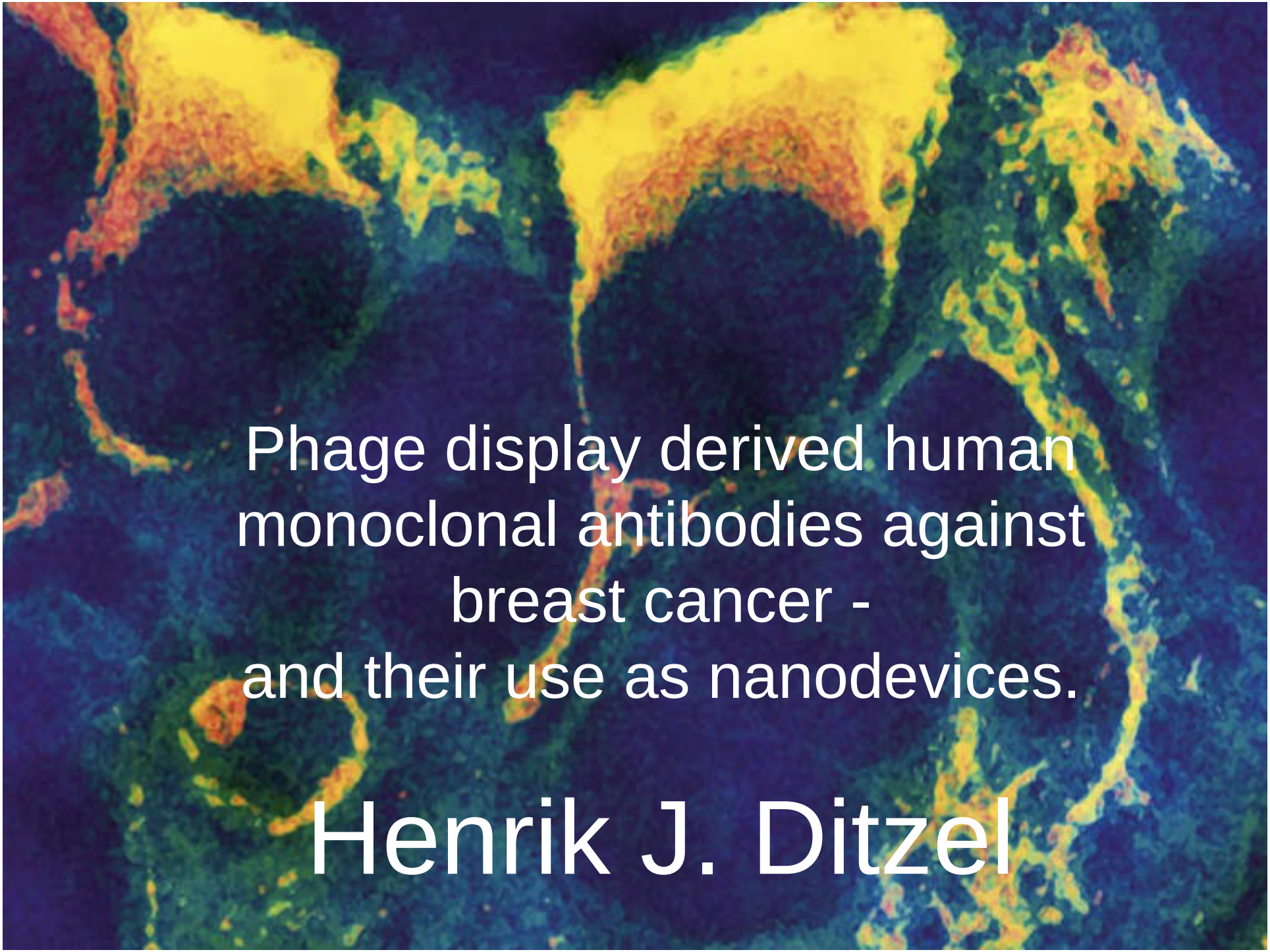

Phage display derived human monoclonal antibodies against breast cancer -
and their use as nanodevices.
Henrik J. Ditzel

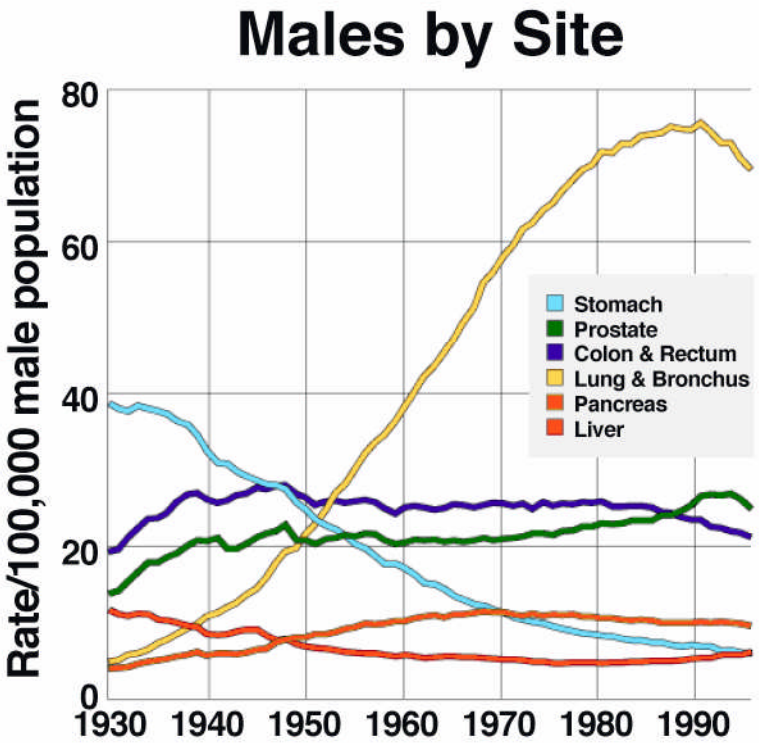
Breast Cancer Statistics

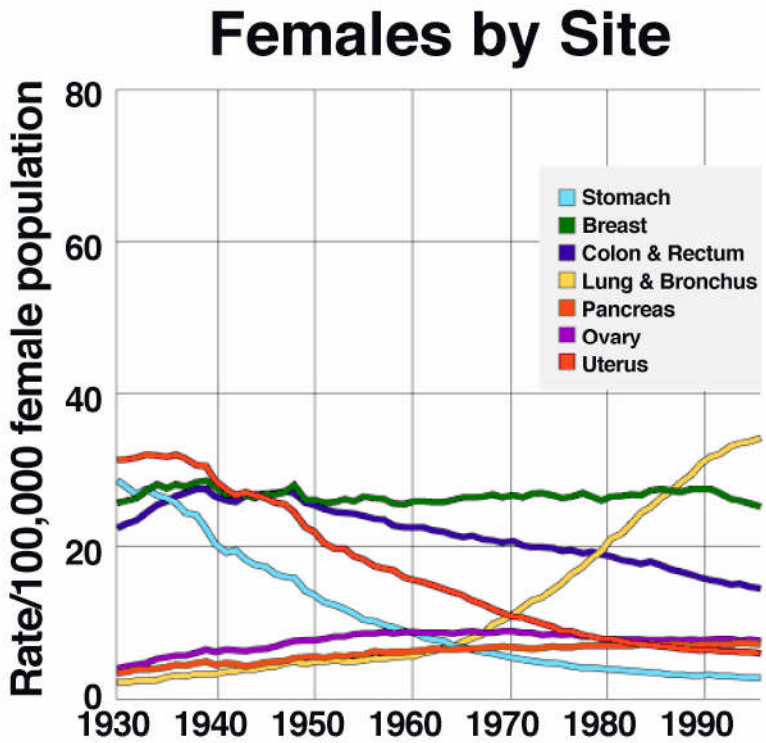
- Every three minutes a woman in the United States is diagnosed with breast cancer. In 2003, it is estimated that about 211,300 new cases of invasive breast cancer will be diagnosed, along with 55,700 new cases of noninvasive breast cancer. And 39,800 women are expected to die from this disease.

- Breast cancer is the leading cancer among white and African American women. African American women are more likely to die from this disease.

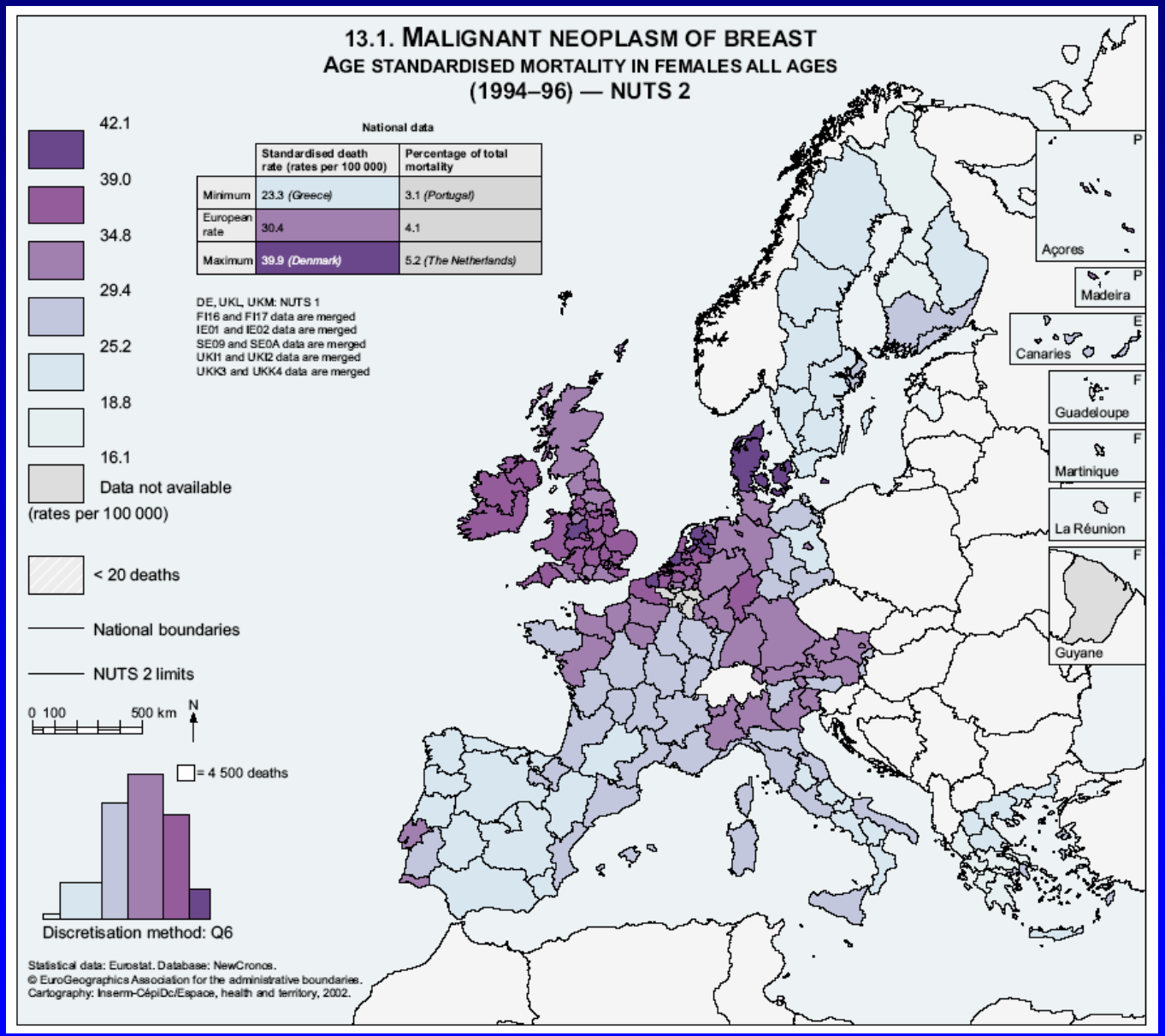
- Breast cancer incidence in women has increased from one in 20 in 1960 to one in eight today.

Age-Adjusted Cancer Death Rates
US, 1930-1995

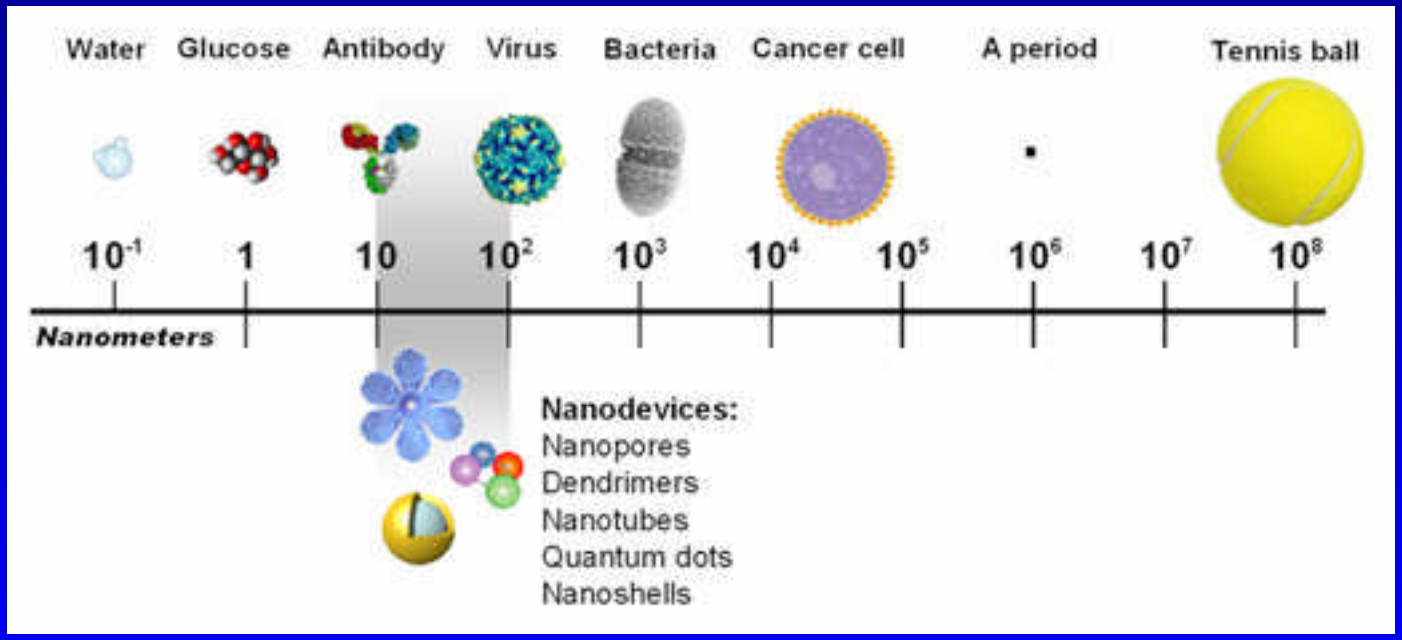




http://www.cancer.org/statistics/cff99/data/
(Downloaded 07/14/99)

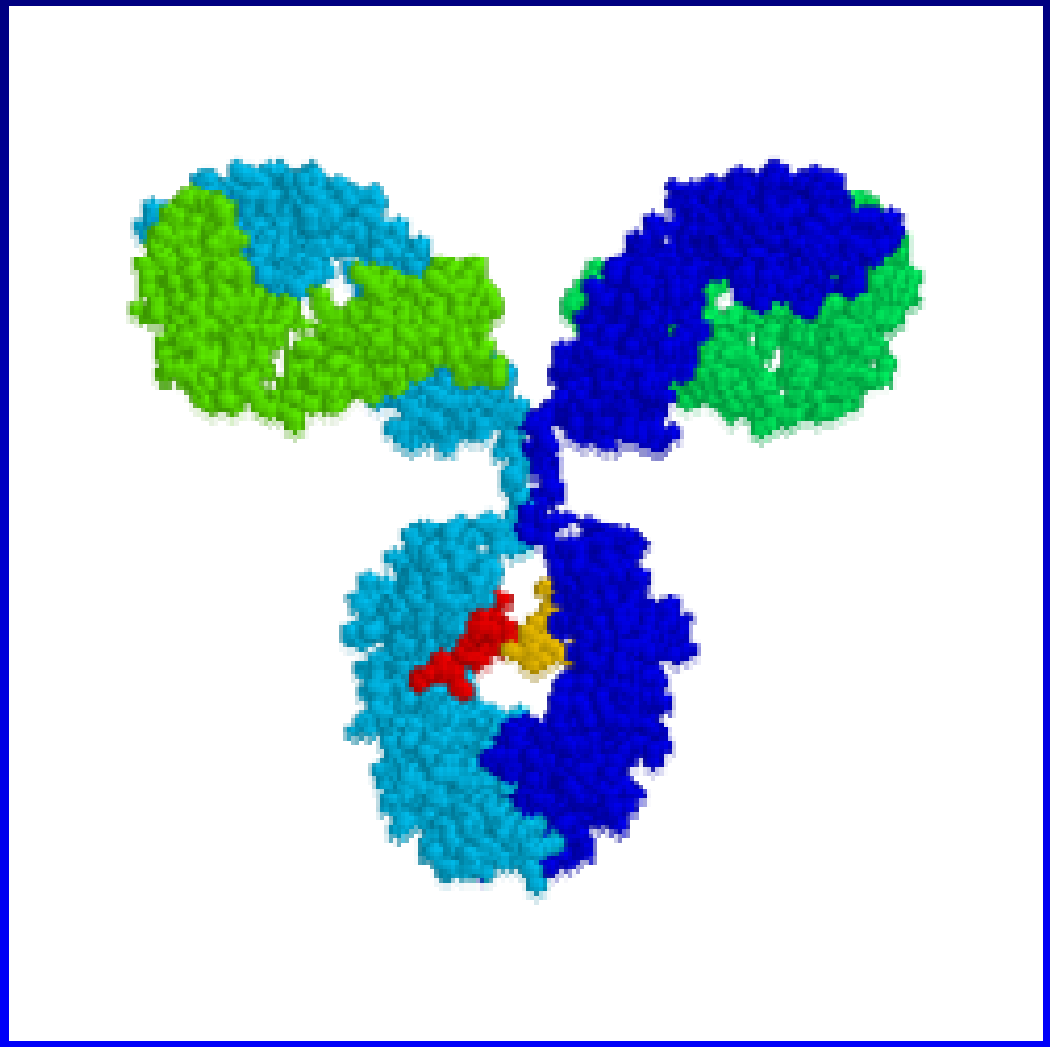

13.1. MALIGNANT NEOPLASM OF BREAST
AGE STANDARDISED MORTALITY IN FEMALES ALL AGES
(1994–96) — NUTS 2
National data
Standardised death rate (rates per 100 000)
Percentage of total mortality
Minimum
23.3 (Greece)
3.1 (Portugal)
European rate
30.4
4.1
Maximum
39.9 (Denmark)
5.2 (The Netherlands)
DE, UKL, UKM: NUTS 1
FI16 and FI17 data are merged
IE01 and IE02 data are merged
SE09 and SE0A data are merged
UKI1 and UKI2 data are merged
UKK3 and UKK4 data are merged
42.1
39.0
34.8
29.4
25.2
18.8
16.1
Data not available
(rates per 100 000)
< 20 deaths
National boundaries
NUTS 2 limits
0 100 500 km
N
= 4 500 deaths
Discretisation method: Q6
Açores
Madeira
Canaries
Guadeloupe
Martinique
La Réunion
Guyane
Statistical data: Eurostat. Database: NewCronos.
© EuroGeographics Association for the administrative boundaries.
Cartography: Inserm-CépiDc/Espace, health and territory, 2002.

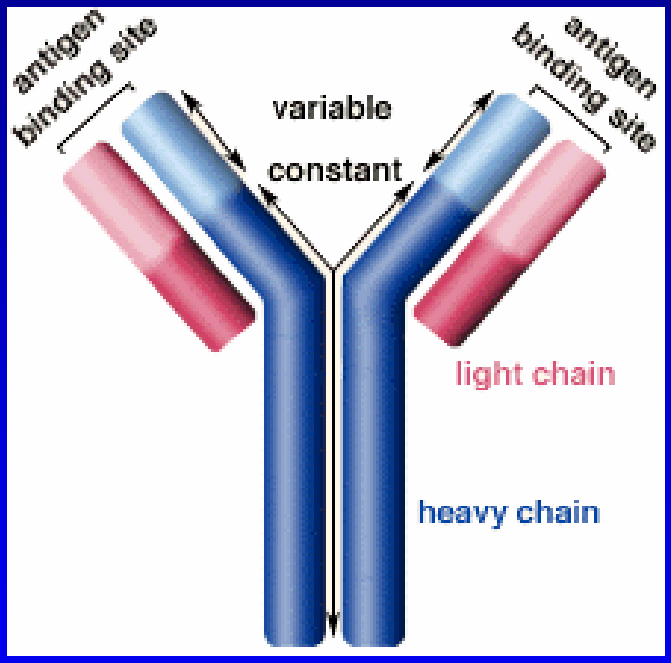
Antibody conjugates in Nanotechnology



- Detection of cancer ex vivo
- Detection of cancer in vivo
- Therapy of cancer

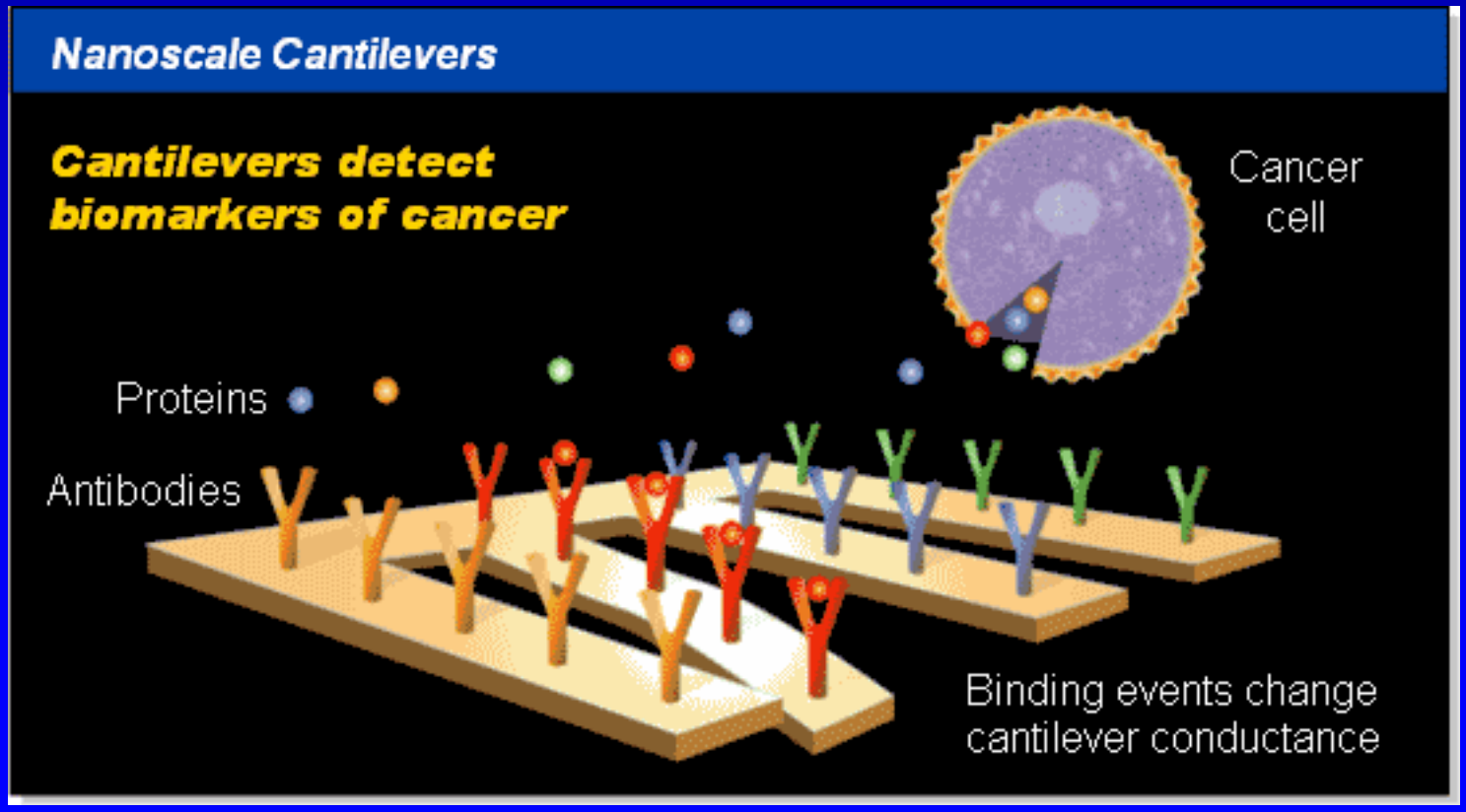
The Structure of an antibody





- Nanoscale cantilevers - rapid and sensitive detection of cancer-related molecules.
- Microscopic, flexible beams built using semiconductor lithographic techniques and coated with antibodies capable of binding to cancer biomarkers.

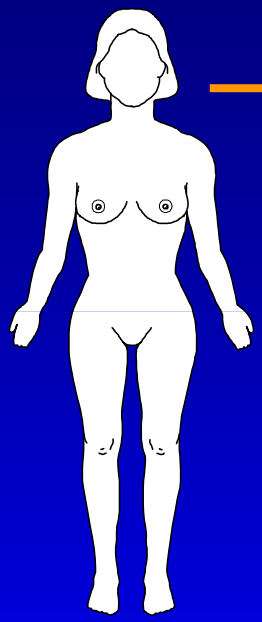
- As a cancer cell secretes its molecular products, the antibodies coated on the cantilever fingers selectively bind to these secreted proteins, changing the physical properties of the cantilever and signaling the presence of cancer. The precise concentration of different molecular marker can be determined.



Current treatment options

- Surgery
- Radiation / chemotherapy
- Anti-hormonal treatment
- Biological Response Modifiers
- Antibody treatment
    - Herceptin® (trastuzumab) antibody against HER-2/neu breast cancer
    - Rituxan® (rituximab) antibody against CD20 Non-Hodgkin's lymphoma

Selection of breast cancer-reactive Fab-phage



Tumor draining lymph nodes, tumor infiltrating B cells, PBLs, bone marrow

RNA

PCR, Library Construction

Recombination of naive antibody genes

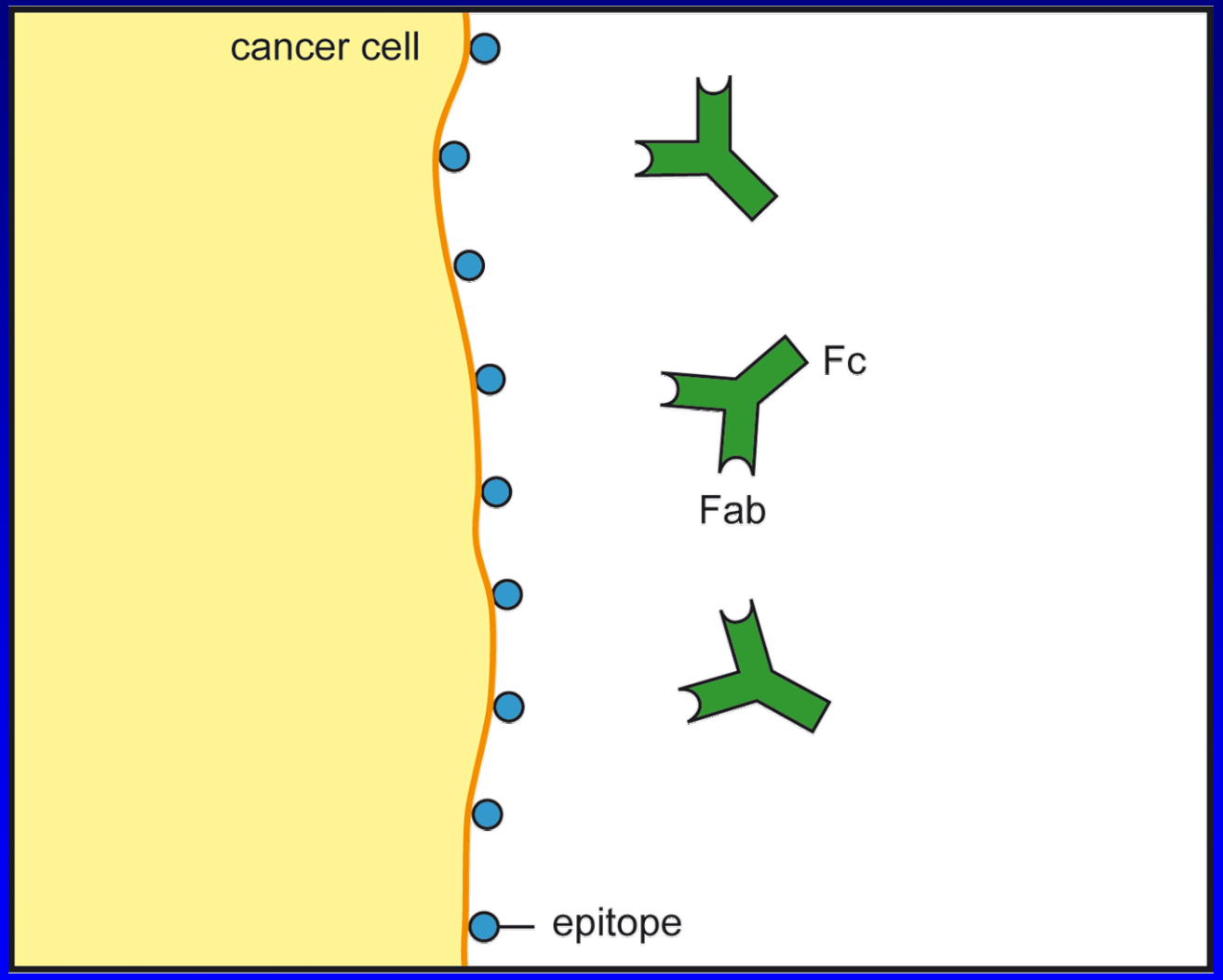
Cancer cells or cancer-associated antigens

Infect Bacteria

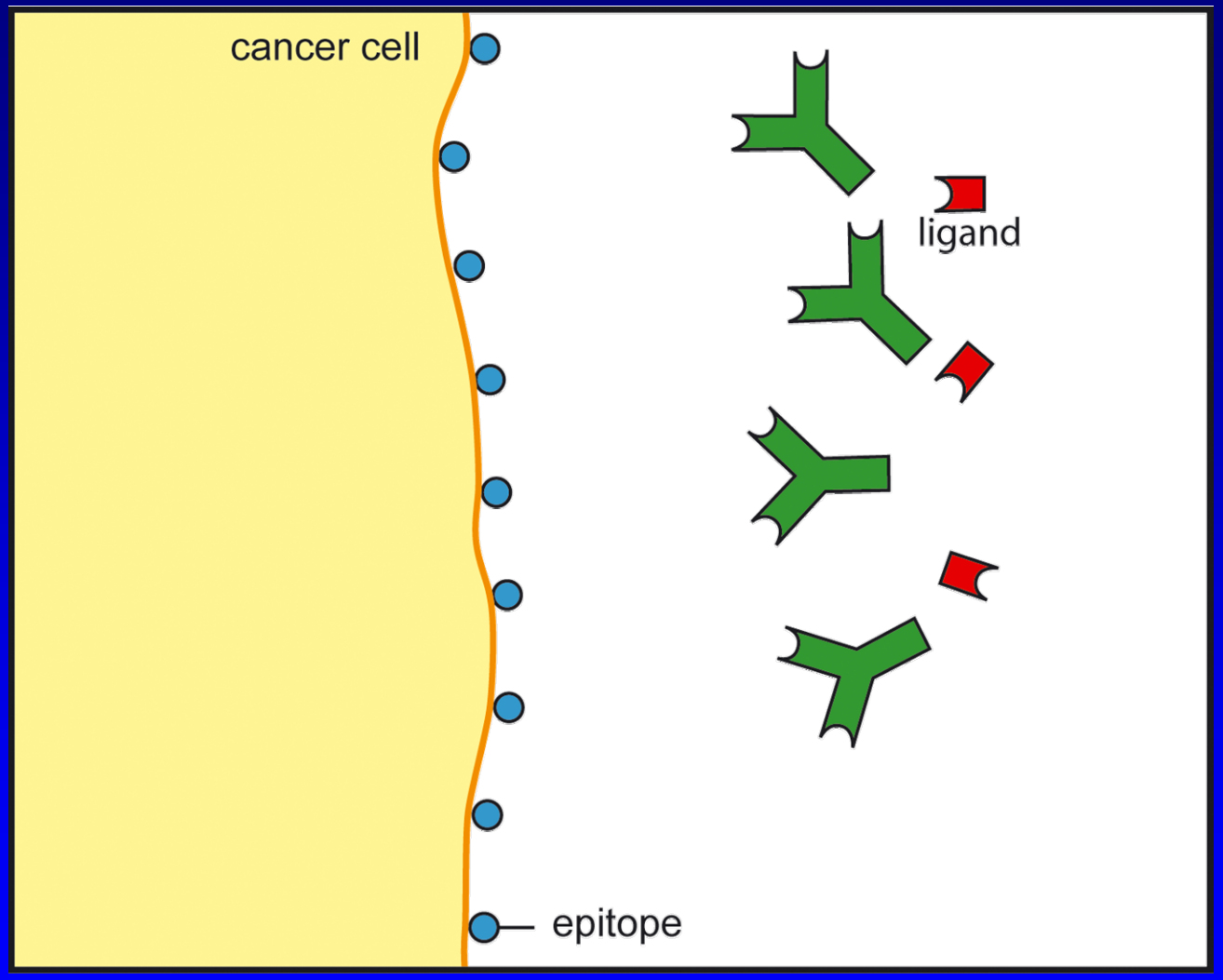
Conversion to Soluble Fab Expressing Phagemid

Characterization of Fab

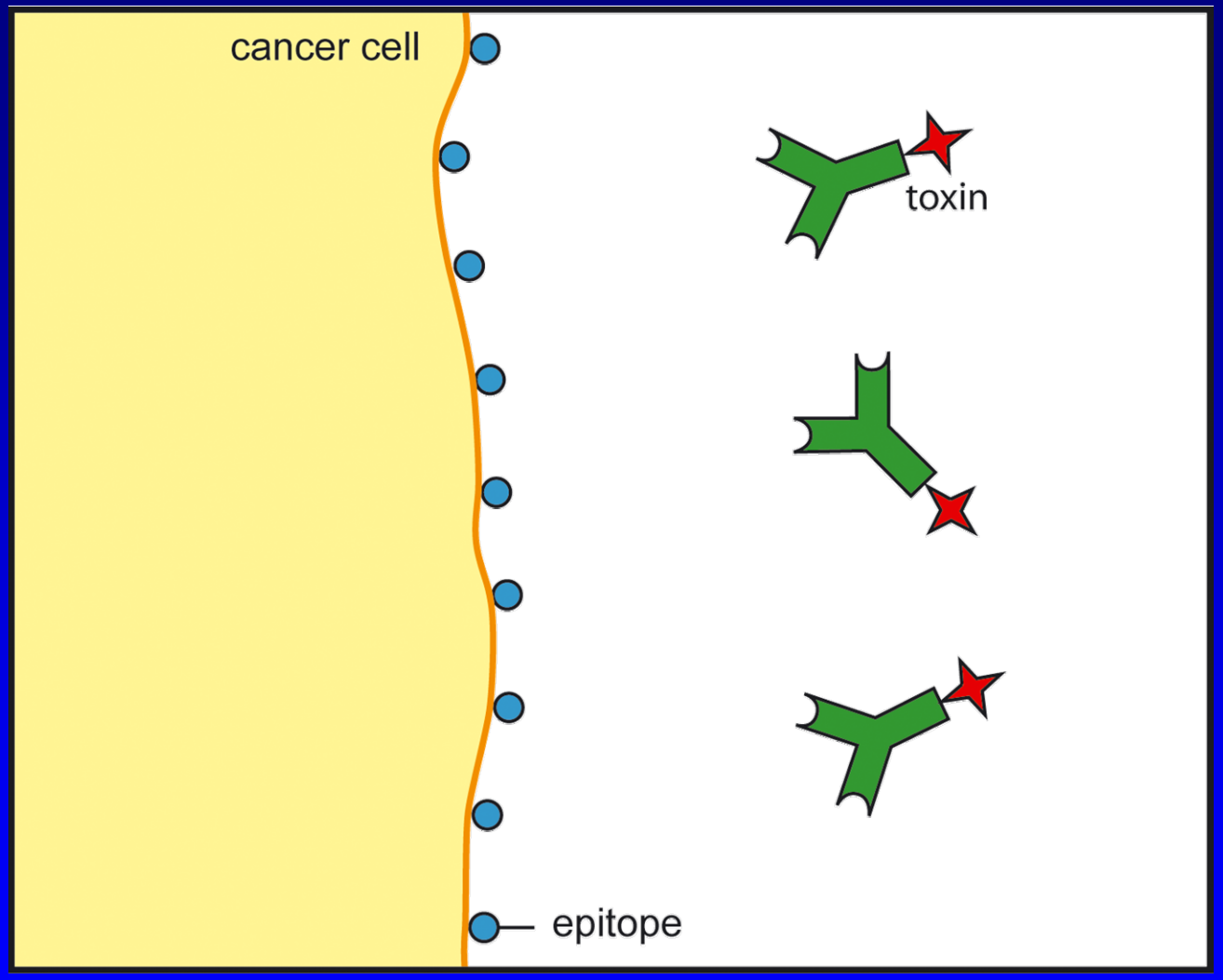
Antibodies that stimulate cell killing: ADCC



Blockage of receptors



Immunotoxins



Antibodies can be used to inhibit the growth of cancer cells in several different ways:

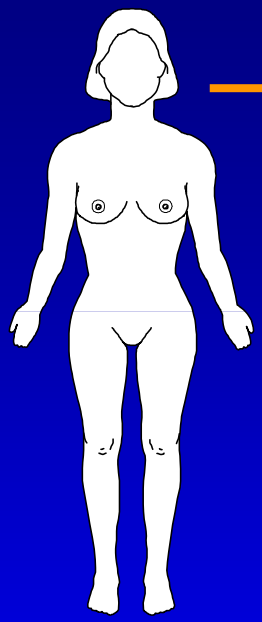
1. Antibodies that stimulate cell killing: These antibodies function by targeting proteins on the surface of cancer cells. The antibodies themselves mark the cell for destruction by cells of the immune system. This process is termed antibody dependent cellular cytotoxicity (ADCC).[1]
2. Blockage of receptors: These antibodies may function as a blockade to the receipt of required growth signals.
3. Immunotoxins: This approach utilizes antibodies to target toxic molecules to the cancer cells. These toxic molecules can be proteins that inhibit cellular activities or radioactive compounds that cause DNA damage and the induction of apoptosis.

Characteristics of cancer cells with high metastatic potential

- Biological functions

- Proliferation
- Adhesion
- Migration
- Invation

Selection of breast cancer-reactive Fab-phage



Tumor draining lymph nodes, tumor infiltrating B cells, PBLs, bone marrow

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PCR, Library Construction

Recombination of naive antibody genes

Cancer cells or cancer-associated antigens

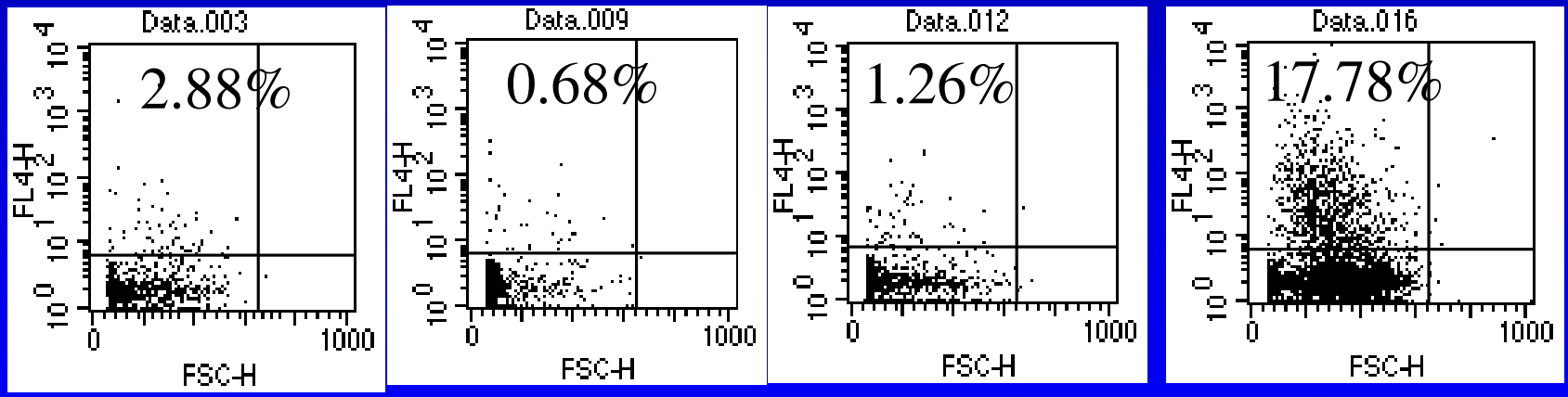
Infect Bacteria

Conversion to Soluble Fab Expressing Phagemid

Characterization of Fab

Fab recognizing metastasizing cell line

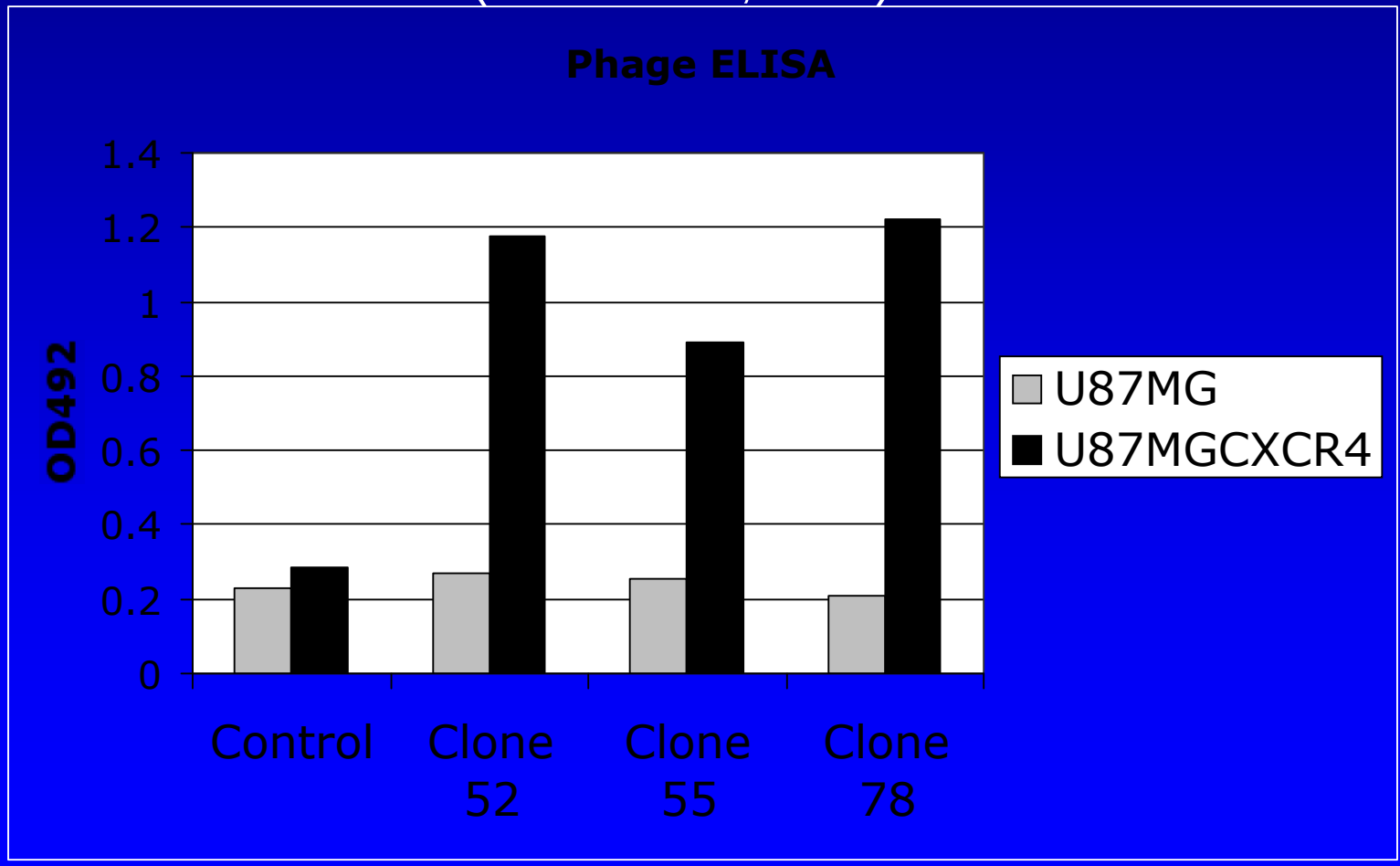
Selection of naïve antibody phage libraries



Human antibodies to CXCR4

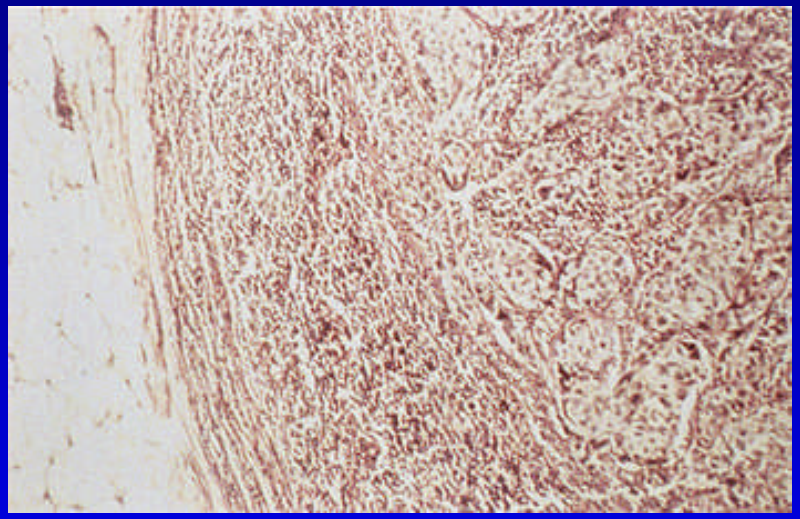
The chemokine receptor CXCR4 has been shown to be highly expressed on invasive breast cancer cell lines, and murine antibodies against CXCR4 have been shown to inhibit invasion

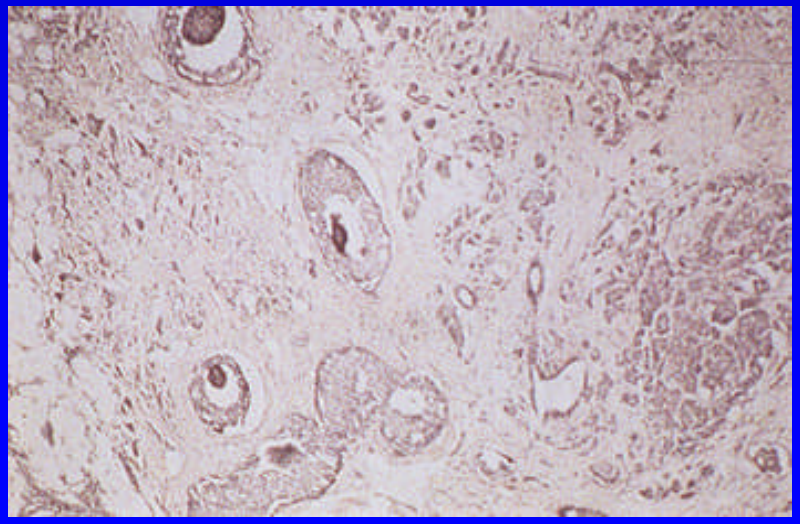
(Müller et al., 2001).



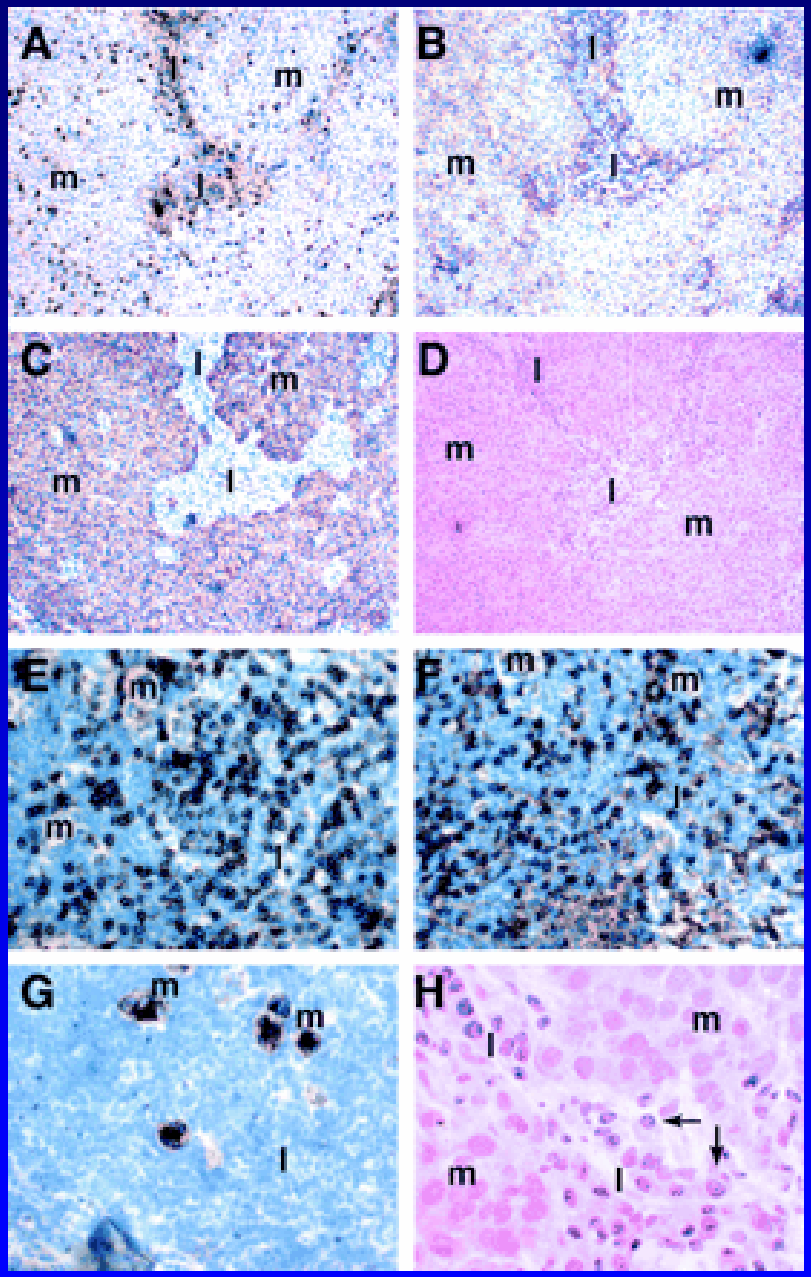
Background: Medullary breast cancer

- ~3-7% of all invasive breast cancers
- Favorable prognosis:
  10-year survival rate 84%





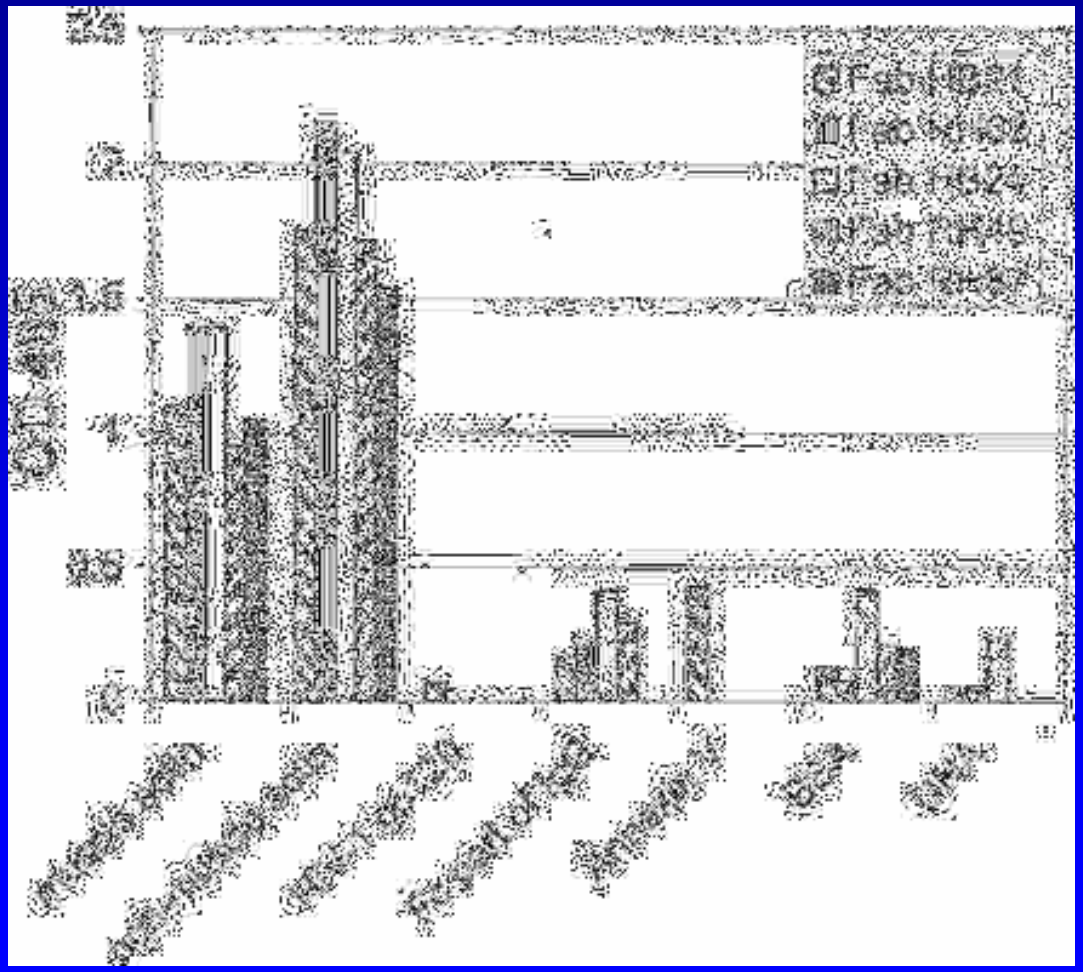
Despite its relatively poor differentiation, this tumor has a better prognosis than does infiltrating ductal carcinoma.



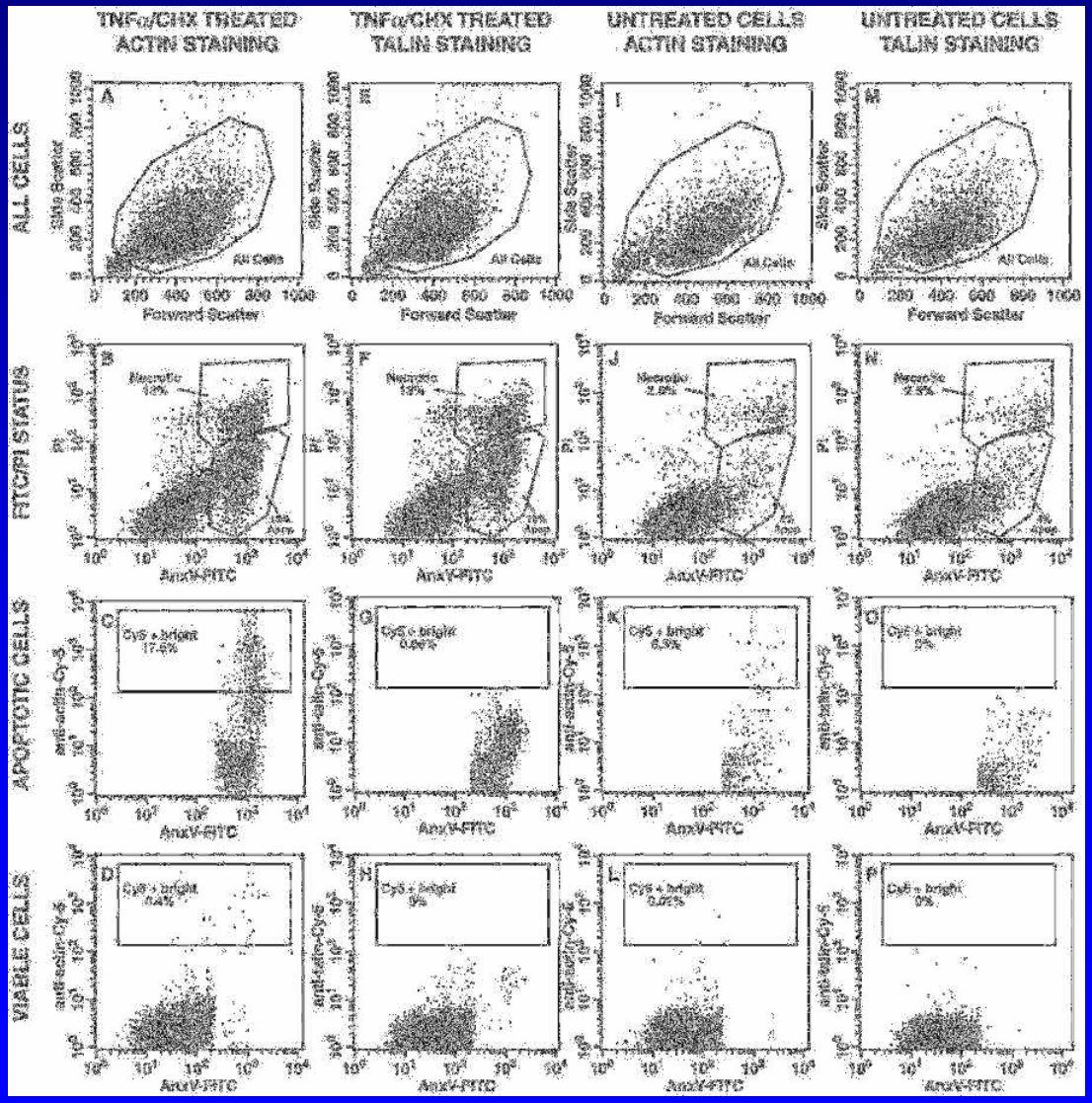
Lymphoplasmacytic cell infiltrate in MCB contain T, B, and plasma cells in intratumoral stroma and within tumor cell nests.

CD8 (A and E)
B cell marker BLA.36 (B and F)
Cytokeratin 18 (C and G)
H&E-stained sections (D and H)
MCB cells (m)
lymphoplasmacytic-rich stromal tissue (l)

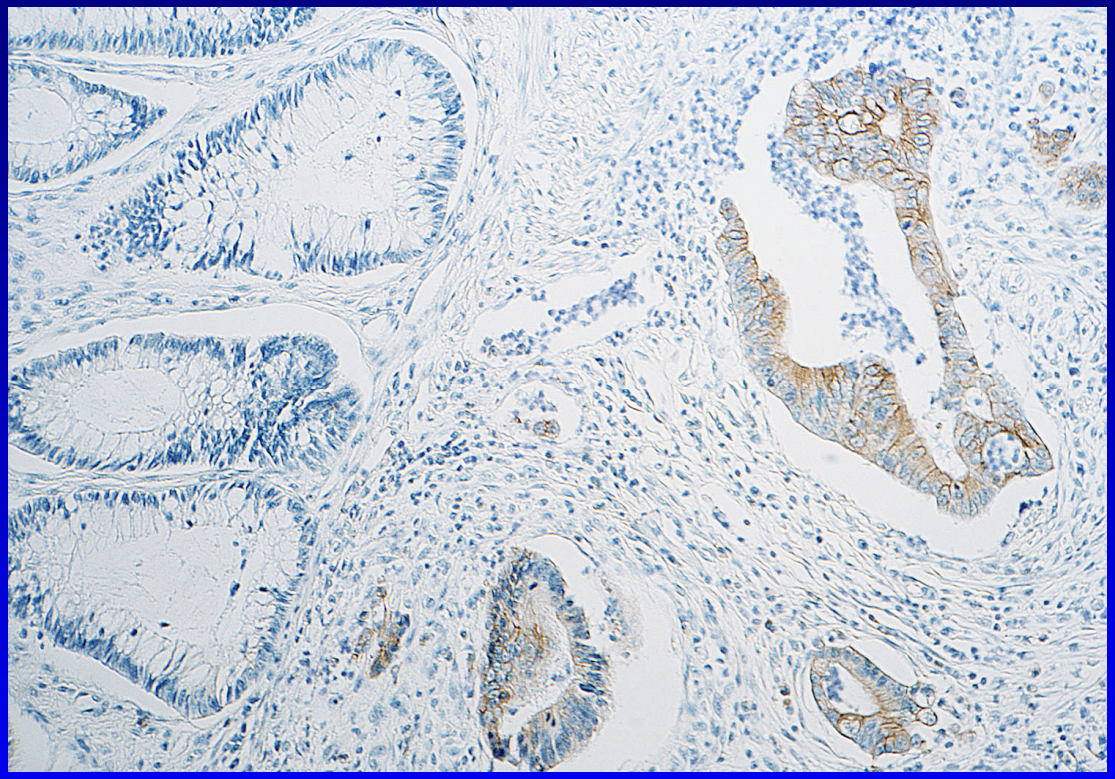
Specificity analysis of human IgG mAb Fabs shows strong binding to actin.



Surface staining of apoptotic MCB cells using anti-actin Fabs

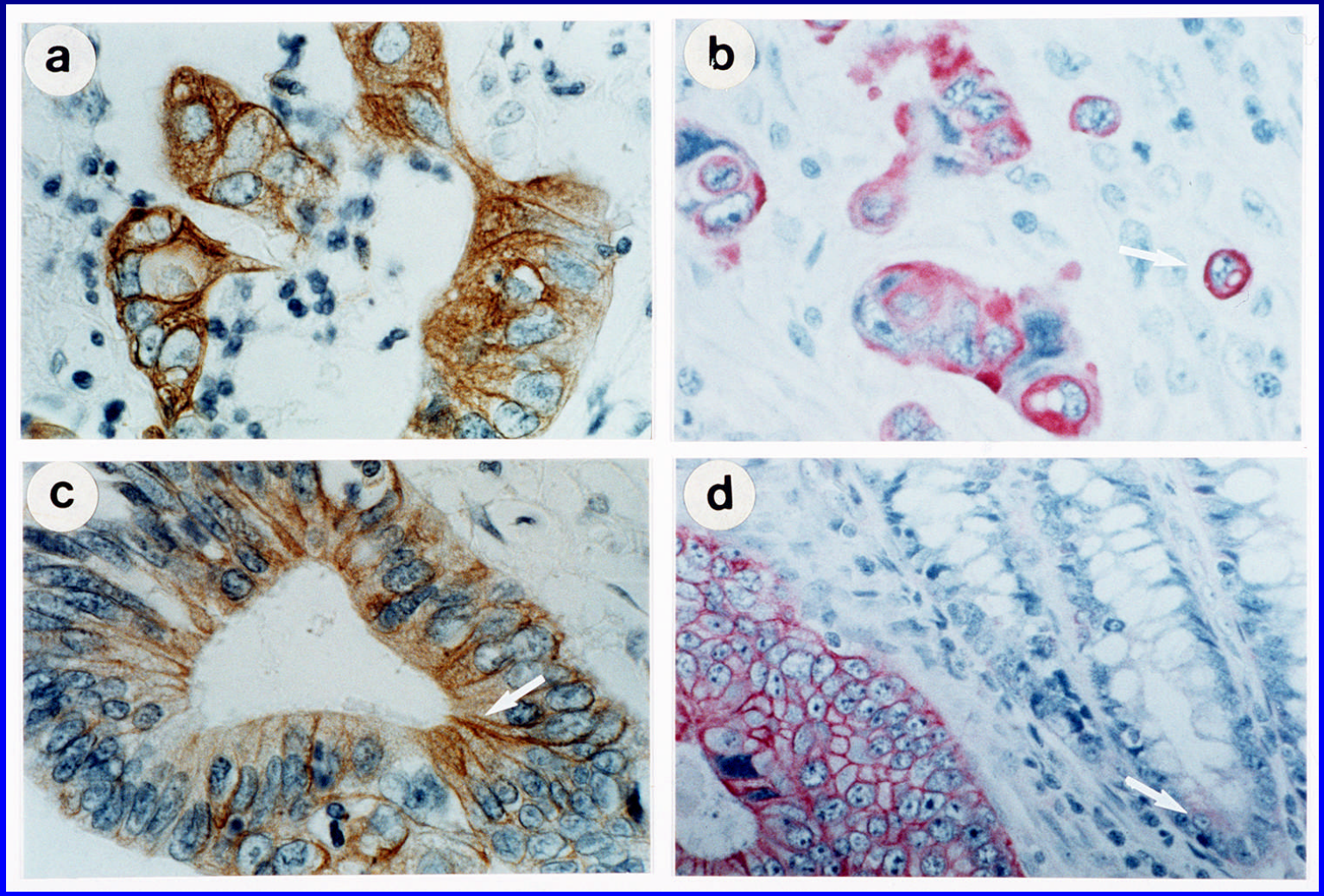


Human monoclonal antibody, COU-1

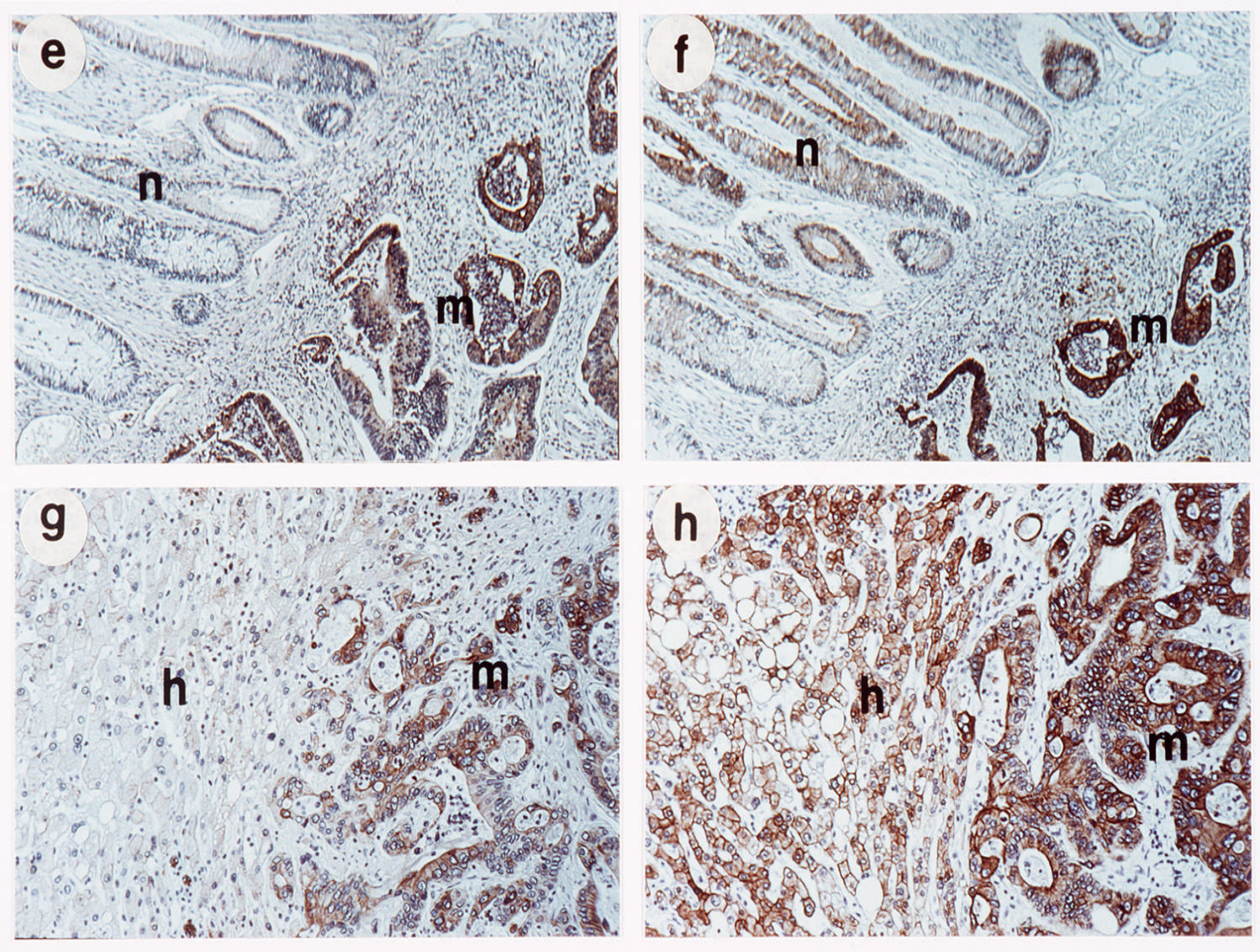


- Produced by human-human hybridoma technology
- using lymphocytes from a tumor draining mesenteric lymph node of a colon cancer patient and the lymphoblastoid cell line WI-L2-729-6-HF2.
- Stable production for more than 10 years.
- Recognized by a pan-adenocarcinoma antigen.

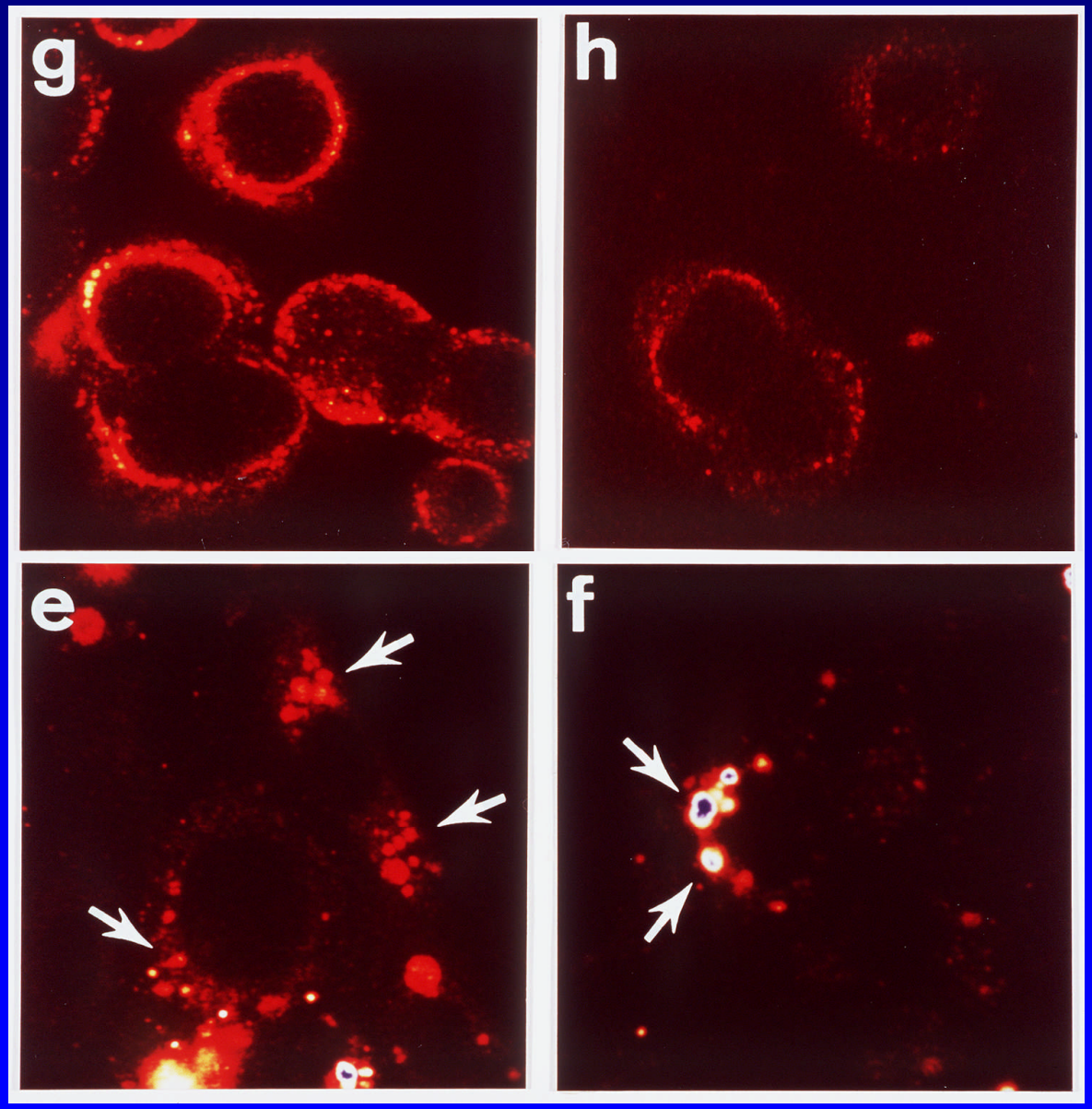
COU-1 staining



Comparison of COU-1 and normal K18 staining in colon and liver



Cell surface binding and internalization of COU-1



Ditzel et al.
PNAS 1997

In vivo tumor imaging

- Imaging agents that can identify tumors that are far smaller than is possible with today's technology, at a scale of 100,000 cells rather than 1,000,000,000 cells.
- Better targeting of imaging agents and generation of a bigger imaging signal, both of which nanoscale devices are capable of accomplishing.

10 patients with suspected colorectal cancer were enrolled. 2 mg COU-1 labeled with 185 MBq ^{131}I.

Immunoscientigraphy using COU-1

Tumors were detected in 7 of 9 cancer patients.

No adverse effects or toxicity

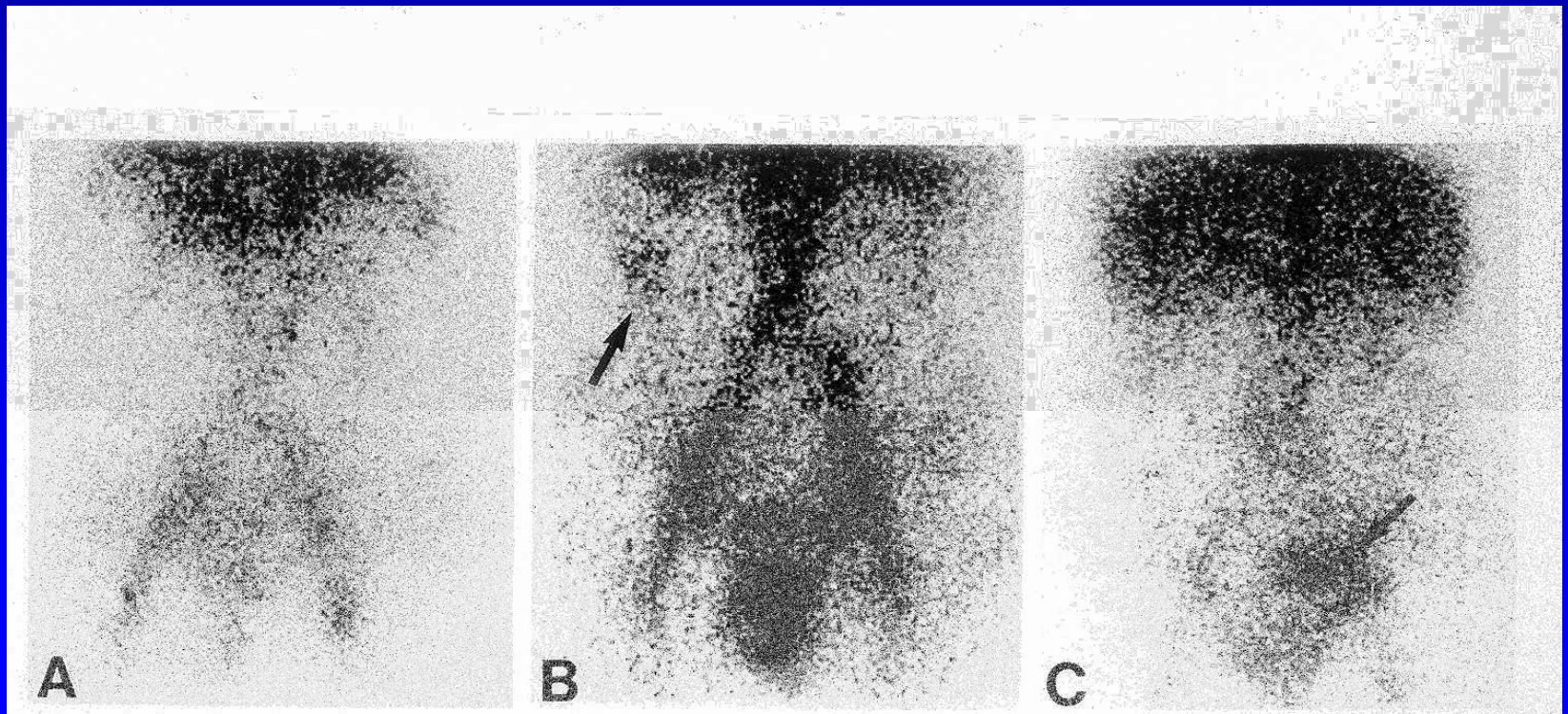
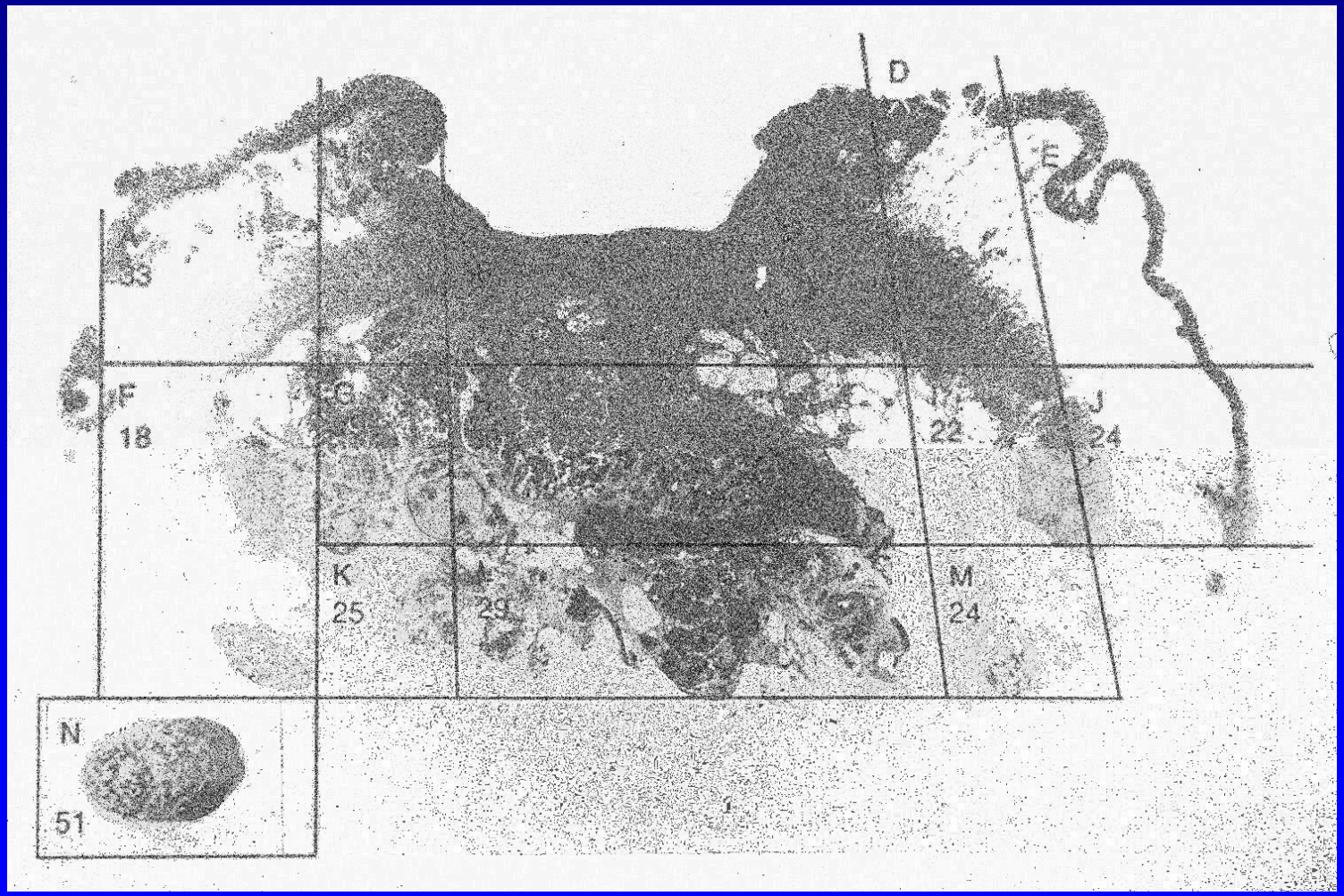


Fig. 2. Anterior abdominal images of patients 7 days after Mab COU-1 infusion. *Arrows*, positions of the cancers. (*A*) Laparotomy of a 71-year-old female with suspected cancer (patient 1) did not reveal cancer, but only adhesions; (*B*) 54-year-old male (patient 5) with a colon carcinoma (diameter of 3 cm) located in the cecum, but no metastasis; (*C*) 68-year-old female (patient 9) with a rectal adenocarcinoma, with no spread.

Distribution of radioactivity within a tumor



Antibody conjugates

Magnetic spheres

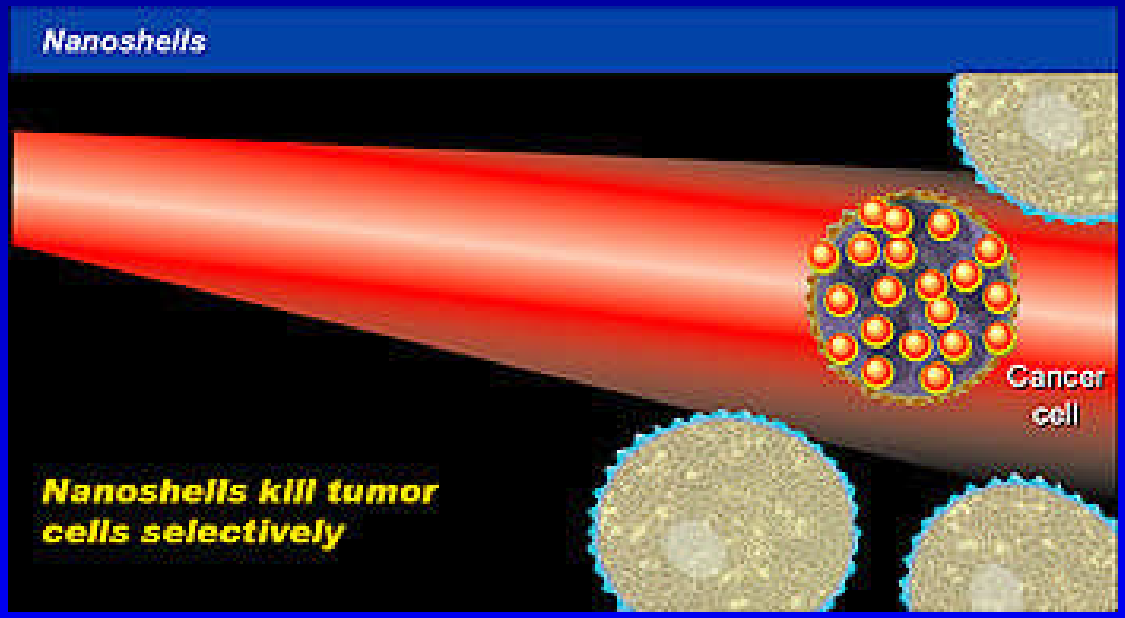
Tiny magnetic spheres delivered systemically with antibodies. The tiny spheres within the tumors are induced to heat by a localized externally applied magnetic field.

Ex. Anti-Ep-CAM antigen monoclonal antibody ING-1 to treat adenocarcinomas
(Triton BioSystems, Inc. and XOMA Ltd.)

Antibody conjugates

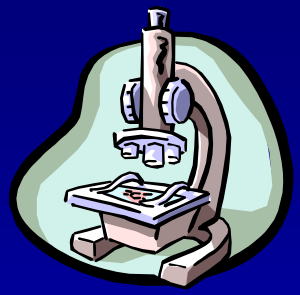
Nanoshells

The nanoshells are linked to antibodies that recognize tumor cells surface marker.



Nanoshells have a core of silica and a metallic outer layer.
Once the cancer cells take them up, near-infrared light is applied that is absorbed by the nanoshells, creating an intense heat that selectively kills the tumor cells and not neighboring healthy cells.

Conclusions



• Phage display is a powerful technique to generate antibody fragments with specific properties.

•Antibodies will be an important component of nanodevices for diagnosis and therapy of cancer.

•The affinity and specificity of the antibodies is important factor as well as the selectivity of the tumor marker.

•The sensitivity of imaging reagents will determine their use in clinical oncology.

•The tumor to normal tissue ratio toxicity is essential for new cancer therapeutic reagents.

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Danish Cancer Society