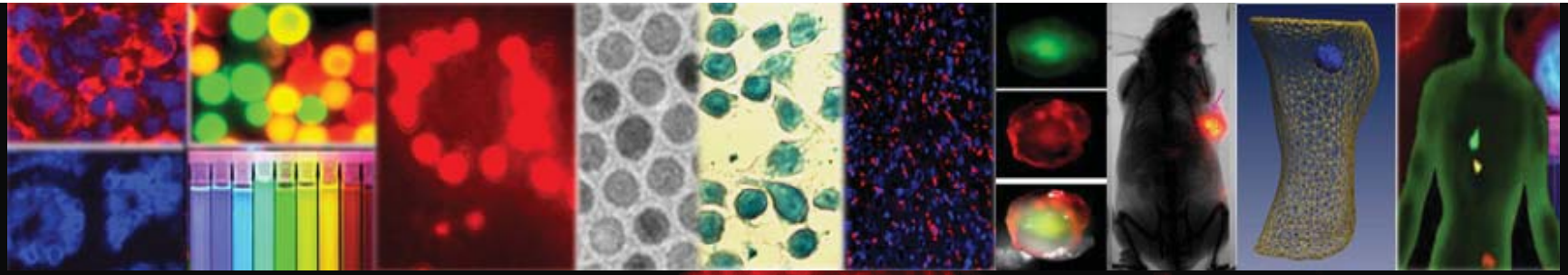

# Nanotechnology for Detection and Treatment of Breast Cancer


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Winship Cancer Institute

Emory University School of Medicine

Atlanta, GA


EMORY

EMORY UNIVERSITY

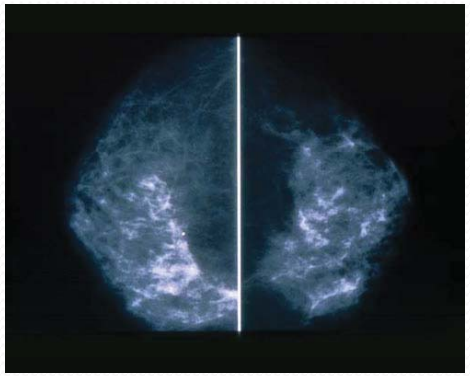
WINSHIP CANCER INSTITUTE

*Accelerating Discovery. Accelerating Hope.*

# Breast Cancer Facts:


- The most common cancer among women.
- The second leading cause of cancer death in women.
- The chance of developing invasive breast cancer at some time in a woman's life is 1 in 8 and about 1 in 35 women will die of breast cancer.
- The American Cancer Society's estimates for breast cancer in the United States (cases/year):
  - 192,370 new cases of invasive breast cancer
  - 62,280 new cases of carcinoma *in situ* (the earliest form of breast cancer).
  - 40,170 women will die from breast cancer
  - 1,990 new cases of invasive breast cancer in men
  - 1.3 million women will be diagnosed with breast cancer annually worldwide
  - 465,000 will die from the disease

# Diagnosis and Treatment of Breast Cancer

**Early Detection:**

- Regular self breast examination
- Screening mammograms
- High risk women: MRI screen in combination with mammograms (recommended by ACS)
- Biopsy

**•Treatment:**

**Surgery:** ***most effective treatment for early stage breast cancer.***

**Post operation radiation** ***to treat possible residual tumor cells in the chest and lymph nodes.***

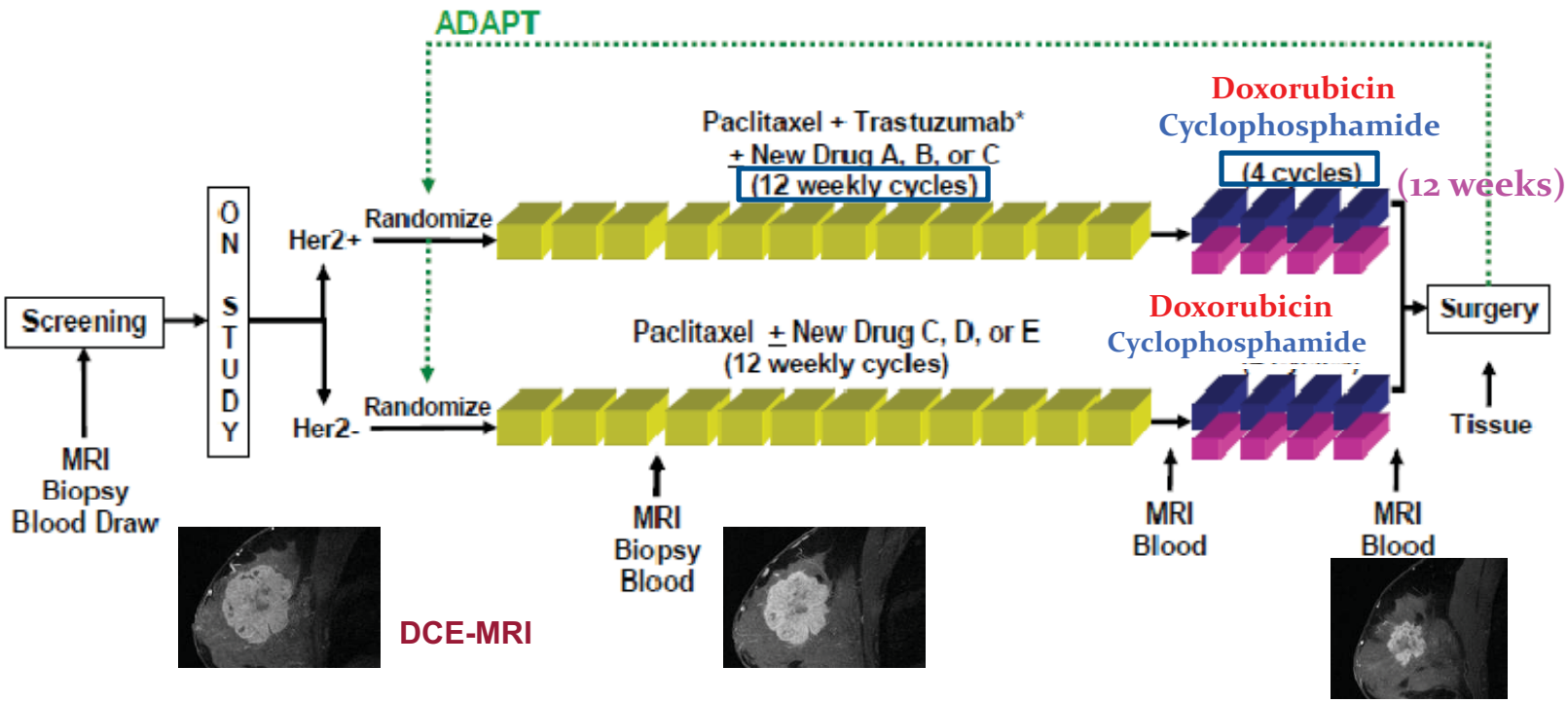
**Systemic treatment** **for invasive and metastatic breast cancers**

**Chemotherapy: Pre-operative neoadjuvant therapy, post operative chemotherapy, and chemotherapy for metastatic breast cancer**

**Hormonal Therapy (Estrogen receptor blocker: tamoxifen)**

**Targeted Therapies: Her-2/Neu (Herceptin) Lapatinib, and Tarceva (inhibitors of growth factor receptor signaling )**

# I-SPY 2 Adaptive Trial Outline

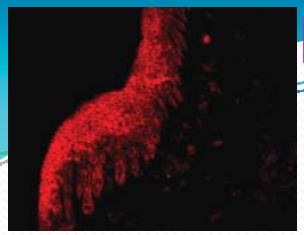



**Accrual:** Anticipate 800 patients over 3–4 years

**Enroll:** ~20 patients per month

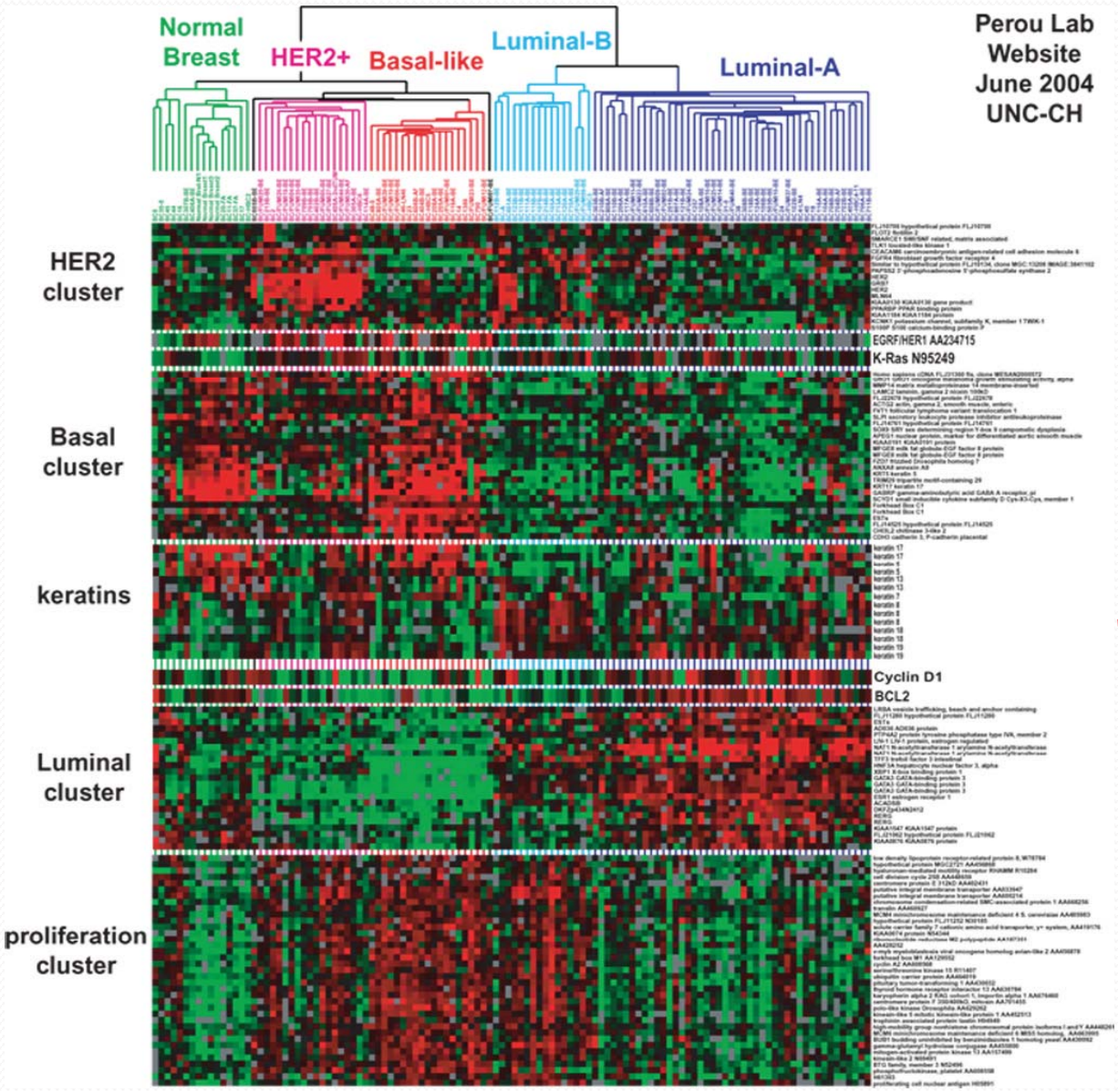
**Participating Sites:** 15–20 across US and Canada

NCI, FDA, FNIH Biomarkers Consortium, Academic and Clinical Partners, Pharma, Biotech, IT, Advocates



# Breast Cancer is a Highly Heterogeneous Disease

## Gene expression profiling breast cancer tissues




**Luminal A**
ER+ PR+ Her-2-

**Luminal B**
ER+ PR+ Her-2+

**Her-2 positive**
ER- PR- Her-2 +

**Triple Negative (Basal-like)**
ER- PR- Her-2-

# Different Subtypes of Breast Cancer Show Differential Response to Chemotherapy

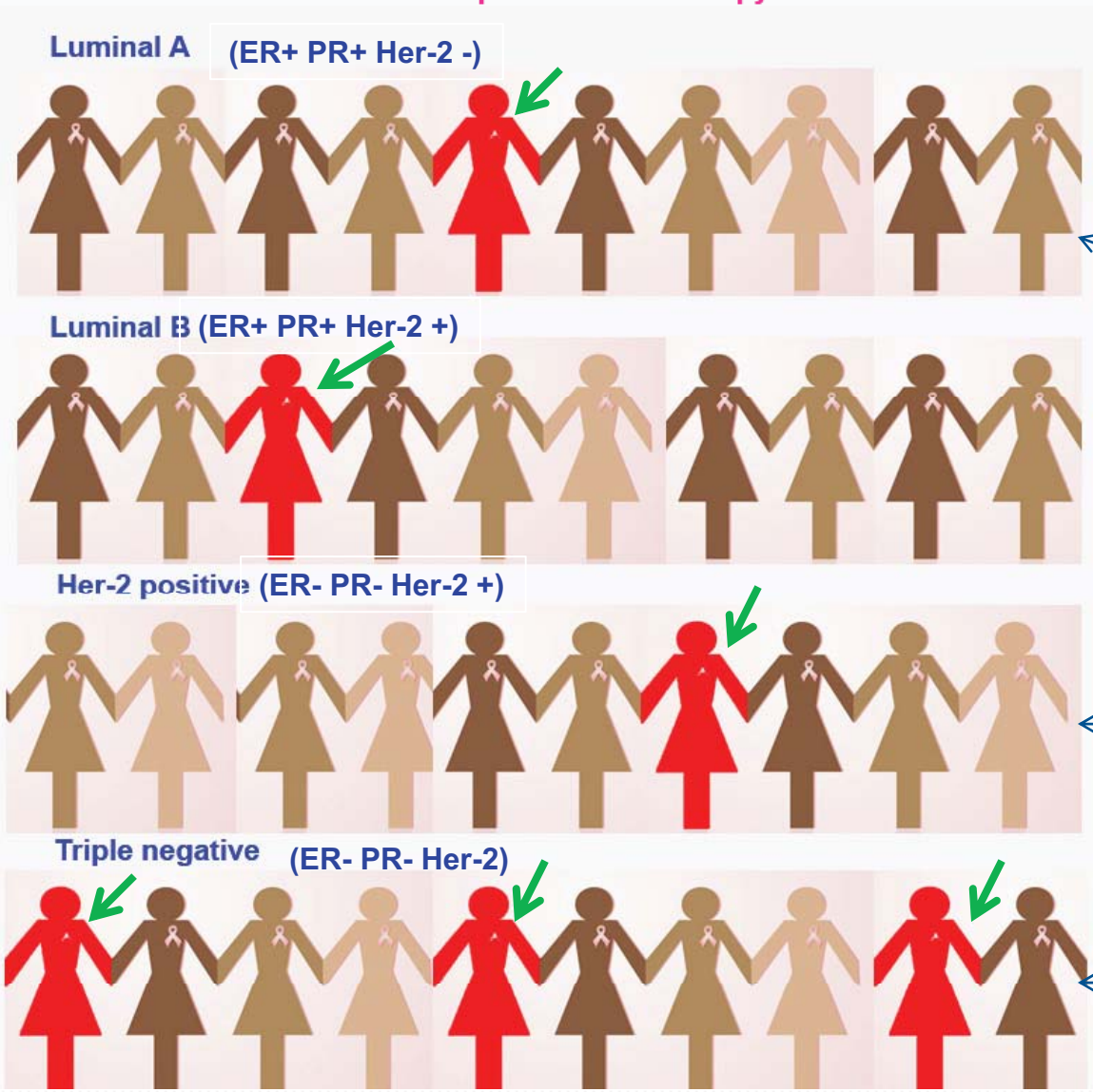
**Table 2.** Breast cancer phenotype and clinical response to anthracycline-based chemotherapy

| | Entire population | Basal-like (*n* = 34) | HER2* (*n* = 11) | Luminal B (*n* = 26) | Luminal A (*n* = 36) | *P* |
|---|---|---|---|---|---|---|
| Clinical response to AC | | | | | | |
| Complete response | 15 (14%) | 10 (29%) | 1 (10%) | 2 (8%) | 2 (6%) | <0.0001 |
| Partial response | 50 (47%) | 19 (56%) | 6 (60%) | 13 (50%) | 12 (33%) | |
| Stable disease | 40 (38%) | 5 (15%) | 3 (30%) | 11 (42%) | 21 (58%) | |
| Progressive disease | 1 (1%) | 0 | 0 | 0 | 1 (3%) | |
| Complete response + partial response | 65 (61%) | 29 (85%) | 7 (70%) | 15 (58%) | 14 (39%) | <0.0001 |
| Pathologic stage post-chemotherapy | | | | | | 0.0004 |
| 0 | 17 (16%) | 9 (27%) | 4 (36%) | 4 (15%) | 0 | |
| I | 26 (25%) | 10 (31%) | 1 (9%) | 8 (31%) | 7 (21%) | |
| II | 33 (32%) | 8 (24%) | 5 (46%) | 8 (31%) | 12 (35%) | |
| III | 27 (26%) | 6 (18%) | 1 (9%) | 5 (19%) | 15 (44%) | |
| IV | 1 (1%) | 0 | 0 | 1 (4%) | 0 | |

*One patient with the HER2+/ER– subtype was not evaluable for clinical response, and three patients did not undergo primary surgery.

Lisa Carry et al. Clin Cancer Res 2007;2329
13(8) April 15, 2007

## Predicative and personalized therapy

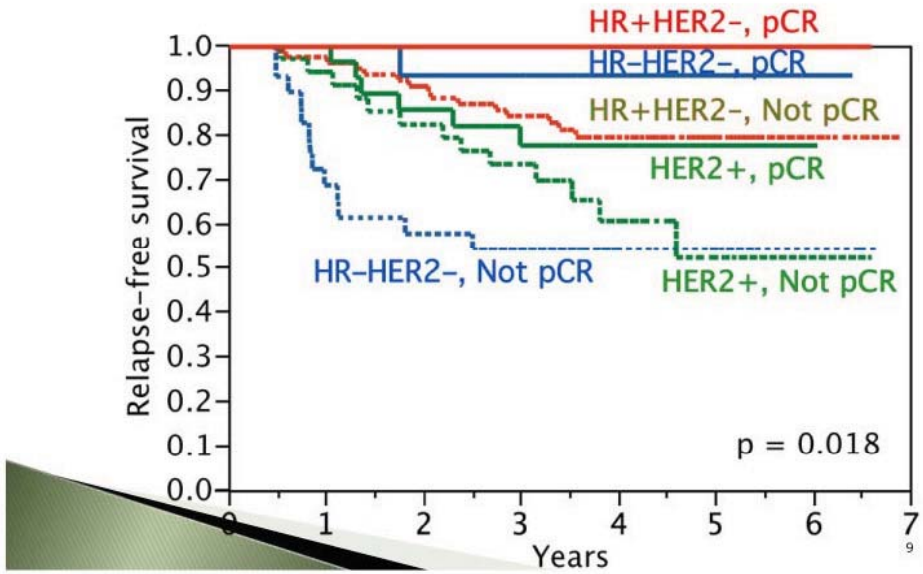



**Tamoxifen + Chemotherapy**

**Herceptin + Chemotherapy Lapatinib**

**Chemotherapy + PARP inhibitor**

## pCR is a Better Predictor by Subtype



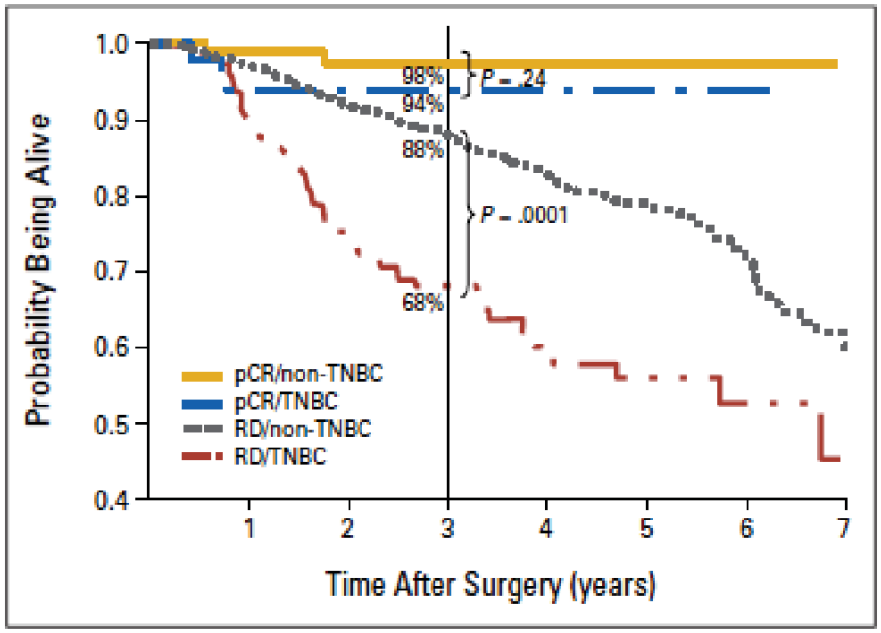




**Fig 2.** Overall survival as a function of response to chemotherapy (pathologic complete response [pCR] *v* residual disease [RD]) and triple-negative status (triple-negative breast cancer [TNBC] *v* non-TNBC).

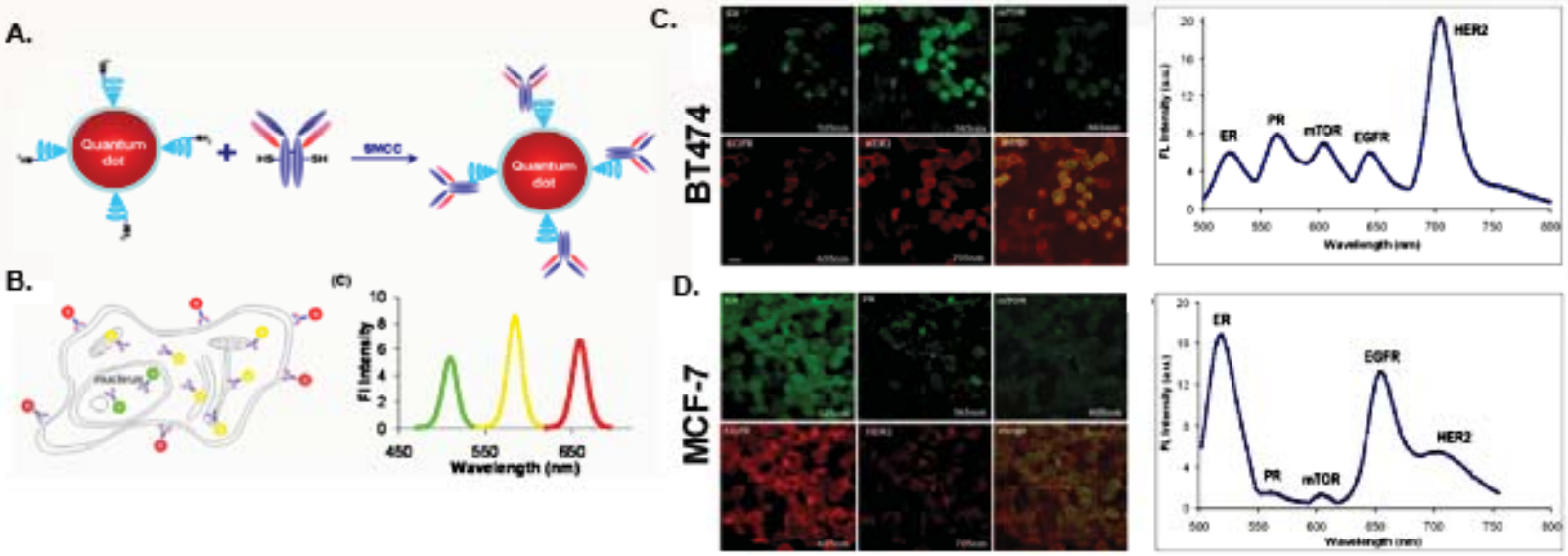
**Cornelia Liedtke et al. JCO 26(8):1275 2008**

# Nanomedicine

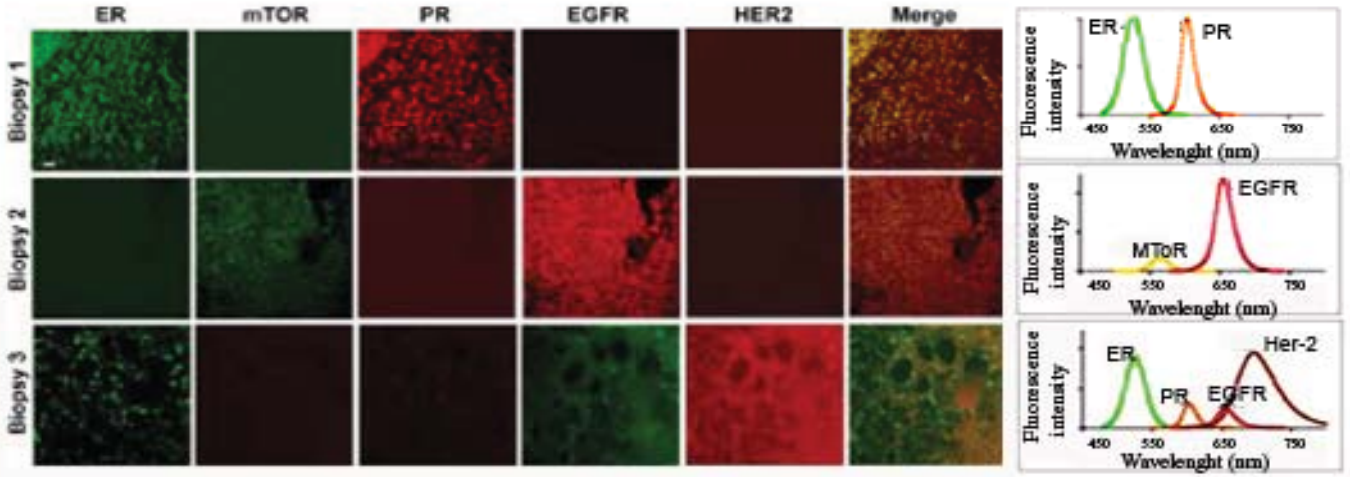
**Applications of Nanotechnology for treatment, diagnosis, monitoring and control of biological systems.**

- Nanoparticals that act as biological mimetics (Functionalized carbon nanotubes)
- Nanomachines (DNA, RNA and DNA Scaffolds)
- Nanofibers and polymeric nanostructures (self-assemble peptides, polymers, peptide-amohiphiles, nanoporous membranes)
- Nanoscale microfabrication-based devices (silicon microchips for drug release, micromachined hollow needles, nanosensors)

# Molecular Profiling of Cancer Tissues for Personalized Medicine Using Antibody-coated QDs

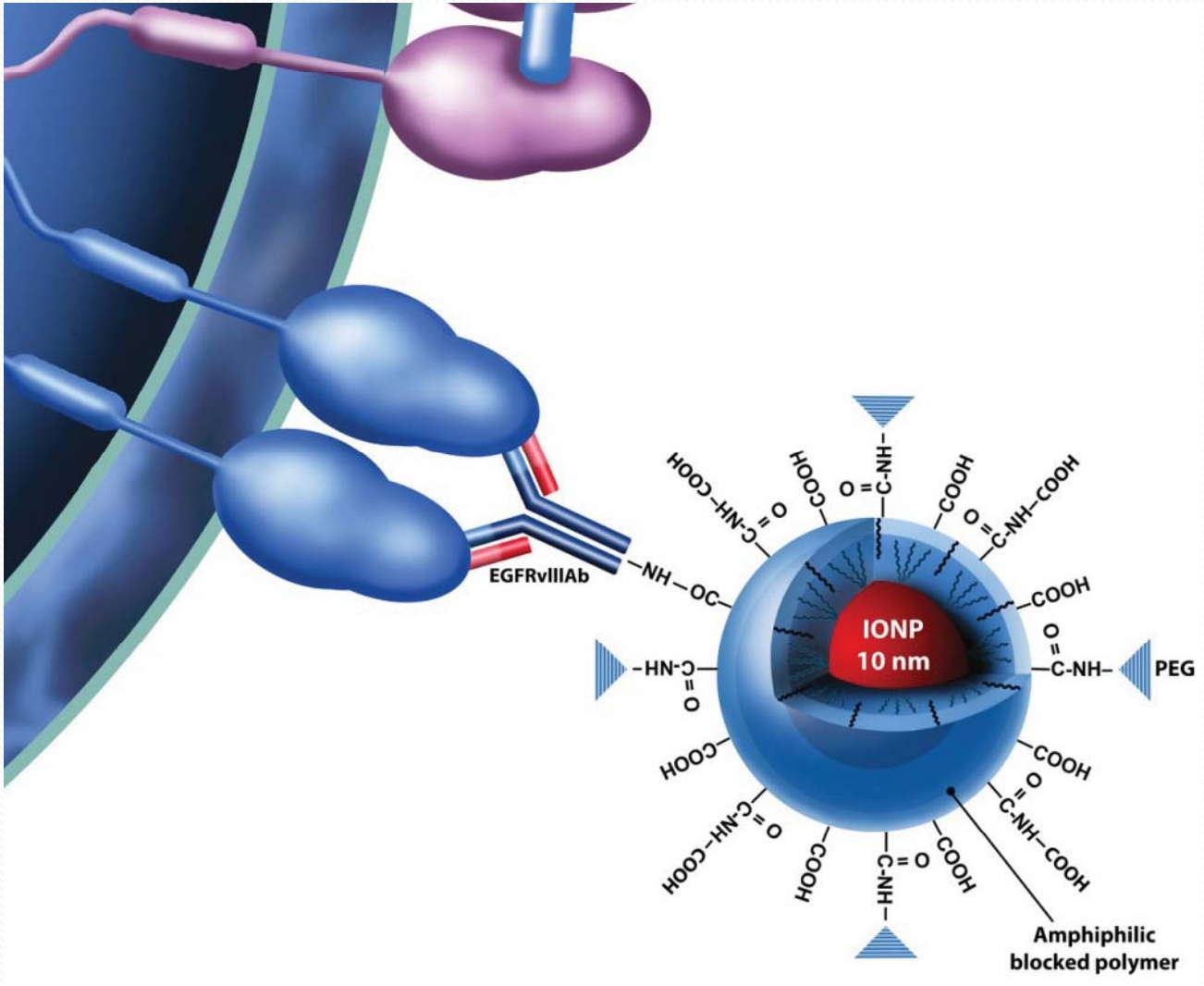


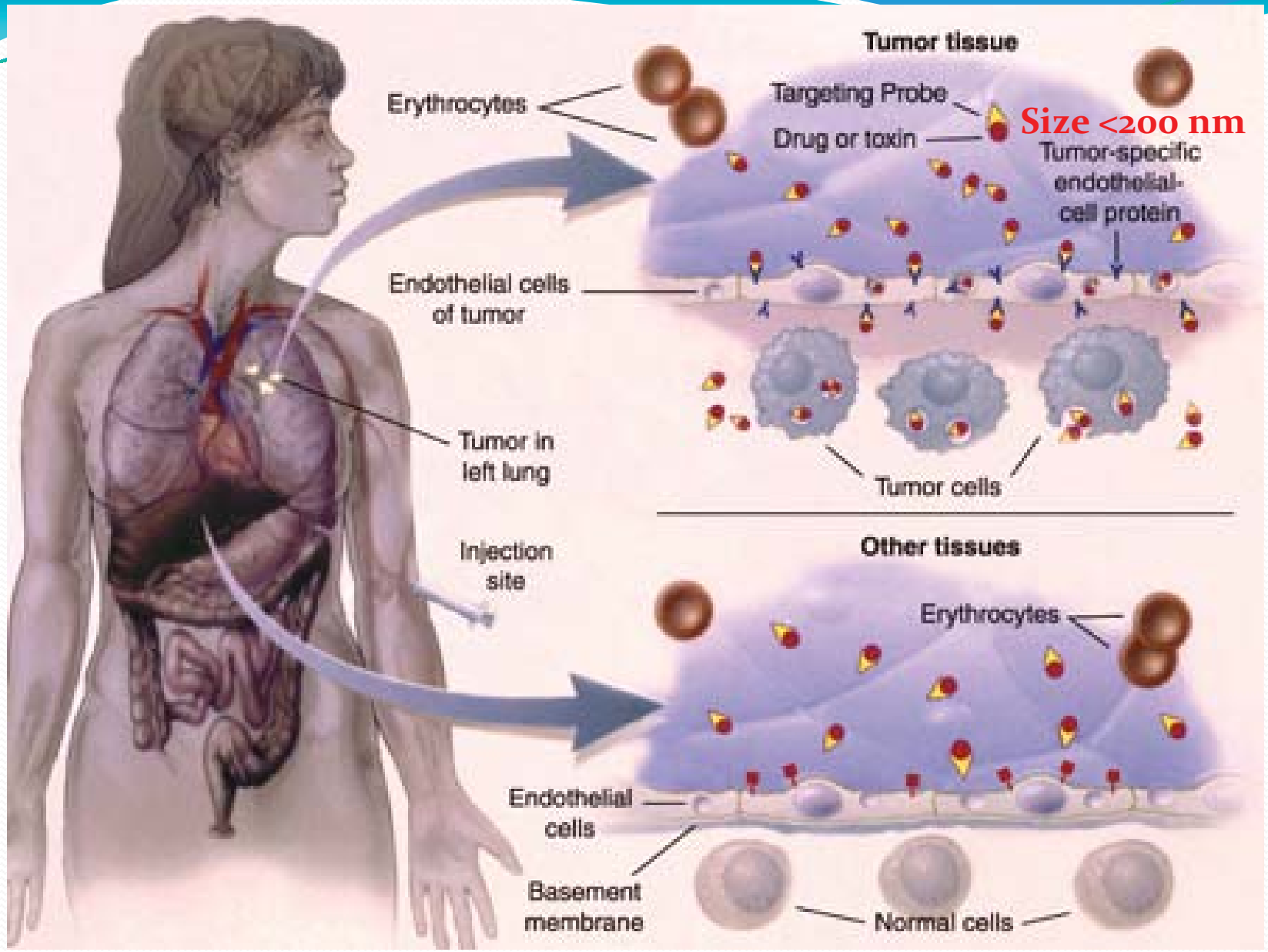

E. Paraffin embeded cancer biopsy samples obtained from breast cancer patients




Drs. Xiaohu Gao and Ruth O'Regan

# Theranostic Nanoparticles for Targeted Therapy and Imaging of Breast Cancer






Dr. Jan Schnitzer, UC San Diego

# Targeted Theranostic Nanoparticles For Treatment of Drug Resistant Cancer Cells

**Advantages of nanoparticle formulated drugs:**

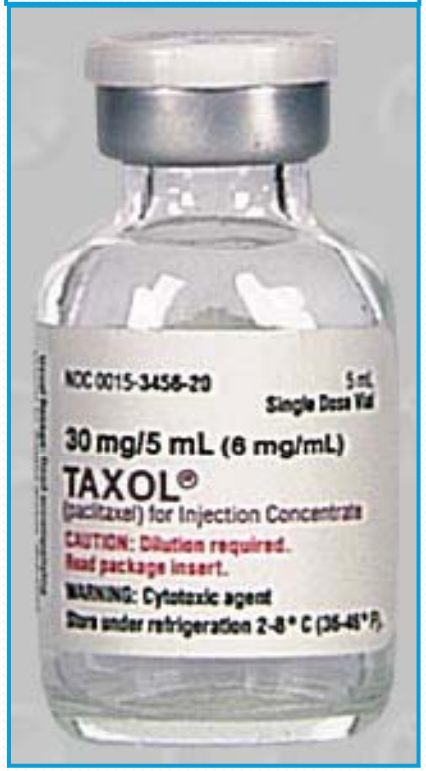
- Increase solubility of the drug and bioavailability
- high capacity for carrying drug molecules (single or multiple drugs)
- Prevent inactivation and rapid clearance of the drug
- Improve intratumoral delivery (passive and active targeting, long blood circulating time)
- Targeted delivery reduces systemic toxicity and allows administrating a higher dosage of drug
- Avoid efflux of drug by p-glycoprotein (MDR) by receptor-mediated endocytosis of nanoparticles
- Timely assess intratuomral drug delivery and response to therapy

# Abraxane (*nab*-paclitaxel) is a Solvent-free 'Nano' Version of Taxol (cremophor-based paclitaxel)

## Abraxane


**Abraxane received FDA Approval January, 2005 for metastatic breast cancer**

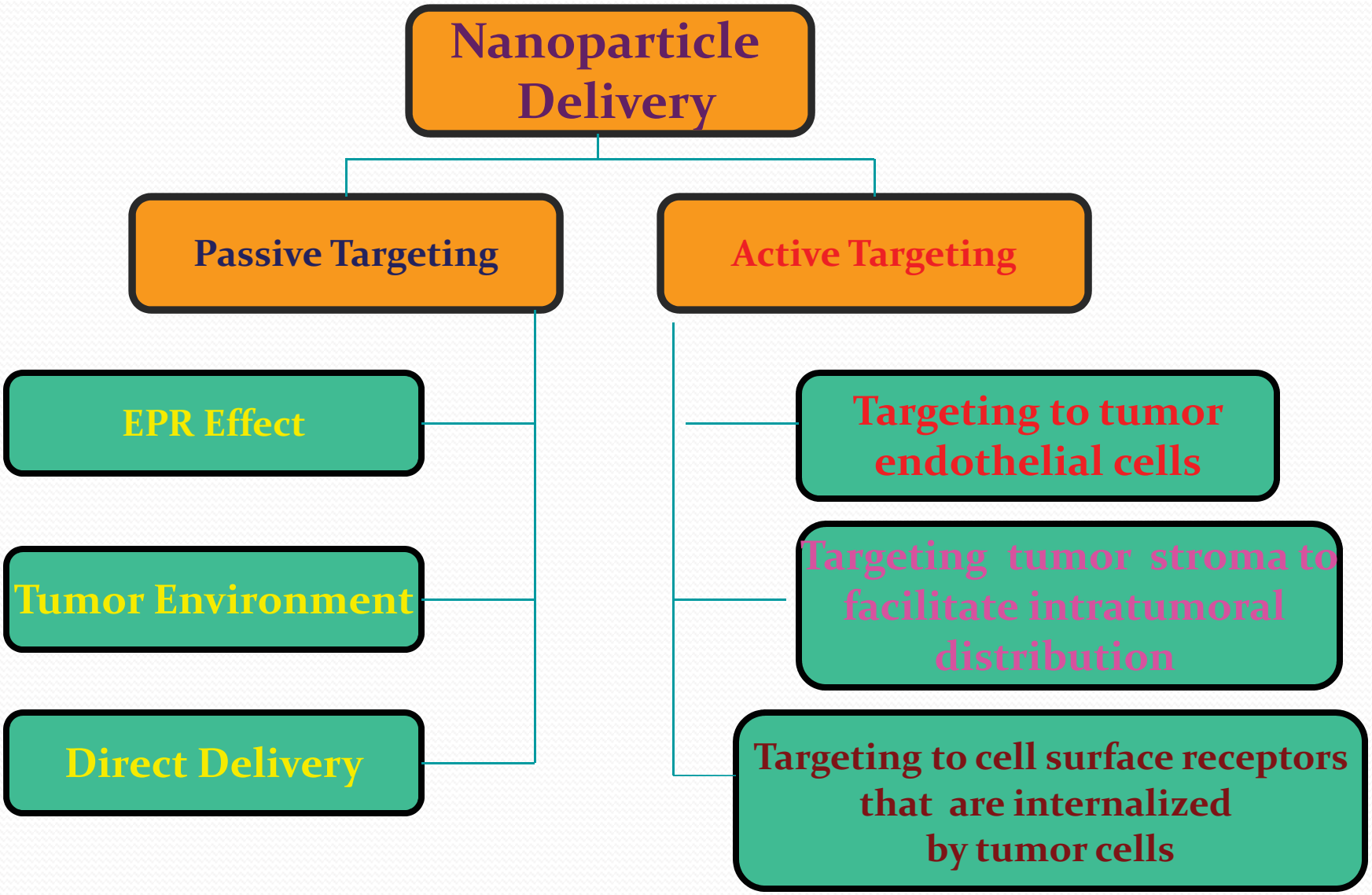
## Taxol

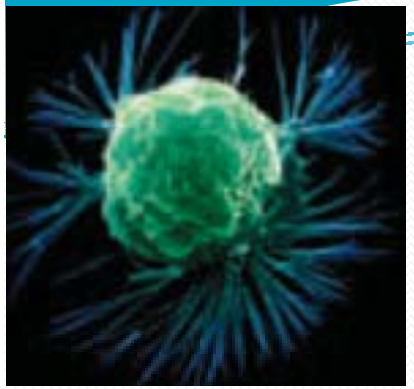



**Contents:**
**100 mg paclitaxel**
**900 mg albumin**
**No Surfactants/Solvents**

**Contents:**
**Paclitaxel 6 mg/ml**
**Cremophor 537 mg/ml**
**Ethanol 396 mg/ml**

# Strategies for Delivery of Theranostic Nanoparticles





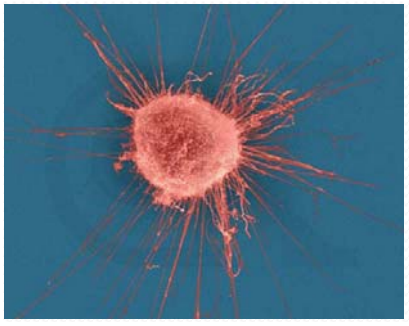
# How to target nanoparticles to tumors?

**Tumor targeting ligands:**

- **Antibody, single chain antibody, Affibody**
- **Recombinant proteins: natural ligands**
- **Peptides: phage display tumor homing peptides**
- **Small molecules**
- **Structured DNA or RNA molecules (Aptamer)**

**Cell surface target molecules**

**Human tumor cells are abnormal growth of normal cells. Cell surface molecular targets that are specific for cancer cells have yet to be discovered.**



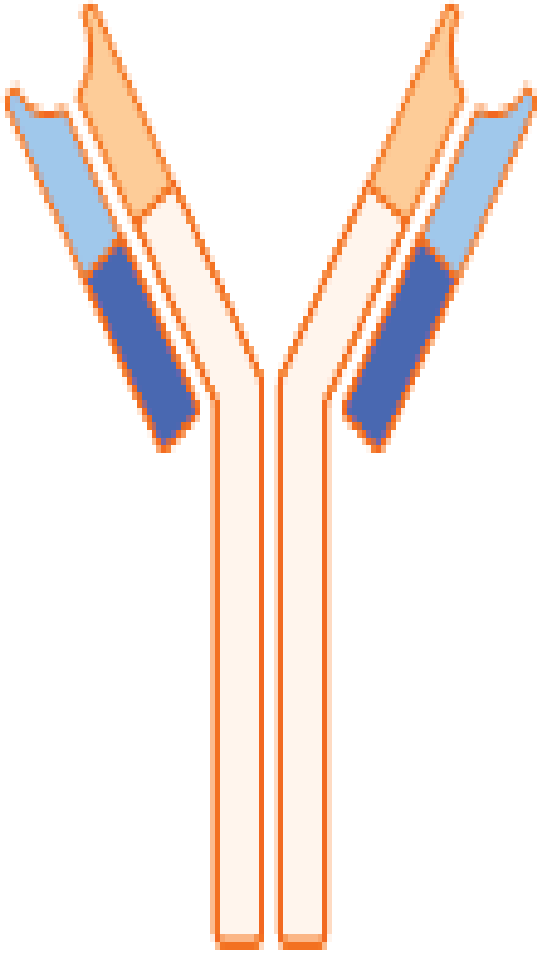
**Identification of appropriate ligand/target systems that are highly expressed in tumor cells and the tumor environment but their expression levels are low in normal cells.**

# Currently Used Molecular Targets

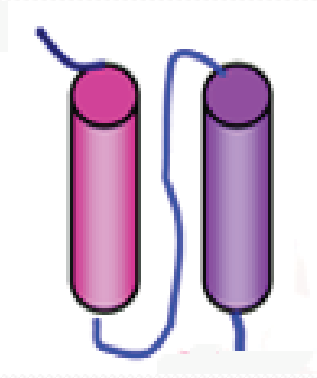
**Her-2/neu**
**EGFR**
**PMSA**
**Folate receptor**
**Transferrin receptor**
**MUC-1**
**$\alpha V\beta 3$ (RGD)**
**Cell surface nucleolin ($F_3$)**
**VEGFR**
**Enzyme activated fluorochromes**

**uPAR**

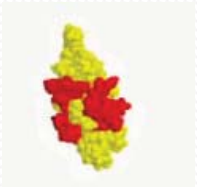
# Size and Binding Affinity of Targeting Ligands



Whole antibody
Size: 150 Kda
Kd: 1 to 100 nM

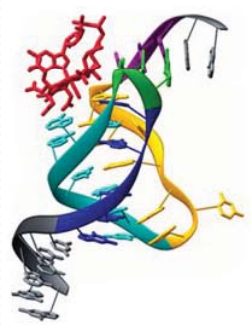


Single Chain antibody
Size: 25 to 27 KDa
Kd: 1 to 10 nM

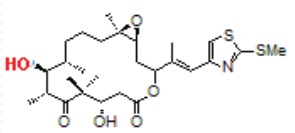


Affibody
Size: 5 to 7 KDa
Kd: pmol

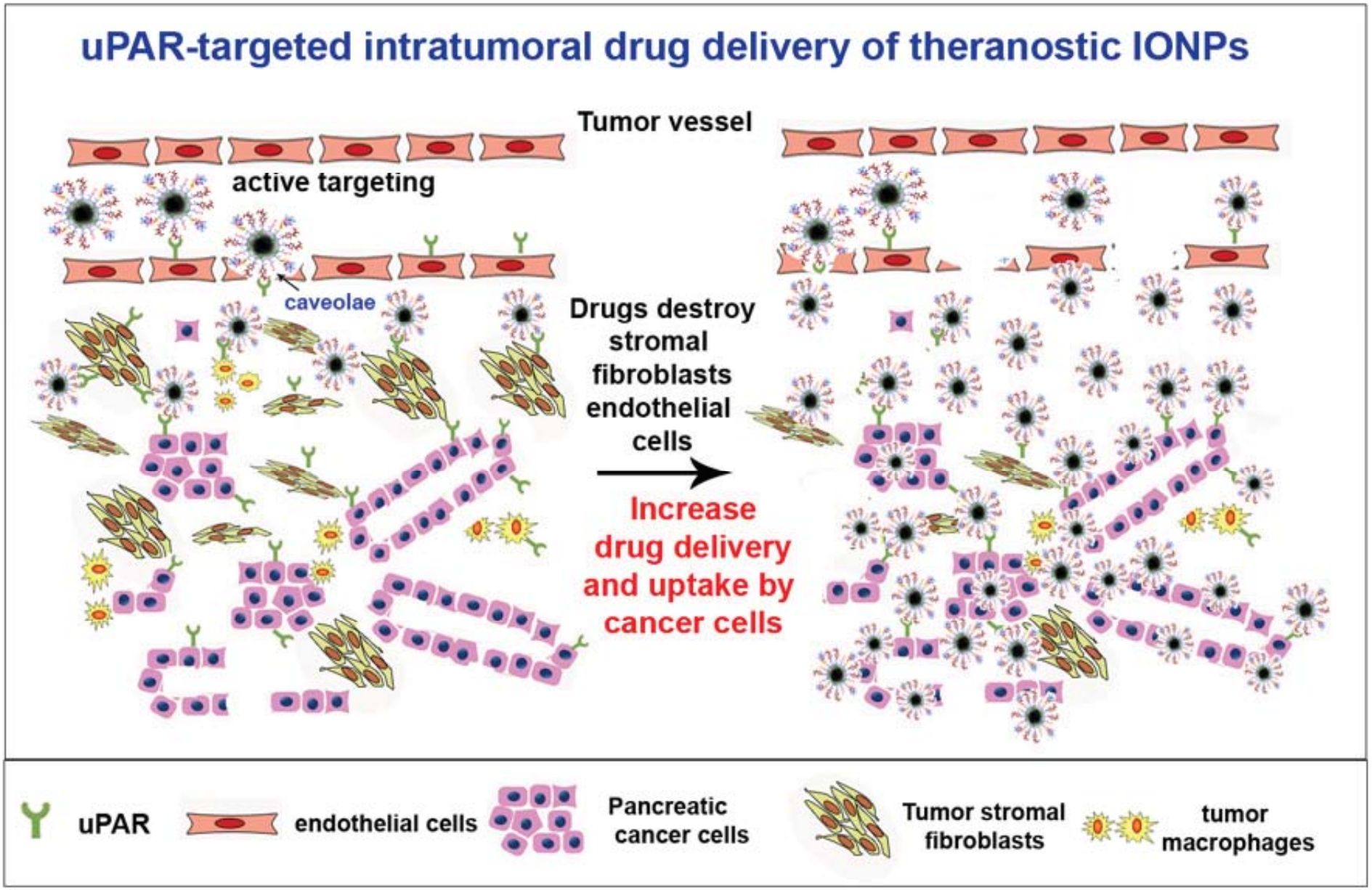
ATF of uPA
Size: 17 Kda
Kd: 0.28 nM



Apatmer
Size: 6 to 40 KDa
Kd: low nM




Folic acid
Kd: < 1 nM

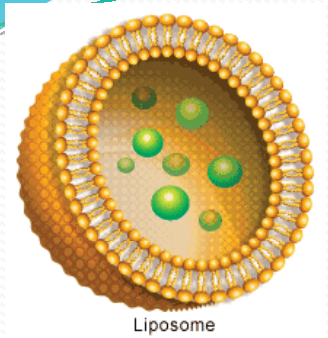

uPAR-targeted intratumoral drug delivery of theranostic IONPs
Tumor vessel
active targeting
caveolae
Drugs destroy stromal fibroblasts endothelial cells
Increase drug delivery and uptake by cancer cells
uPAR
endothelial cells
Pancreatic cancer cells
Tumor stromal fibroblasts
tumor macrophages

# Challenges in Developing Targeted Nanoparticles for Cancer Therapy and Imaging

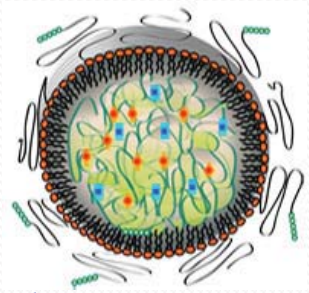
- Develop powerful nanomaterials that can generate strong imaging signals or contrast;
- Have a high capacity to carry therapeutic agents and can efficiently release the agents into tumor cells;
- Develop a delivery system to direct the imaging probe and therapeutic agents into the targeted tumor;
- Have a low or non-toxicity and biodegradable;
- Sensitive imaging techniques for non-invasive tumor imaging, detection of intra-tumoral drug delivery, and monitoring the response to therapy.

# Various Nanoparticles for Targeted Imaging and Drug Delivery

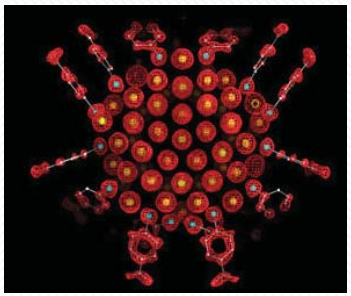
## Metallic nanoparticles



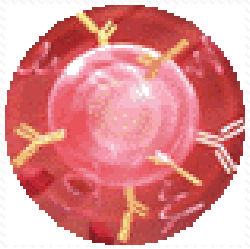

Liposome



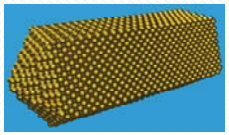
Polymeric NP



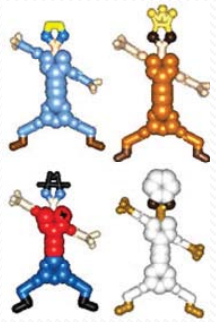
Gold NP



QDs

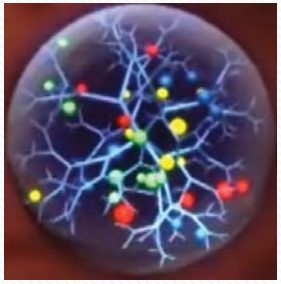


Nanorods

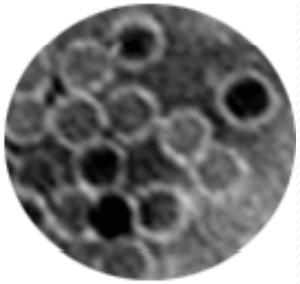


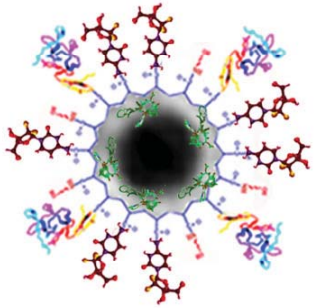
Nanokids

Micelle

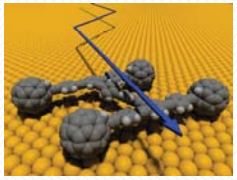


Dendrimer

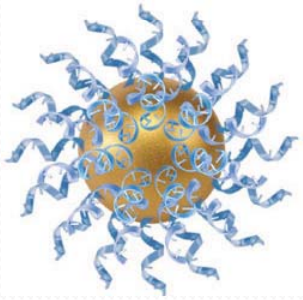




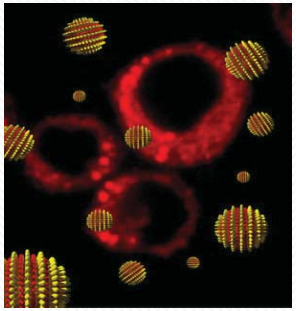
Magnetic iron oxide (IO)NP



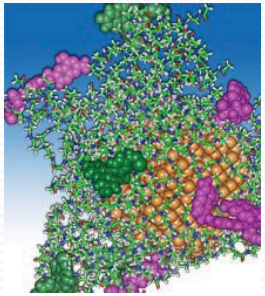
Nanocar



Gold-DNA

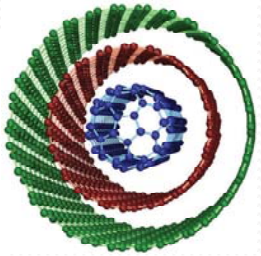


Gold-Drug

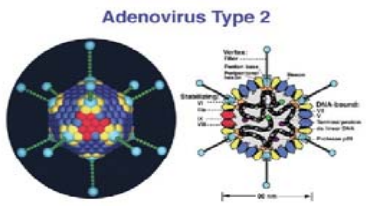


Dendrimer Gold

Self-assemble peptides

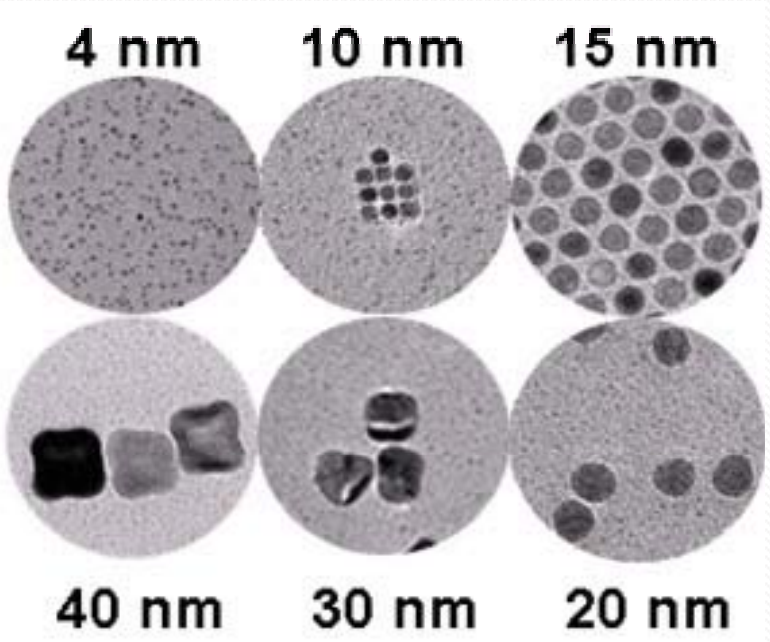


carbon nanotube



# Magnetic Iron Oxide (IO) Nanoparticles as MRI Contrast Agents and Delivery Vehicles

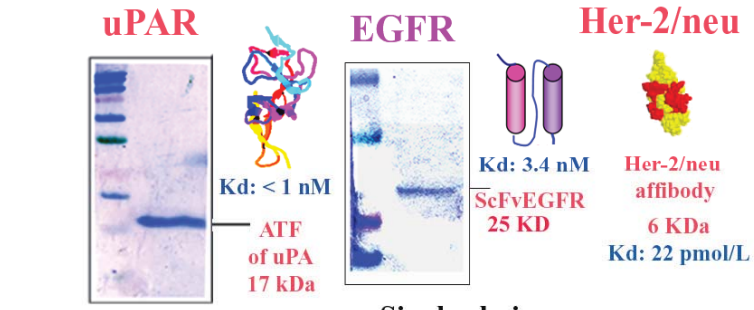
- Controllable IO nanoparticles with uniform sizes ranging from 4-40 nm;
- Stable and can be activated for surface functionalizations;
- Favorable pharmacokinetics: Long blood retention time, low toxicity, biodegradability, human applications;
- Have unique paramagnetic properties, generating strong susceptibility T2 weighted MRI contrast;
- Low cost and large production.




Dr. Y. Andrew Wang
Ocean Nanotech, LLC

# Development of Tumor Targeted Nanoparticles (magnetic iron oxide nanoparticles: IONP)

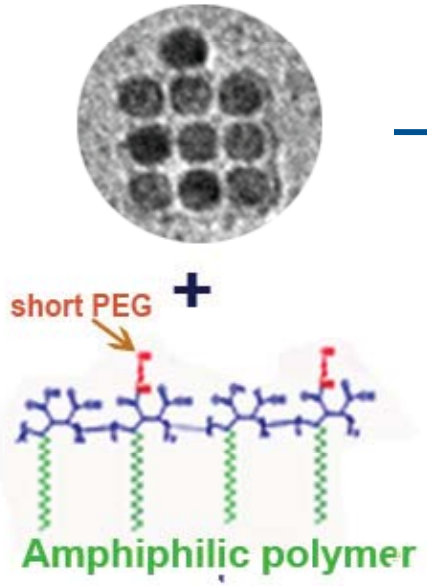
**Recombinant Targeting ligands**
**(High affinity, small size, large production)**

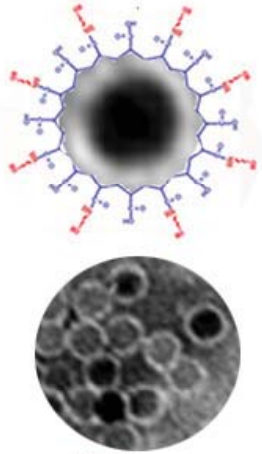



Receptor binding domain of a natural ligand

Single chain antibody

Affibody



EM image

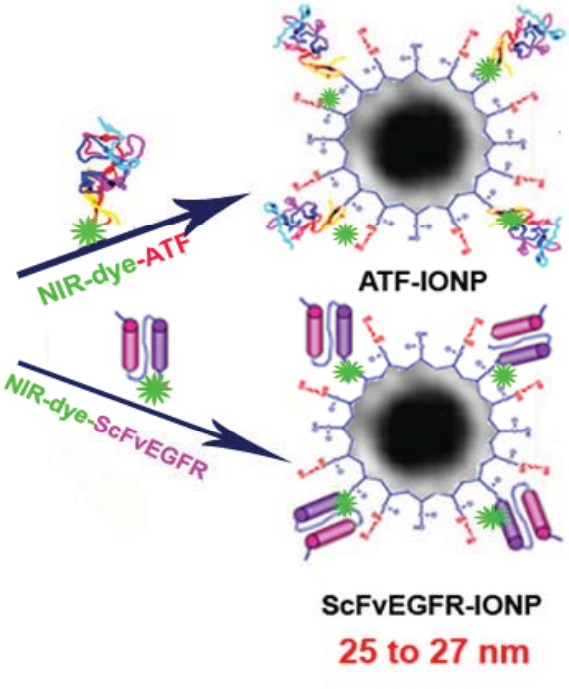
2 nm polymer coating

Polymer-coated IONP size: 15 nm

MRI signal strength

R2: $124 \pm 7.2$ ($S^{-1}.mM^{-1}$)

R1: $3.6 \pm 0.3$ ($S^{-1}.mM^{-1}$)

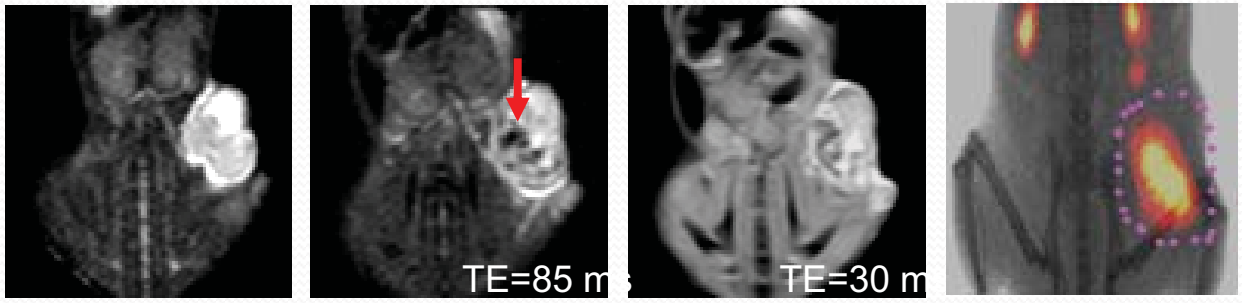



ScFvEGFR-IONP

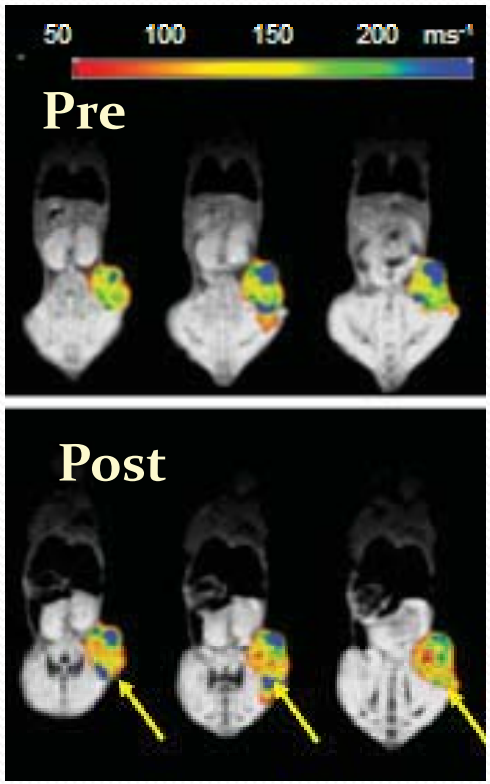
25 to 27 nm

**Targeted MRI and optical imaging probes**

# Detection of Breast Cancer using uPAR-targeted MRI



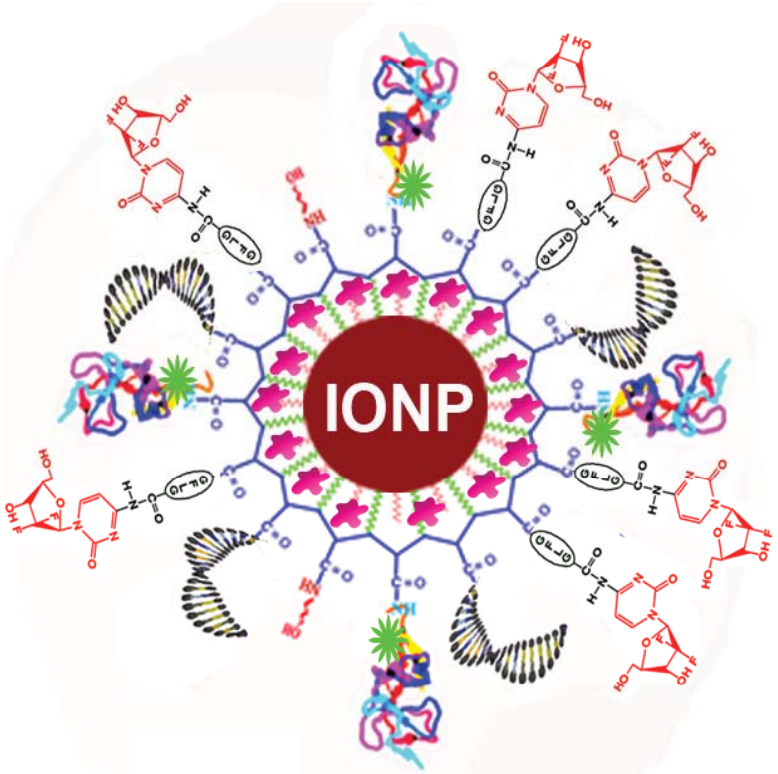

Pre contrast Post 24 Hrs Dual Echo T2W FSE Cy5.5 NIR




**T2-Map**

In vivo MRI of peritoneal tumor metastasis using ATF-IO nanoparticles

# Multifunctional Theranostic Nanoparticle



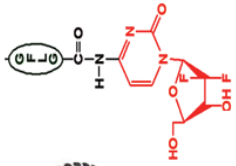

**IONP Magnetic Iron Oxide Nanoparticle**
**(MRI Contrast and Drug Carrier)**

**Targeting ligand (ATF or ScFvEGFR)**
**Targeted Drug Delivery**

**Short PEG chain**

1). 

**Hydrophobic drugs (encapsulation)**

2). 

**Hydrophilic drugs (conjugation and protease-activated drug release)**

3). 

**siRNA expressing DNA cassette**

**Near infrared dye labeling for optical imaging**

# Theranostic Nanoparticles for Clinical Applications

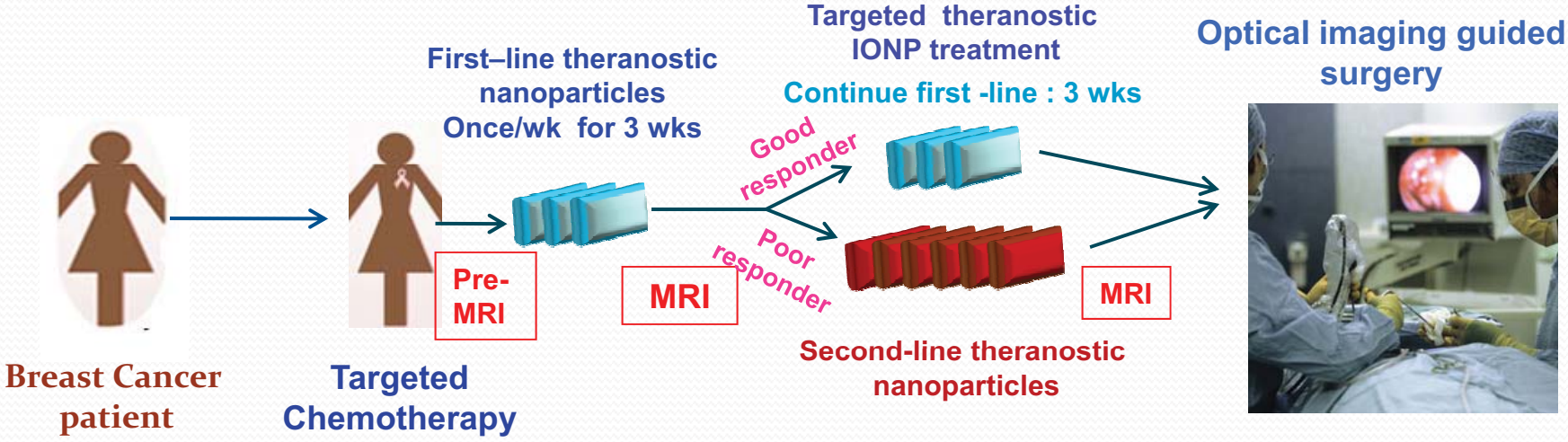
- **Preoperative targeted neoadjuvant chemotherapy and MRI monitoring drug delivery and response to therapy**

  - To reduce primary tumor size and local invasion:
    Mastectomy to Breast Conservative Surgery

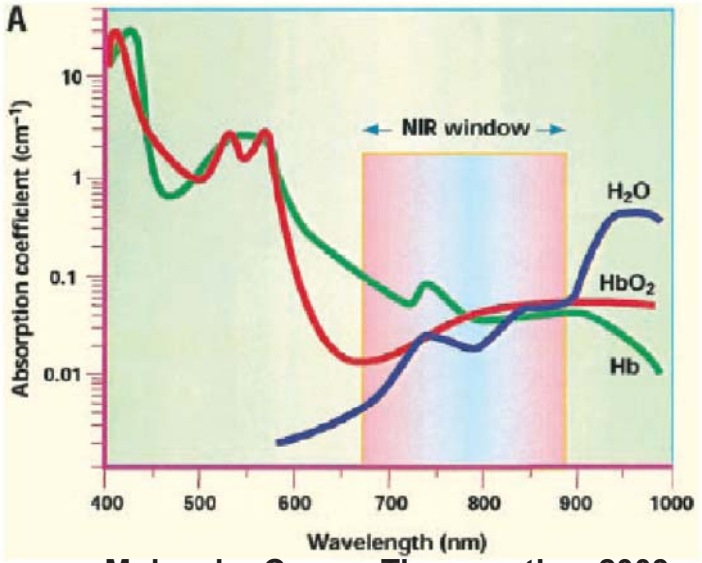
  - To treat distant micrometastasis:
    Reduce incidence of development of tumor metastasis

  - MRI monitoring therapeutic response that allows timely replacing an ineffective drug to avoid un-necessary systemic toxicity

- **Intraoperative optical imaging-guided surgery**
  - Complete removal of residual tumors to prevent local and distance recurrence
  - Reduce removal of normal tissues



# NIR Optical Probes for Intraoperative Imaging of Breast Tumor margins




Molecular Cancer Therapeutics, 2003

*Fig. 7.* Absorption of light *versus* wavelength. Given the decreased absorption of light in th **Umar Mahmood[1] and Ralph Weissleder** h visible light (~400–650 nm) and infrared light (>800 nm), tissue penetration of NIR photons may be up to 10–15 cm. Fluorochromes used in the reviewed imaging studies fluoresce in this window of opportunity.

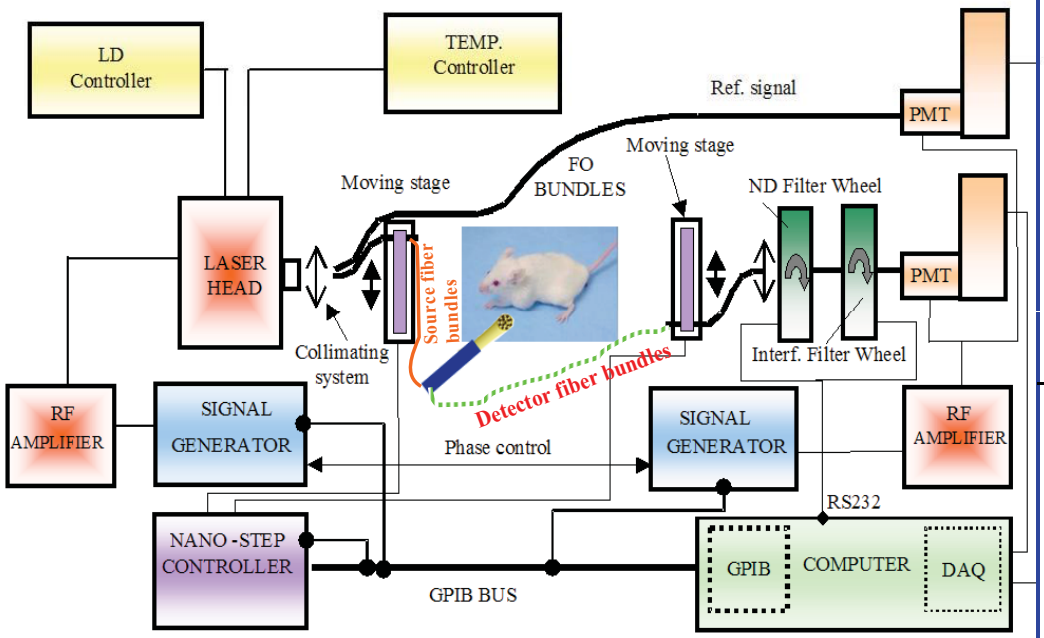
**Indocyanine green (ICG): FDA approved , A high level of plasma protein binding, Need further modification for targeting.**

**Cy5.5: Ex/Em 675nm/694 nm, high body background.**

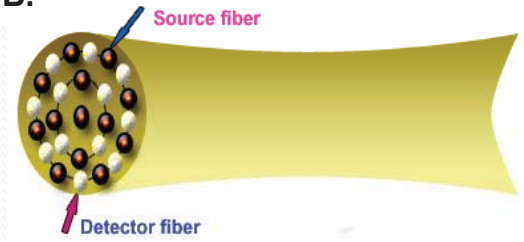
**IRDye 800 (LI-COR Bioscience) low quantum yield and stay in the liver for long time.**

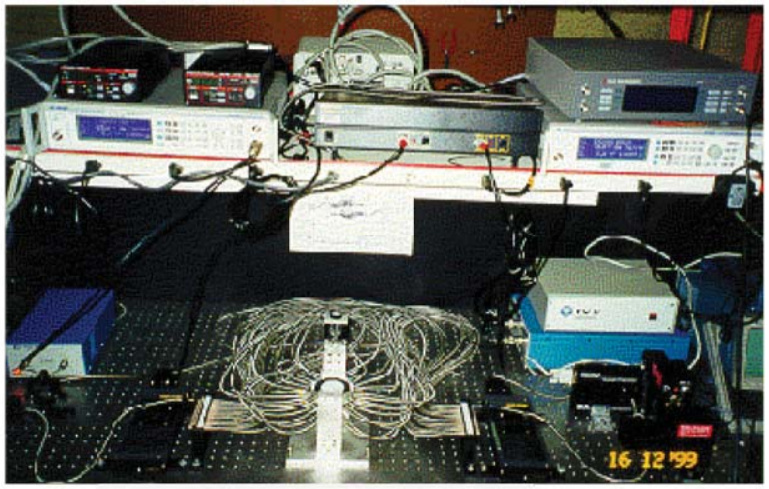
# Automated Multi-channel Diffuse Fluorescence Tomography (DFT) Imaging System

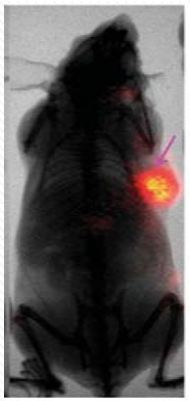
A. Basic Components of DFT Imaging Set-up

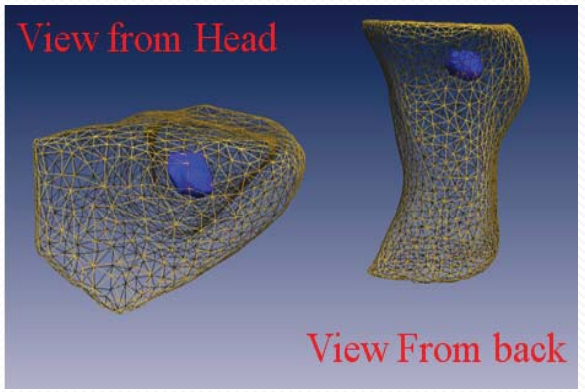



B.






3D-diffuse fluorescence tomography

Dr. Huabei Jiang, University of Florida

# Issues to be addressed for translation of those multifunctional nanoparticles for clinical applications


"If you increase the magnification another million times you can see the safety regulations."

## Summary of Results

- We have developed receptor targeted biodegradable nanoparticles using uPAR, EGFR or Her-2-targeted ligands conjugated to magnetic iron oxide nanoparticles (IONP) .

- Systemic delivery of the targeted IONP is able to detect primary and metastatic tumor lesions in animal tumor models by MR and near infrared optical imaging.

- We have developed theranostic nanoparticles for targeted tumor imaging , drug delivery, and monitoring therapeutic response.

- uPAR-targeted theranostic nanoparticles are multifunctional nanoparticles that bind to tumor endothelial cells and stromal fibroblasts to facilitate intratumoral delivery of imaging and therapeutic nanoparticles, are internalized by tumor cells for drug delivery to produce anti-angiogenesis and anti-tumor effects, and are able to monitor therapeutic effect by non-invasive tumor imaging.

- Our receptor targeted, multifunctional theranostic nanoparticles are promising drug delivery vehicles that have potential to break physical and intrinsic barriers of drug resistance in cancer cells and tissues, and enhance therapeutic effects in drug resistant cancer cells.

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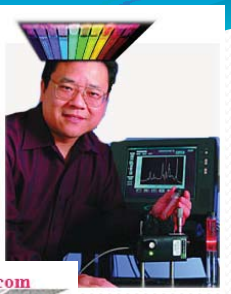
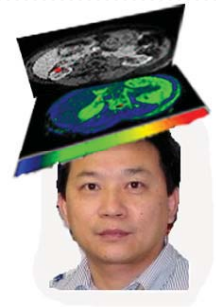
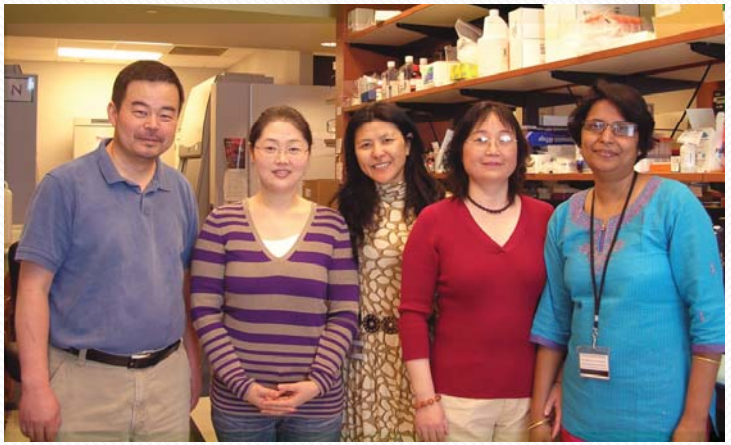




**Ocean NanoTech, LLC**
**Y. Andrew Wang, PhD**

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