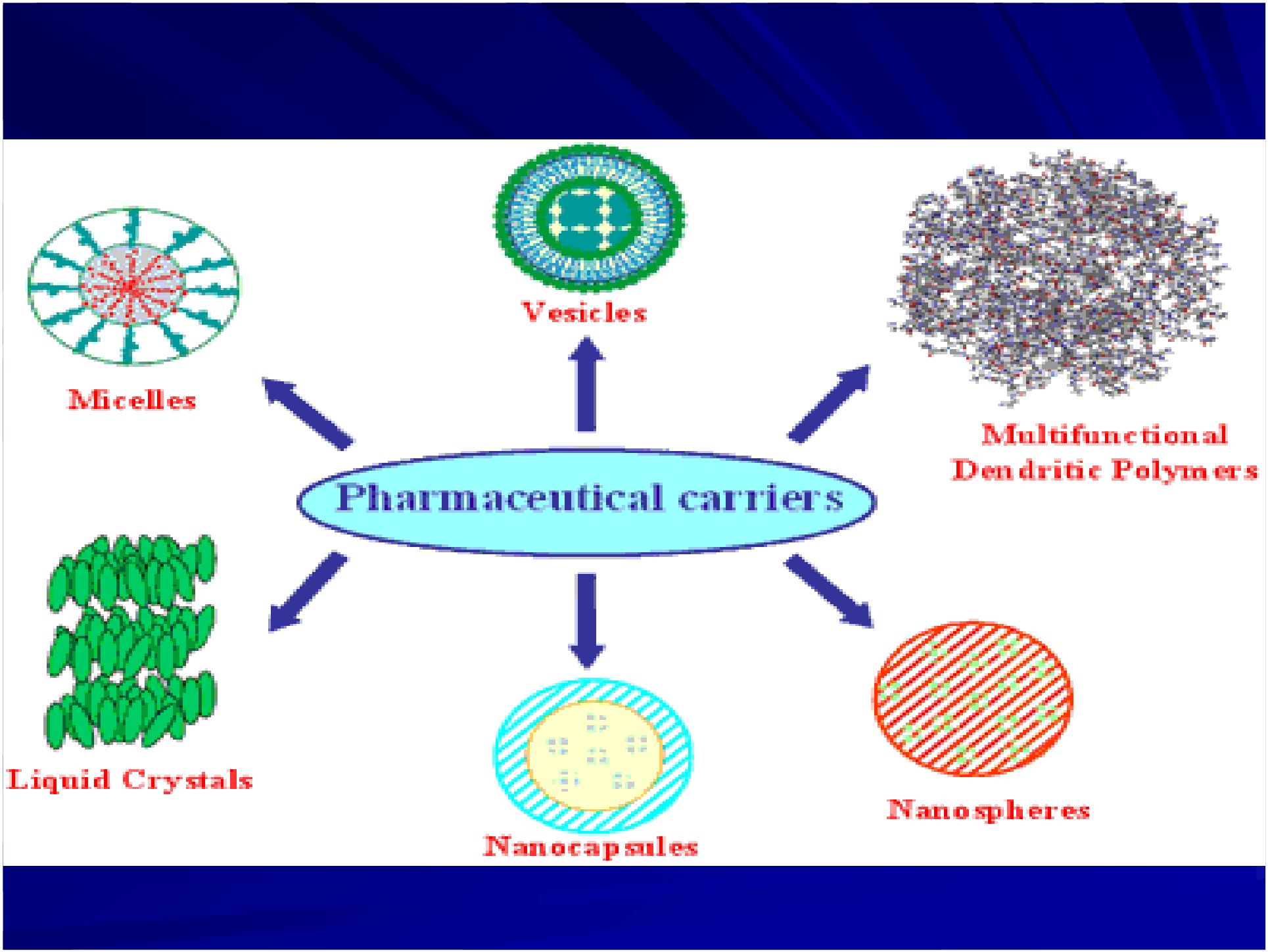
Lokalization



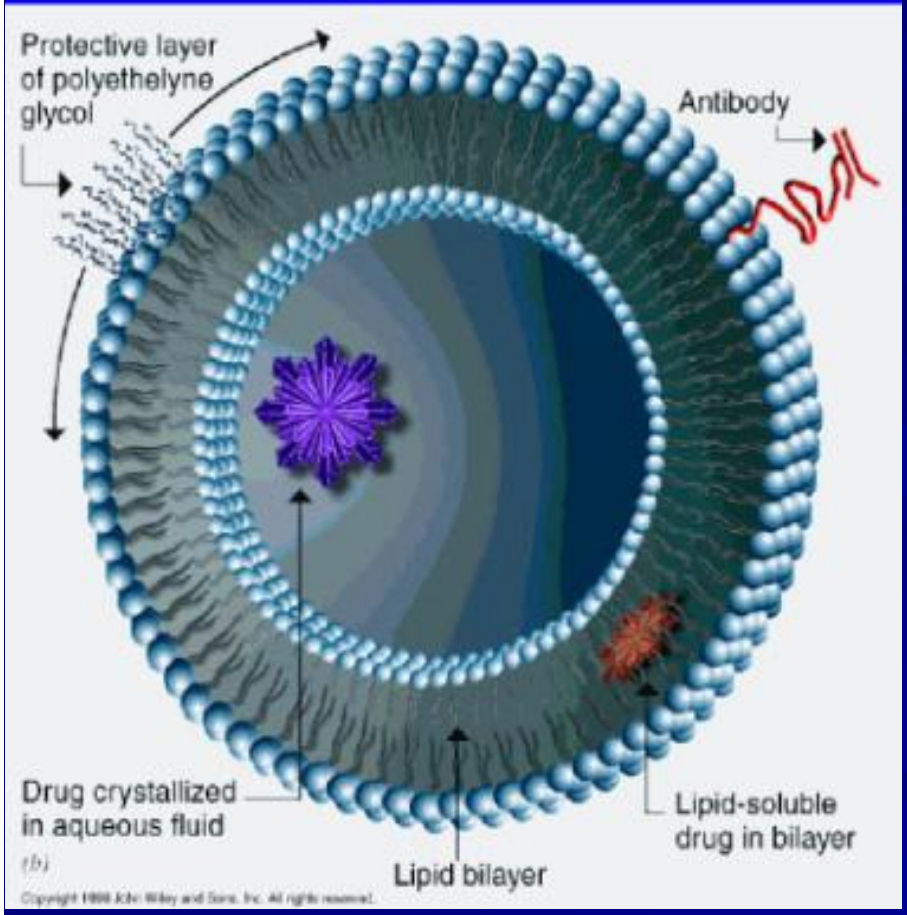

0 hour
3 hour
6 hour

Nanoparticles for drug delivery

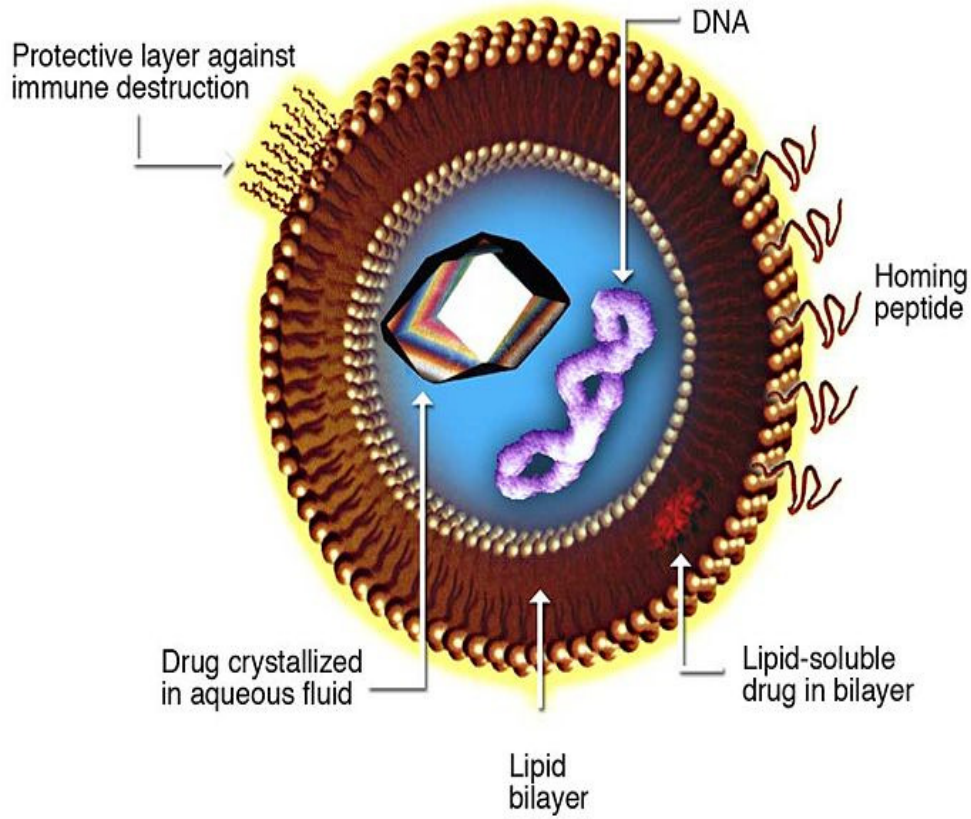
- Metal-based nanoparticles
- Lipid-based nanoparticles
- Polymer-based nanoparticles
- Biological nanoparticles


Pharmaceutical carriers
Micelles
Vesicles
Multifunctional Dendritic Polymers
Liquid Crystals
Nanocapsules
Nanospheres

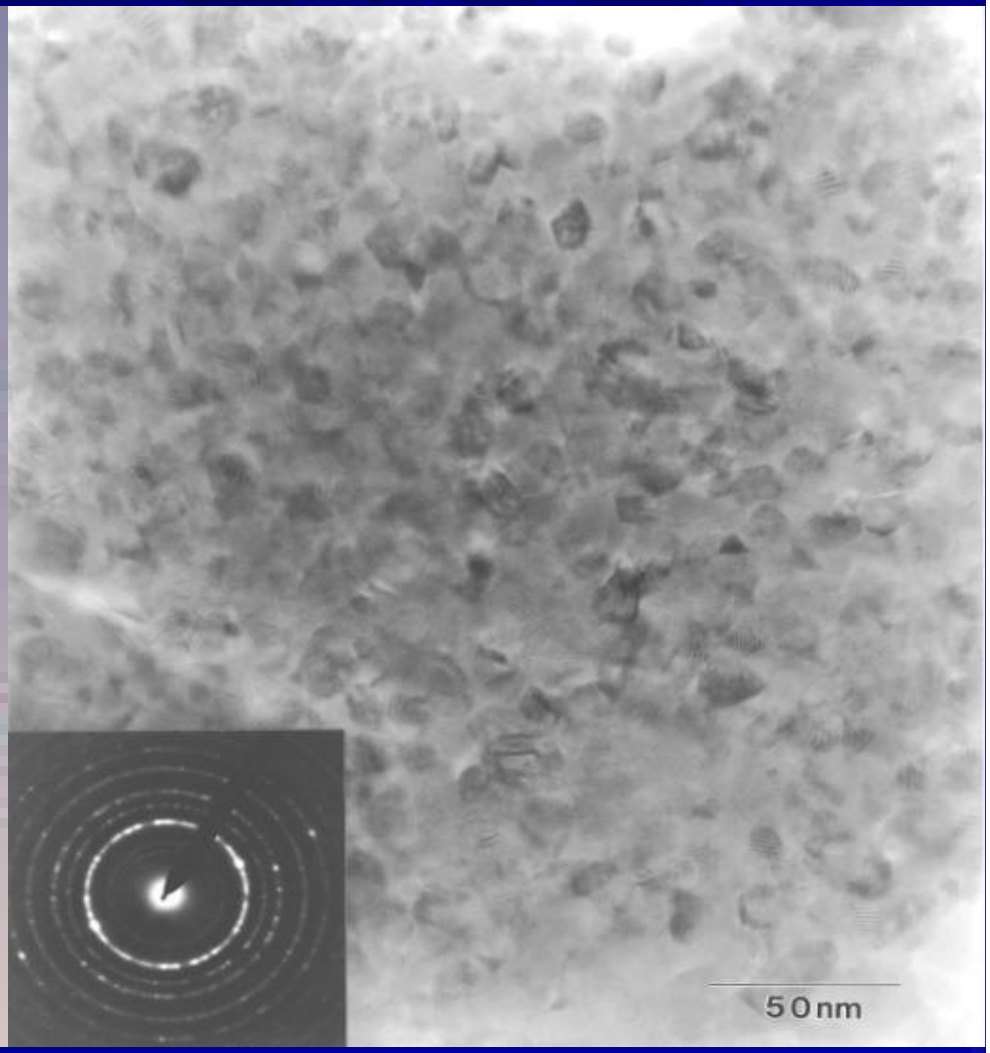
Liposomes



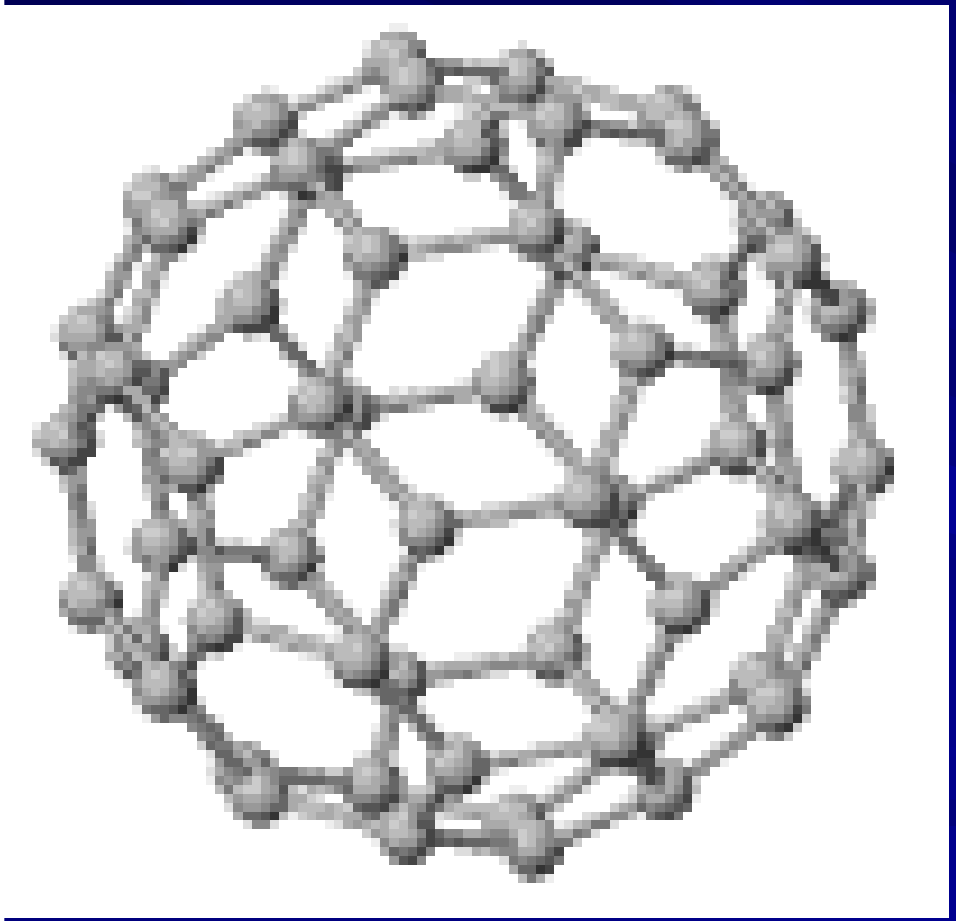
Liposome for Drug Delivery

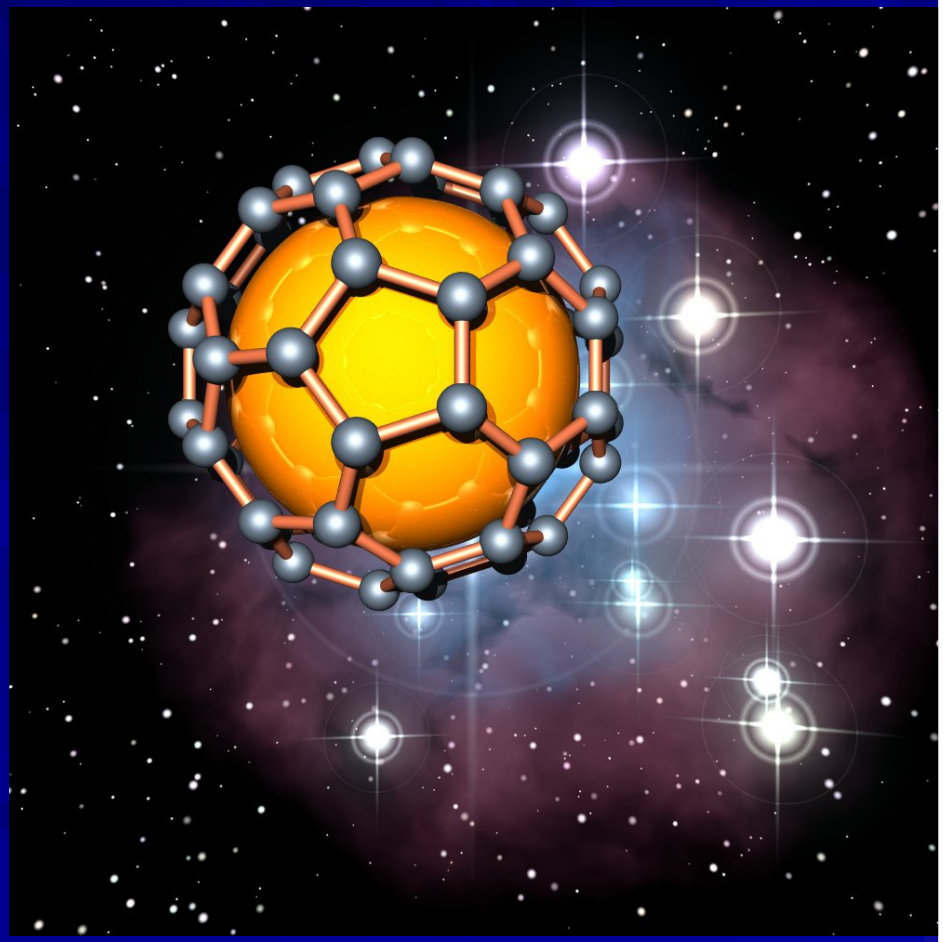


„Nanopowders”



Carbon 60



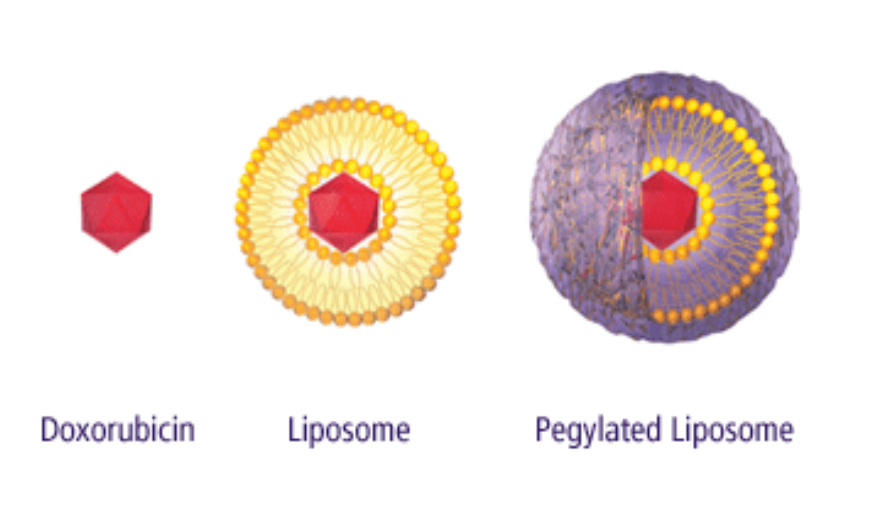


- Two FDA approved nano drugs:

  1. Abraxane®: albumin-bound paclitaxel 130 nm). FDA: 2005 January.

  2. Doxil®: Liposome covered doxorubicin (100 nm). FDA: 2005 February.

http://www.abraxane.com/images_charts/vhs_tape_box.gif



http://www.doxil.com/images/clientChart.gif

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