

## Immunoliposomes for Targeted Radionuclide Therapy

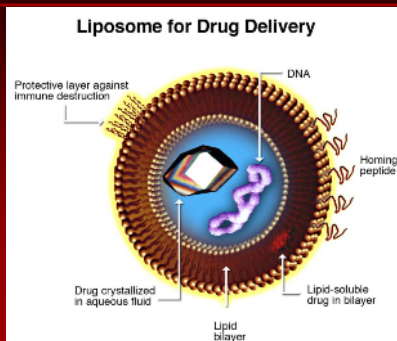
George Sgouros, Ph.D.

Russell H. Morgan Dept of Radiology  
& Radiological Science  
Johns Hopkins University, School of Medicine  
Baltimore MD

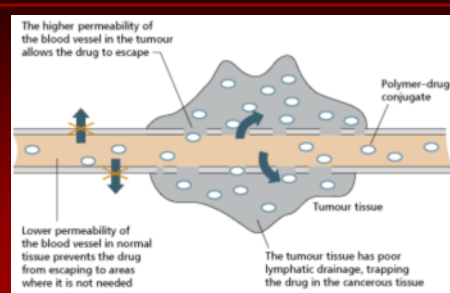
## Outline

- **Liposomes**
  - Overview of structure and function
  - Enhanced Permeability and Retention (EPR) effect
  - PEG, size, charge
  - Doxil, Myocet
- **Immunoliposomes**
  - Composition and structure – different types
  - Mode of targeting/contrast w/ untargeted liposomes
- **Immunoliposomes in targeted alpha-emitter therapy**

## Liposome - structure



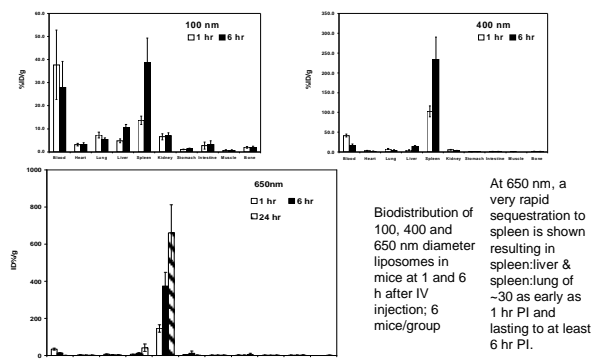
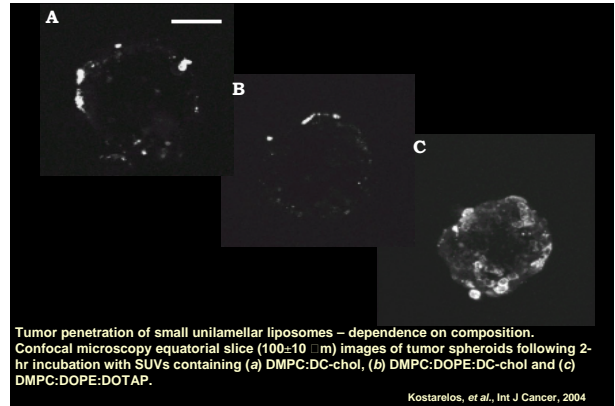
## Liposome – tumor localization



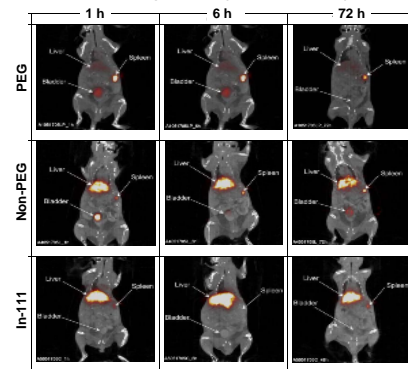
Enhanced Permeability and Retention (EPR)

## Liposomes – Composition

- **Phospholipids chosen**
  - Surface Charge - interaction with cells, cluster penetration
  - Membrane fluidity - rigidity
- **Size**
  - Diameter > 250 nm, localize to spleen
- **long polymeric chains on surface (PEGylated)**
  - Evade RES system, increase circulation half-time
  - Typically 5% mole of 2000 MW PEG
  - Too much (>10%) – micelles, too little - phagocytosed



### Biodistribution of Liposomes (PEG vs nonPEG) vs In-111

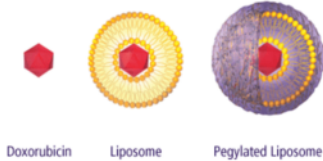


## Doxorubicin

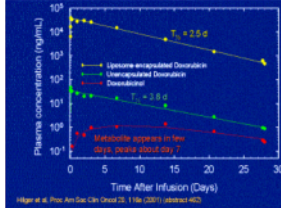
### Cardiac Toxicity 4 to 20 Years After Completing Anthracycline Therapy

Laurell J. P. *Journal of Clinical Oncology* 2005; 23: 4475-4481. DOI: 10.1200/JCO.2005.03.4475

**Doxil®**

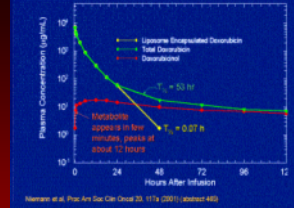


### DOXIL Pharmacokinetics



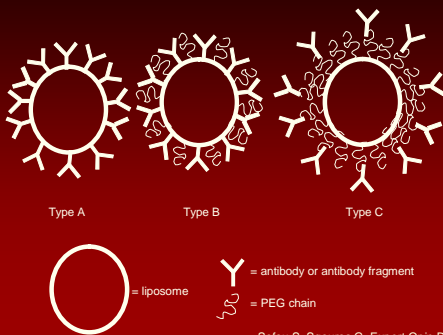
Size = 100 nm  
Type = large unilamellar vesicles  
Hydrogenated soy phosphatidyl choline (PC), cholesterol, PEG-2000, distearoyl phosphatidyl ethanolamine  
Drug/lipid weight = 0.125  
Drug loading: ammonium ion gradient  
Sulfate salt precipitate – “striated gel”

### Myocet Pharmacokinetics



Size = 180 nm  
Type = large oligolamellar vesicles  
Egg phosphatidyl choline, cholesterol, Drug/lipid weight = 0.27  
Drug loading: hydrogen ion gradient  
Citrate fiber bundles

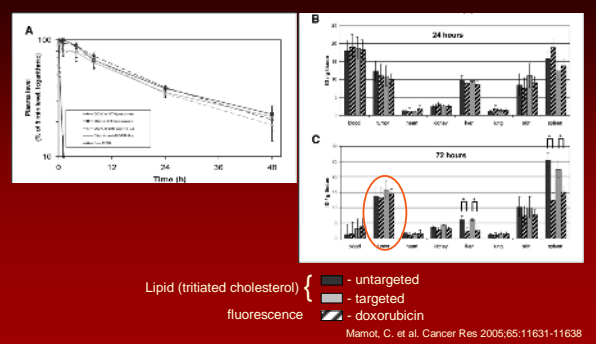
## Immunoliposome - types



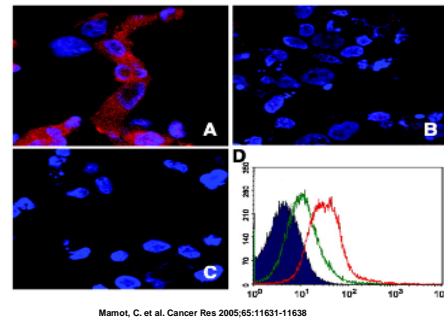
## Immunoliposomal - targeting

- No difference in overall tumor concentration
  - Gross localization to tumor based on EPR effect
- Significant difference in drug delivery
  - Increased interaction with target cell population
  - Increased drug delivery to target cell population

# Immunoliposome - targeting



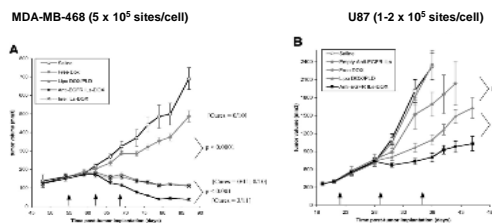
In vivo uptake of liposomes and immunoliposomes in the U87/EGFRvIII tumor xenograft model



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Cancer Research

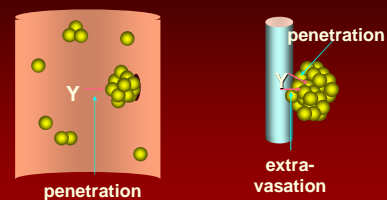
Therapeutic efficacy of anti-EGFR immunoliposome-doxorubicin in EGFR-overexpressing tumor models



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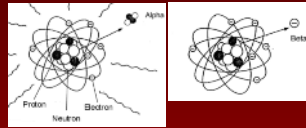
## Ab targeting – Metastatic dissemination



## Alphas vs Betas

### alphas

- He nucleus (4 amu)
- 80 keV/ $\mu$ m
- 2 to 3 tracks kill cell
- Irreparable DNA damage
- potent single cell, cluster kill

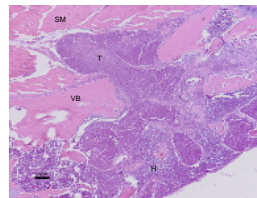


### betas (electrons)

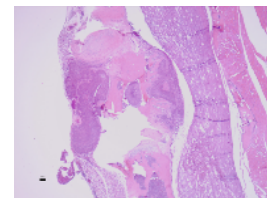
- 0.2 keV/ $\mu$ m
- $10^3$  to  $10^4$  tracks to kill cell
- DNA damage is repaired
- cross-fire required



## Animal Model: LCV injection

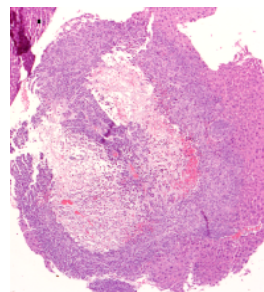
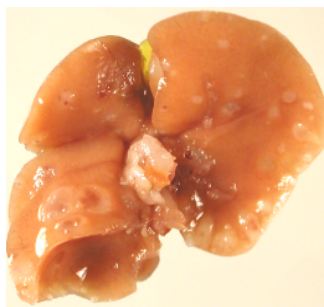


B3 vertebra lysis 50X

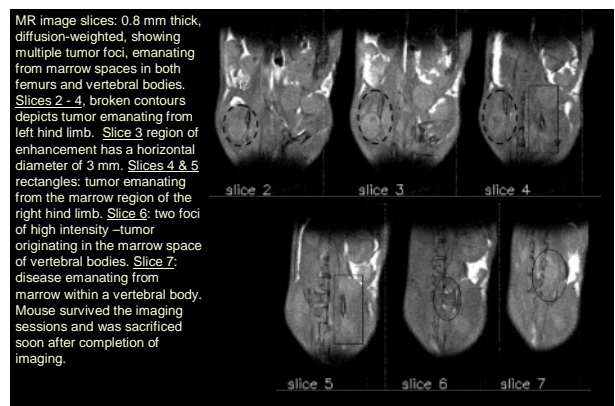


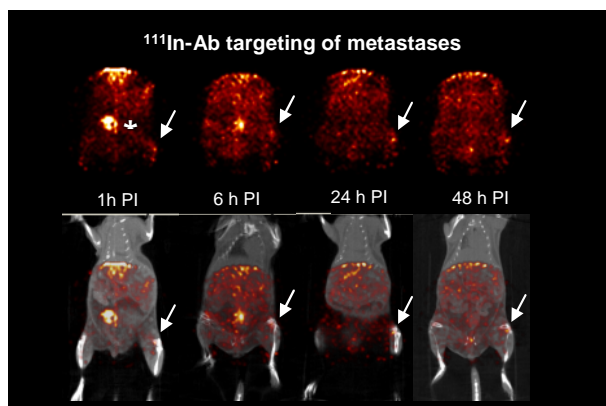
B4 vertebra lysis and impinge spinal cord 25X

16 N12 cells, bone, liver metastases, survival for 3.5 to 4.0 weeks, 90% successful inoculation. Bright red arterial blood indicates successful inoculation. TB = trabecular bone; VB = vertebral bone; BV = blood vessel; SC = spinal cord; SM = skeletal muscle; T = tumor cells; H = hematopoietic cells



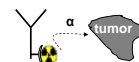
Left: Image of excised liver, 4-wks after LCV injection. Right: Section of the liver containing a metastatic tumor mass. At the periphery of the mass is a thick rim of viable tumor cells that infiltrate and compress adjacent hepatic parenchyma. At the center, a large area of tumor necrosis with remnants of congested vasculature and lesser amounts of hemorrhage and inflammatory cells.





## $\alpha$ -Radioimmunotherapy $\alpha$ and $\beta$ radiation

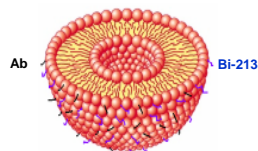
	$\alpha$ particles	$\beta$ particles
Dimension / range ( $\mu\text{m}$ )	35-100	3,000-8,000
LET in tissue ( $\text{keV}/\mu\text{m}$ )	80-150	0.1-3.0
# of DNA hits required to inactivate a cell	1-5	100-1,000
Therapy	short penetration and short half life	wide penetration and extended damage



**Specific activity of antibody**  
1 Bi-213 / ~1900 antibody

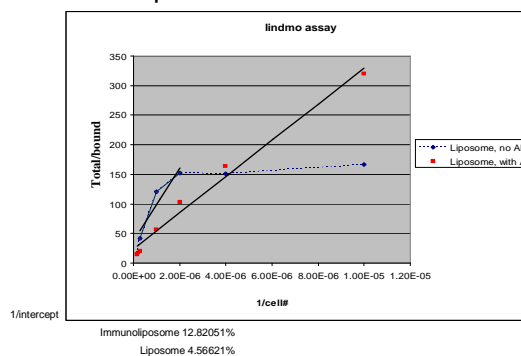
## Alpha-Immunoliposome construction

DMPE + Cholesterol + DSPE-PEG  $\xrightarrow{55^\circ\text{C extrusion, 100nm membrane}}$  Liposomes (~100nm)  
 Liposomes + DTPA  $\xrightarrow{\text{pH } 8-9}$  Liposomes-DTPA  
 DSPE-PEG-maleimide + thio-Ab  $\xrightarrow{\text{overnight}}$  DSPE-PEG-Ab  
 Liposomes-DTPA + DSPE-PEG-Ab  $\xrightarrow{55^\circ\text{C, 1h}}$  Immunoliposomes-DTPA  
 Immunoliposomes-DTPA + Bi-213  $\xrightarrow{\text{pH } 4}$  Immunoliposomes-Bi-213

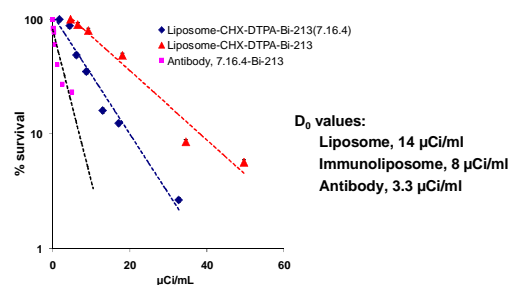


Specific activity of 0.1 Bi-213/liposome  
>90% stability after 4h in 10% serum

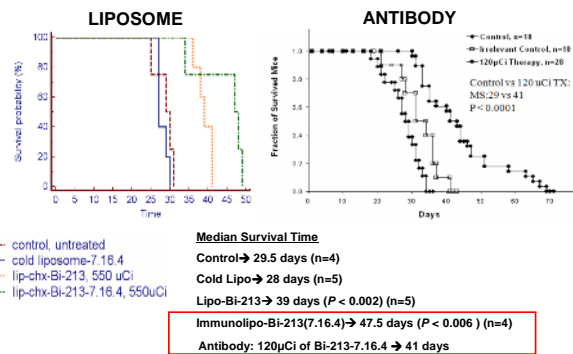
## Liposome Immunoreactive fraction



## In-vitro Cell Kill Assay



## Metastatic BCa Targeted alpha-radiotherapy



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