

# Critical Path Research Opportunities in the Development of Nanotechnology-Based Medicine – Lessons Learned from DOXIL<sup>®</sup> Development

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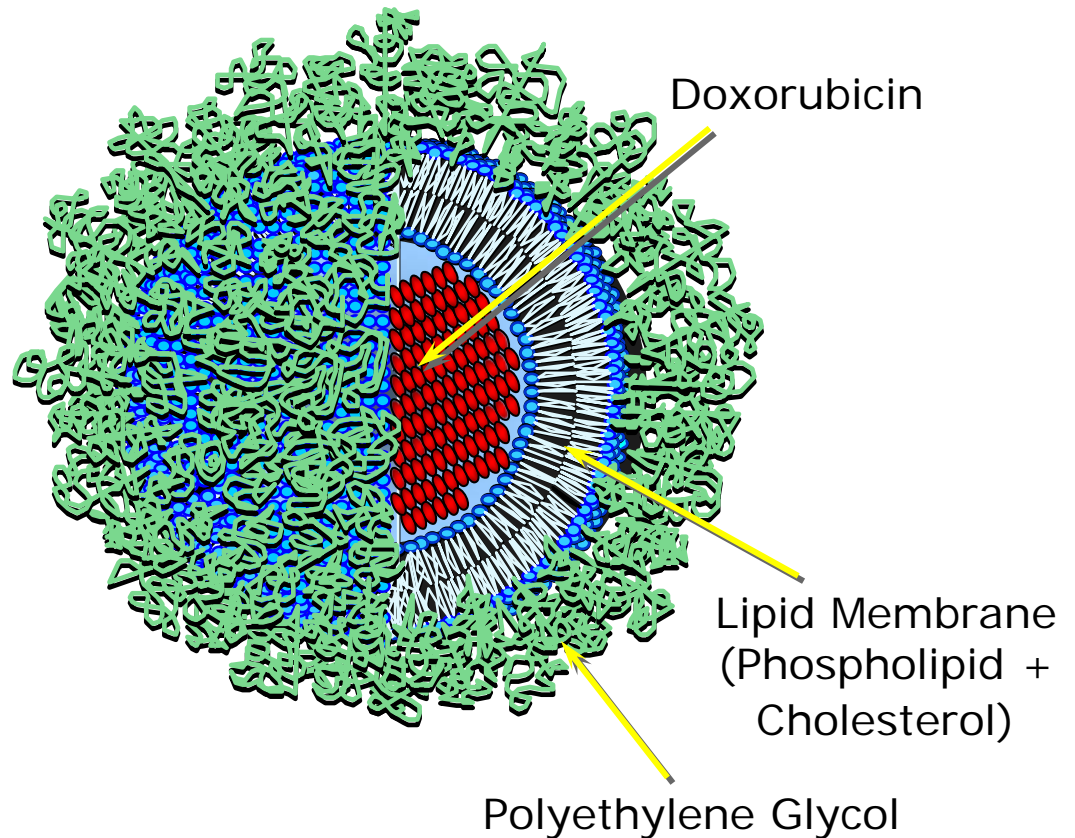


# Agenda

- Design features and pharmacological properties of DOXIL<sup>®</sup>
- Critical path challenges in the development of DOXIL<sup>®</sup>
  - Safety and efficacy
  - Manufacturing
- Potential critical path research opportunities for nanoparticle medicines with similar mechanism of action as DOXIL<sup>®</sup>

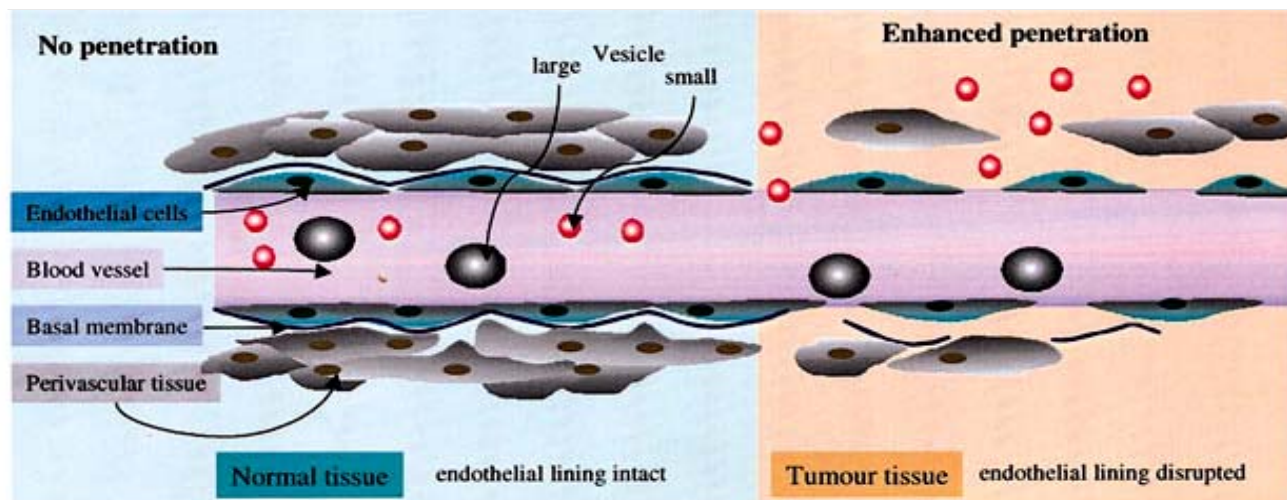
# DOXIL® Structure Doxorubicin Encapsulated in STEALTH® Liposomes

- Small (~100 nm)
- Pegylated



# Enhanced Permeability & Retention (EPR) of Nanoparticles in Tumor Tissues

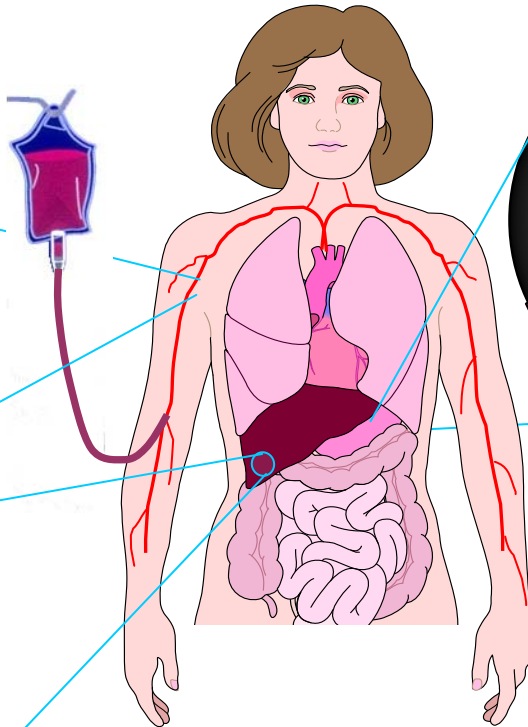
- Enhanced permeability of tumor vessels
  - Related to angiogenesis
  - Incomplete formation of vessel walls and basement membrane
  - Gaps and defects in endothelium
- Retention
  - Deficient lymphatic drainage
- Particle extravasation, retention, drug release in solid tumors



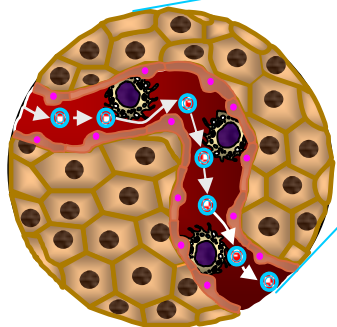
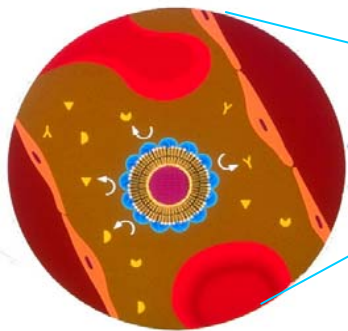
# Pharmacological Basis for DOXIL<sup>®</sup> Action

Key attributes: PEGylation, stable drug encapsulation & optimal size

- Evade MPS
- Long circulating
- Reduced healthy tissues uptake

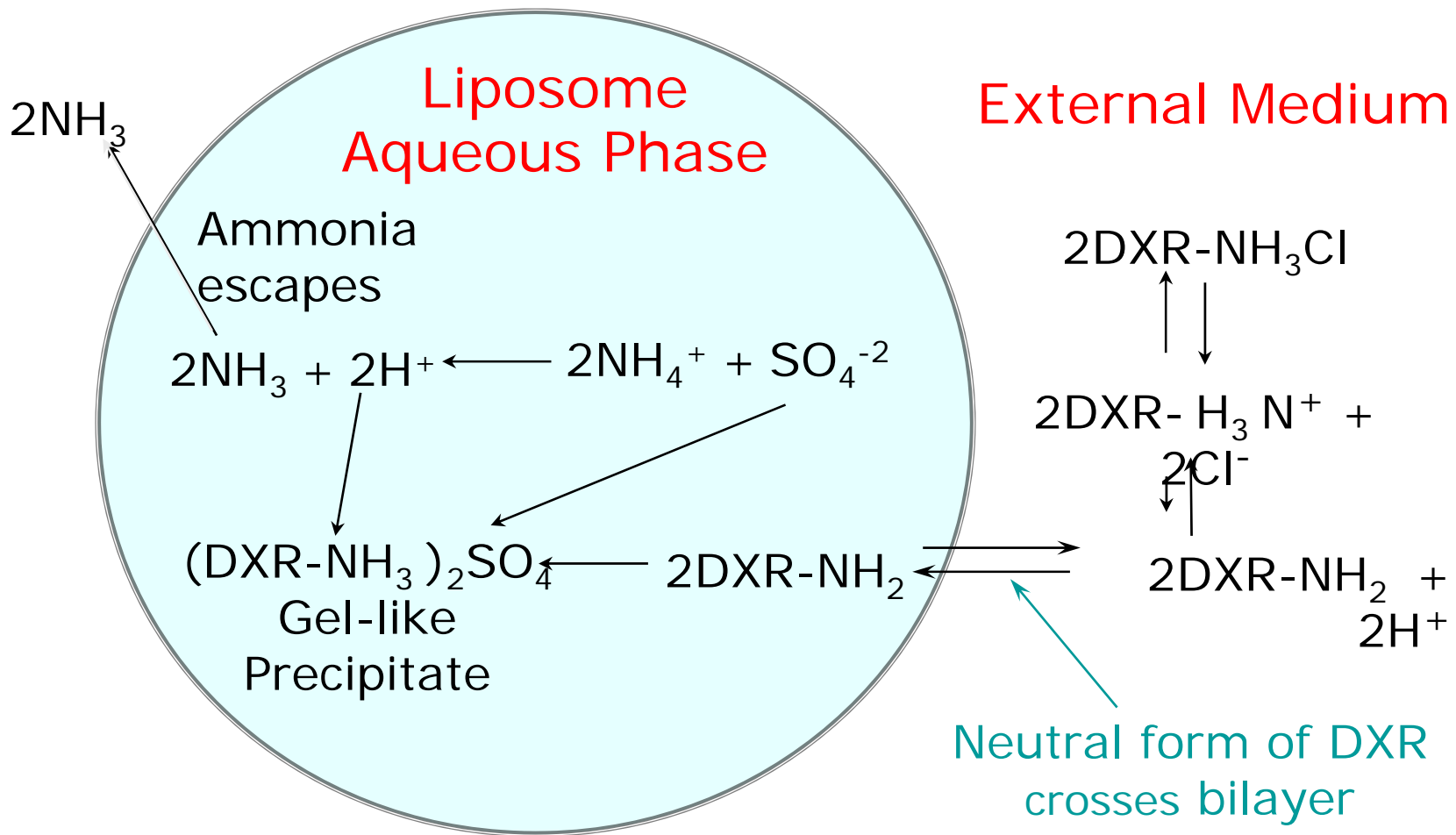


Extravasation  
Tumor accumulation



- Slow drug release
- Enhanced efficacy
- Reduced toxicity

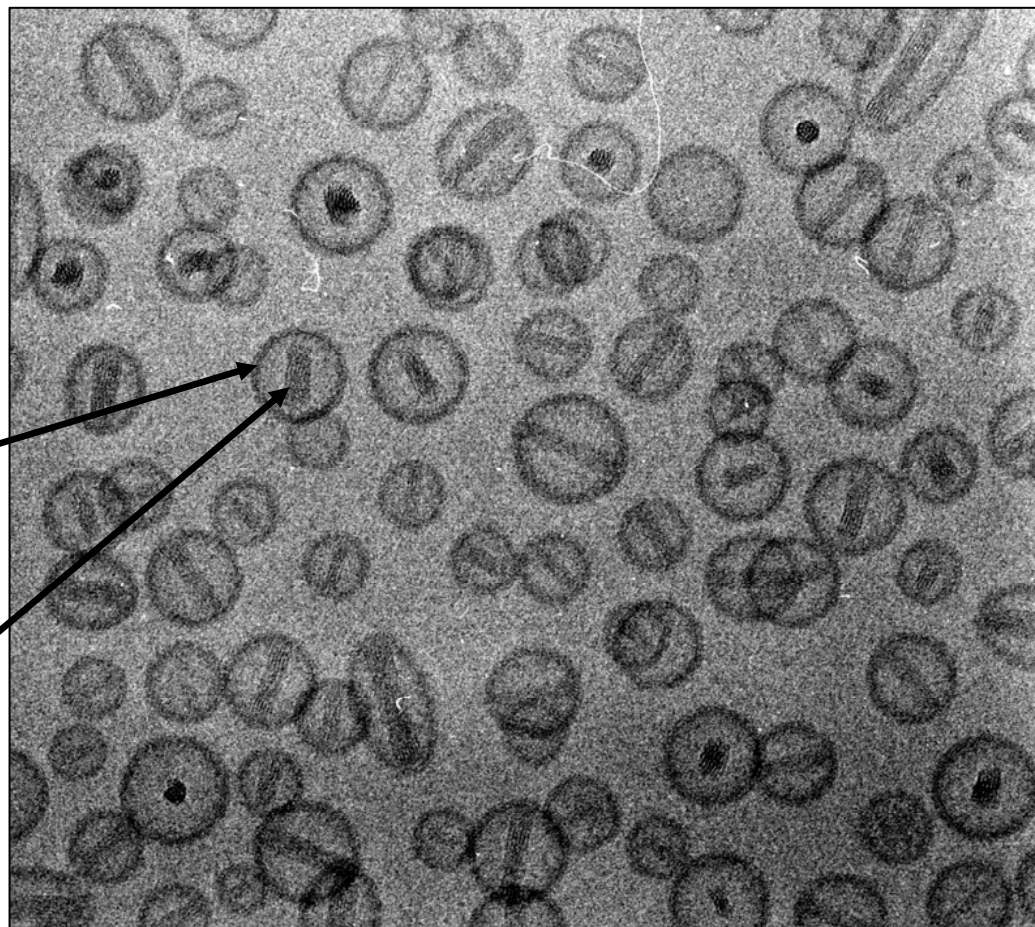
# Critical Design Feature: Drug Loading Method in DOXIL® Manufacturing





# Morphology of DOXIL® Liposomes

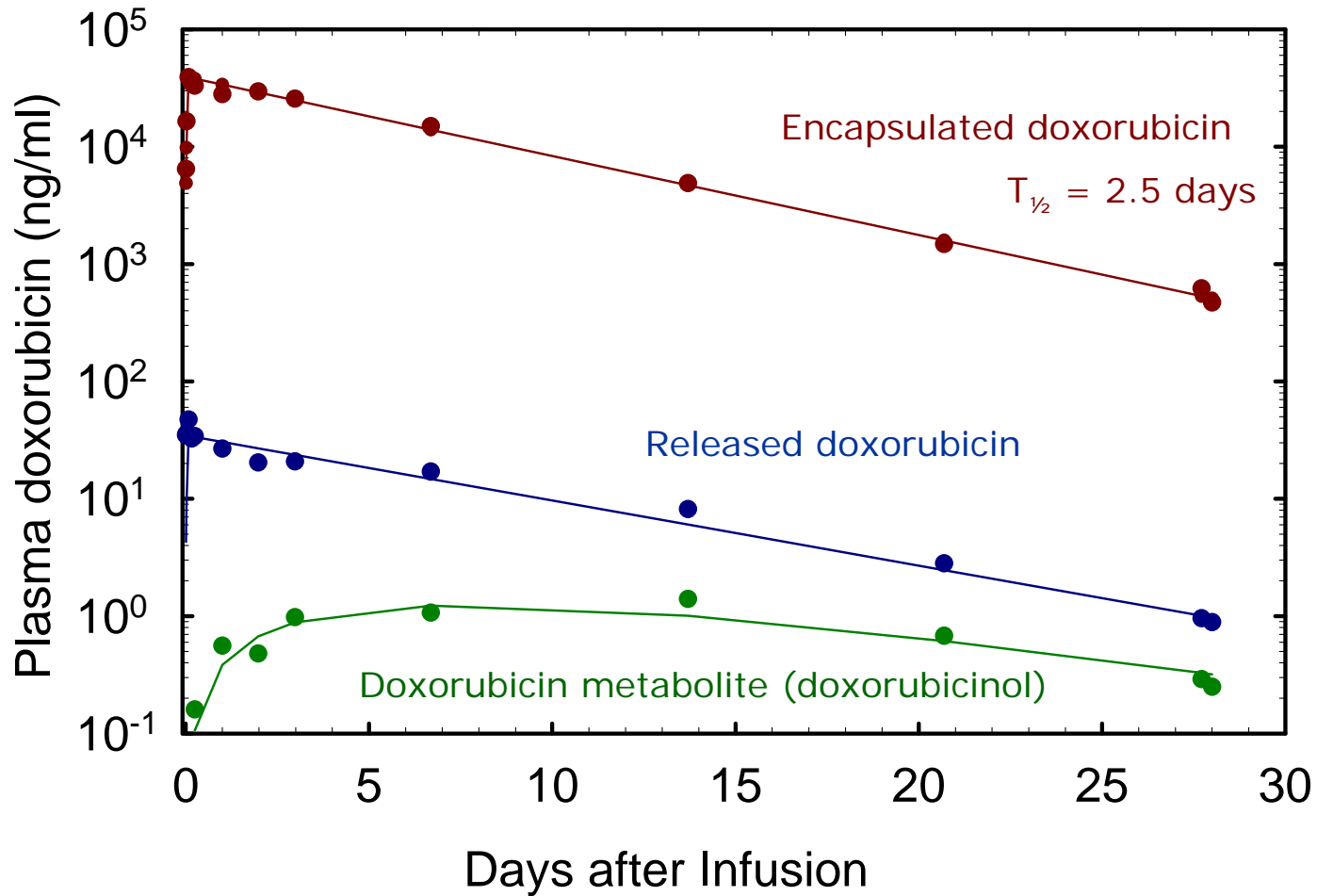
## Cryo-Electron Micrograph of DOXIL



liposome

doxorubicin sulfate  
precipitate

# STEALTH<sup>®</sup>: Enables DOXIL<sup>®</sup> to Circulate for Days

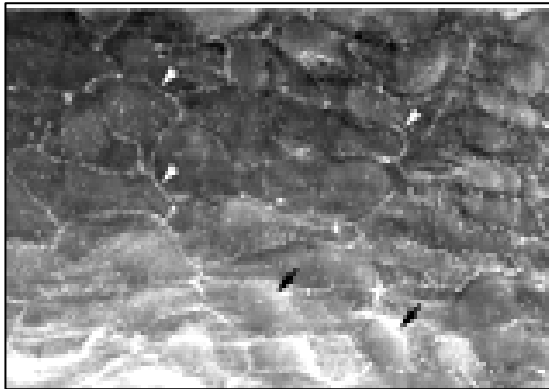


Single 60 mg/m<sup>2</sup> dose in cancer patients n = 9  
Vail et al. Seminars in Oncology 31(6) suppl. 13; 16-35 (2004)

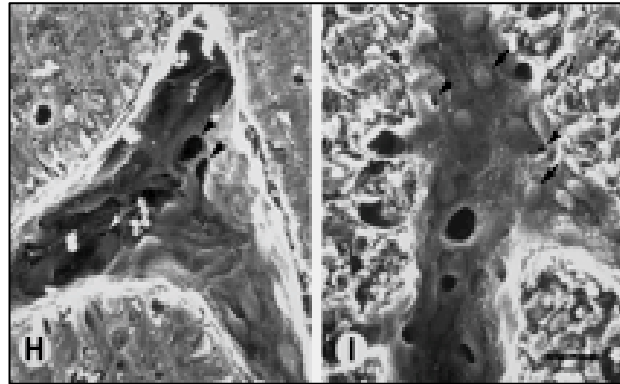


# Extravasation of STEALTH<sup>®</sup> Liposomes

## Normal Endothelium    Gaps in Tumor Endothelium

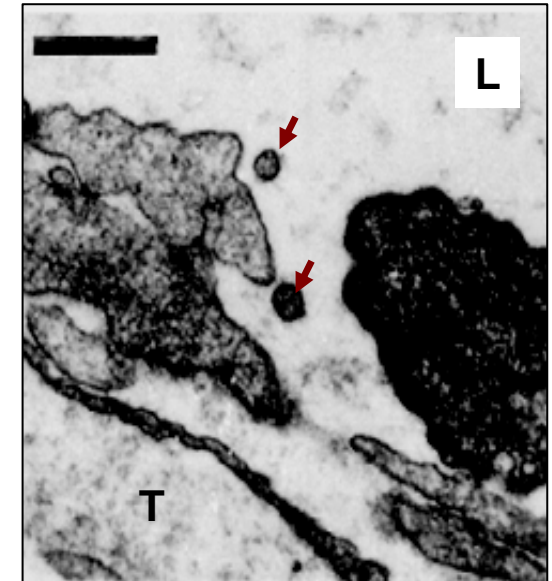


Flat edges, cobblestone pattern, tight junctions



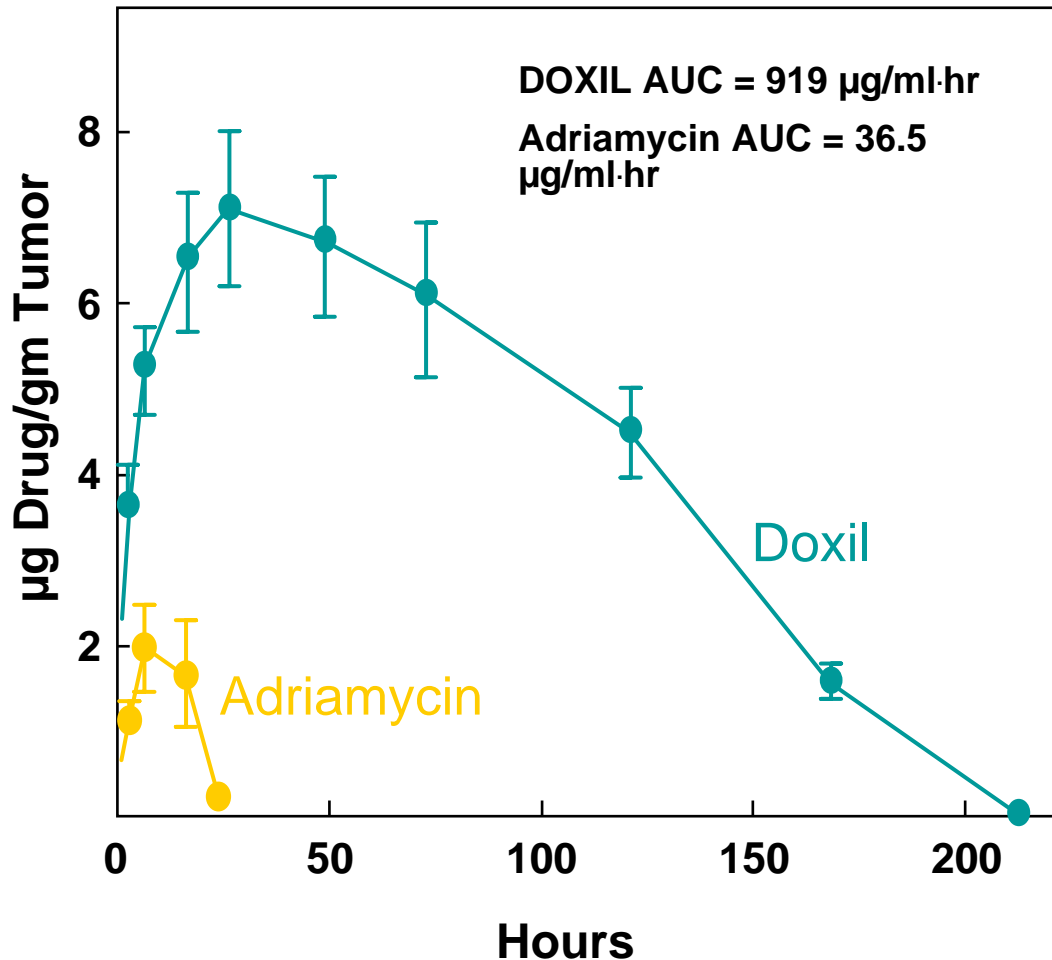
Tumor vessels often have defects, gaps as large as several hundred nanometers, with poorly formed or missing basement membrane

## Liposome Extravasation



STEALTH<sup>®</sup> (arrows) can pass from lumen (L) through endothelial gaps and basement membrane into tumor parenchyma (T)

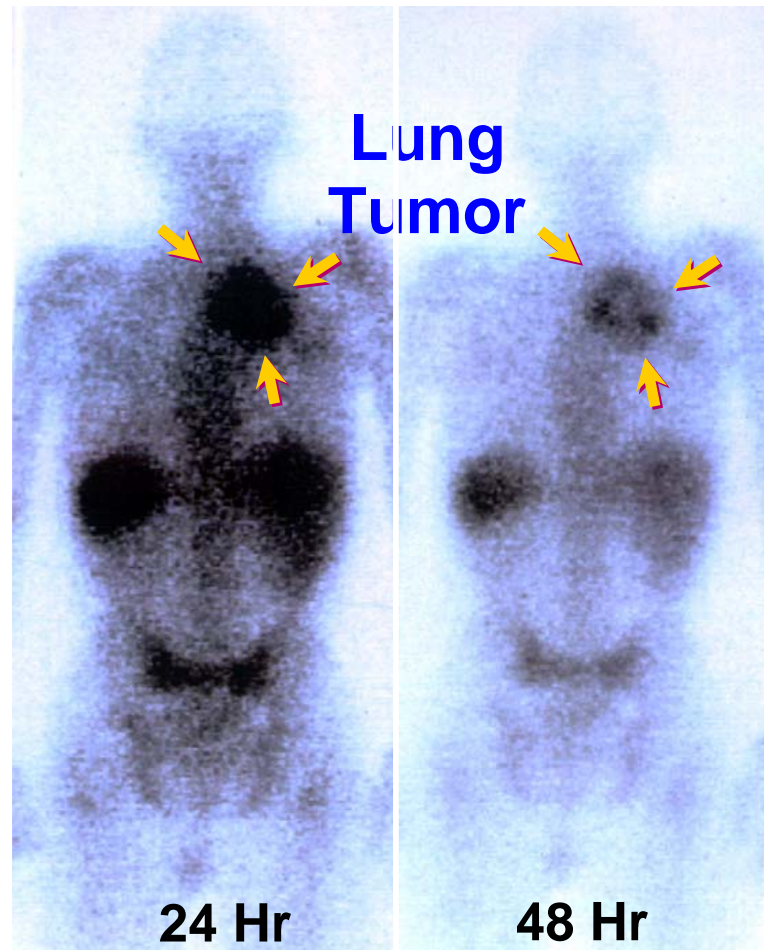
# High Drug Accumulation in Human Prostate Carcinoma Xenograft with DOXIL




Quantified by microfluorometry of DOXIL (adjusted for the autoquenching factor 2.8) and adriamycin in 0.3 g tumors. Each mouse received 9.0 mg/kg drug intravenously at 0 hour.

Vaage J, et al. *Cancer Research* 73(5);1994

# Localization of STEALTH<sup>®</sup> Liposomes in Lung Tumor




Gamma scintigraphy post injection of  $^{111}\text{In}$ -labeled STEALTH<sup>®</sup> liposomes



## DOXIL<sup>®</sup> - Approved Indications

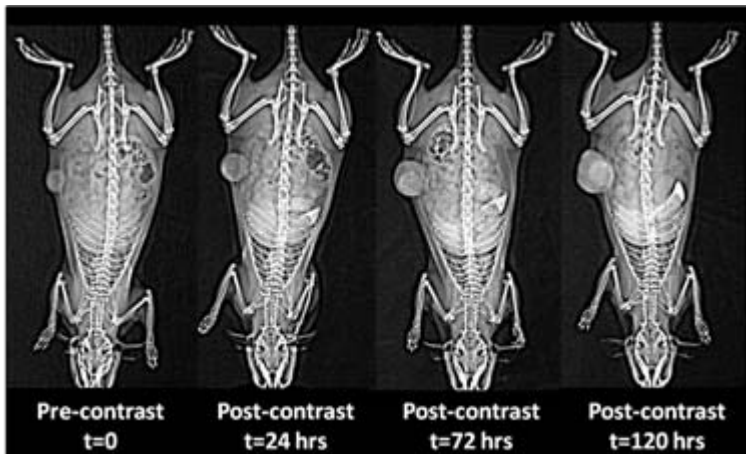
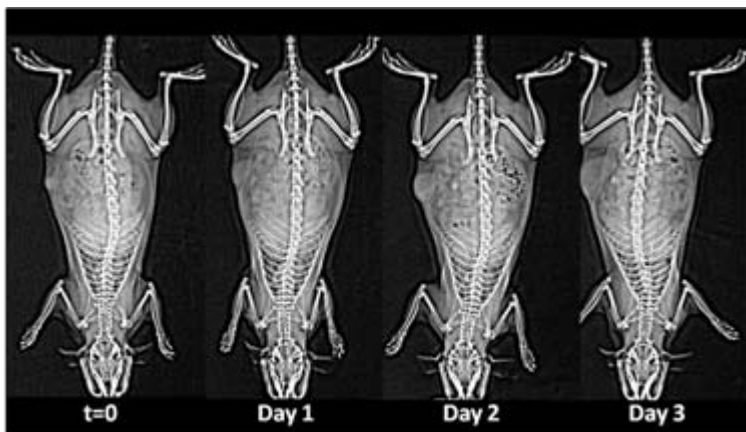
- Treatment of patients with ovarian cancer whose disease has progressed or recurred after platinum-based therapy
- Treatment of AIDs-related KS patients with disease progressed on or intolerant to prior combination chemotherapy
- In combination with bortezomib for treatment of patients with MM who have not previously received bortezomib and have received at least one prior therapy



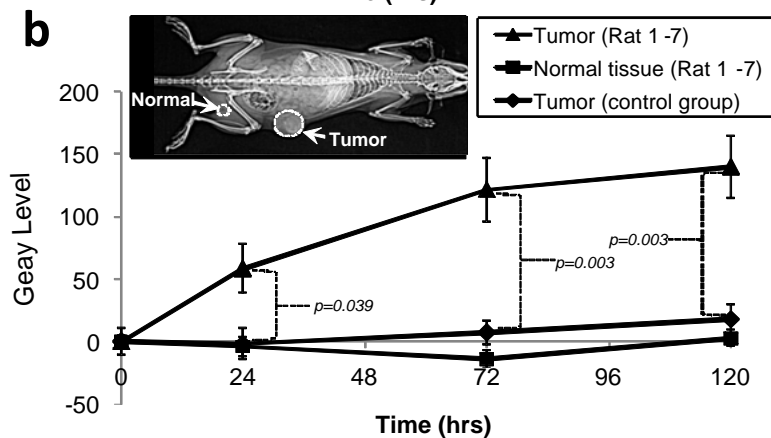
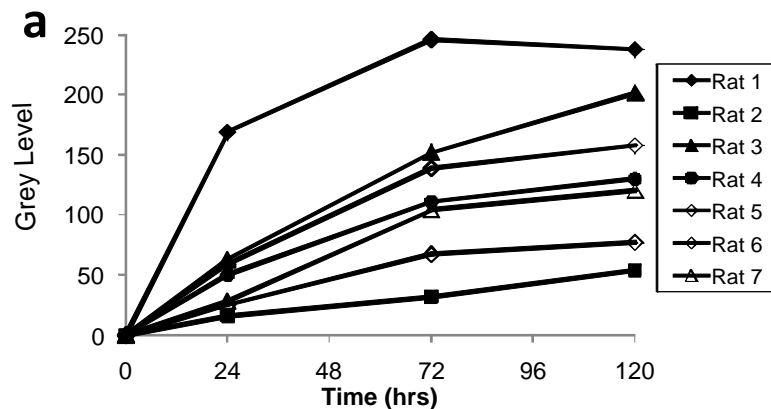
# Critical Path Challenges in DOXIL<sup>®</sup> Development

- Safety and Efficacy (“Medical Utility”) Perspectives:
- Liposome-associated and free drug components:
  - Greatly complicated plasma and tissue pharmacokinetic study design and data analyses
  - Total drug level provides insufficient information
- Particle delivery complicated pharmacological considerations:
  - Efficiency of particle/drug delivery to tissues
  - Drug release rates in tissues
  - Intrinsic drug activity
- Lack of knowledge in species-to-species and patient-to-patient variations in particle clearance and extravasation efficiency:
  - Uncertainties in translating pre-clinical data to clinical setting
  - Difficulty in predicting patient-to-patient variations

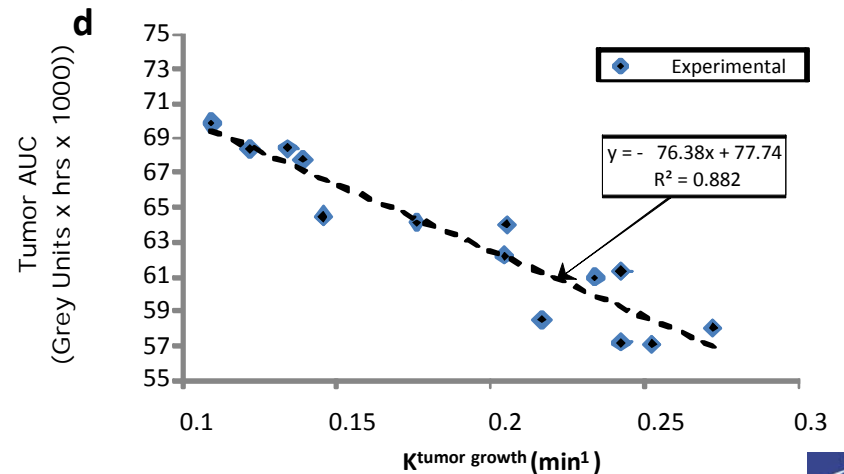
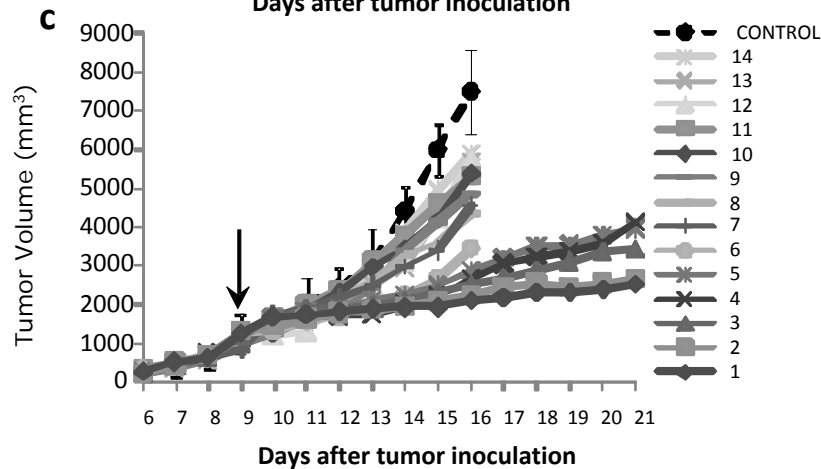
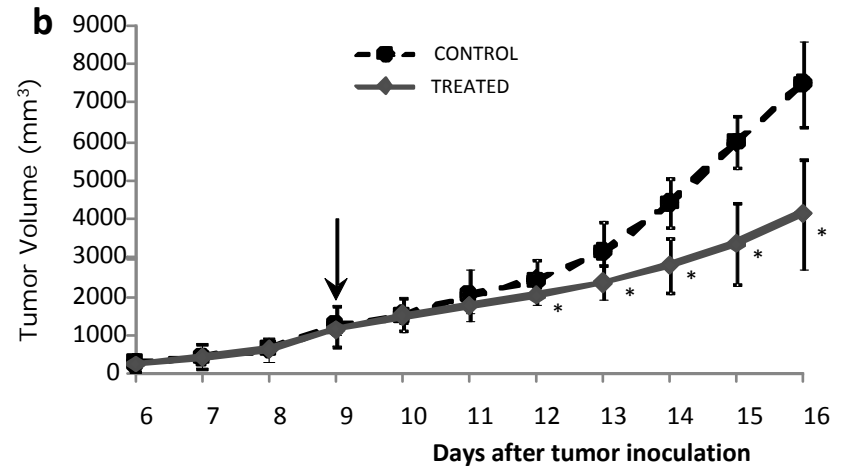
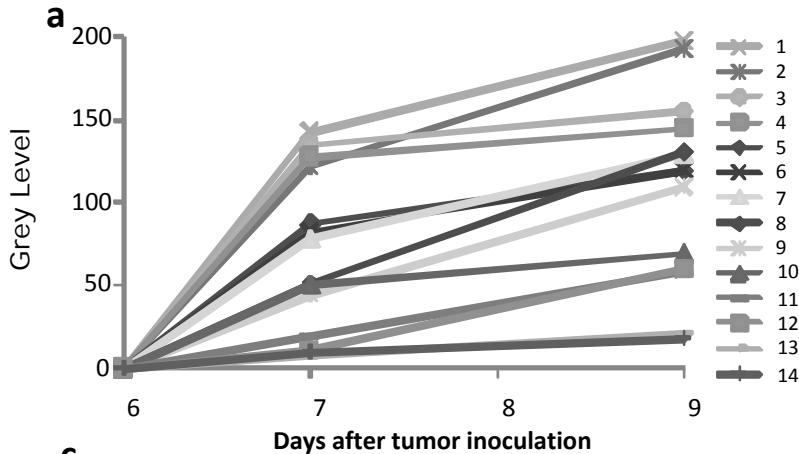
# Differential Tumor Uptake of STEALTH<sup>®</sup> Liposomes in Rats



CT Imaging of STEALTH<sup>®</sup> liposomes containing an iodine containing contrast agent



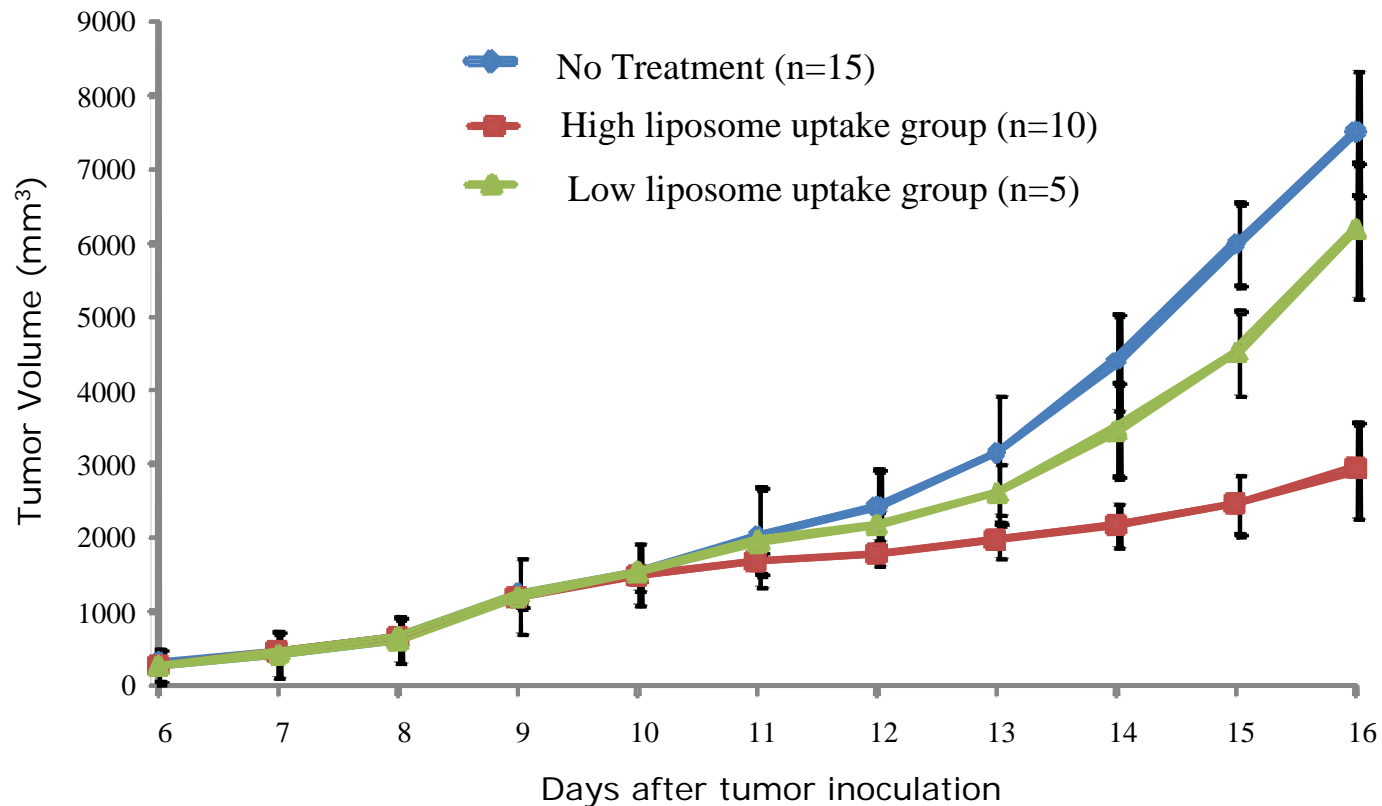
# Tumor Localization and anti-Tumor Effect of Doxorubicin STEALTH<sup>®</sup> Liposomes



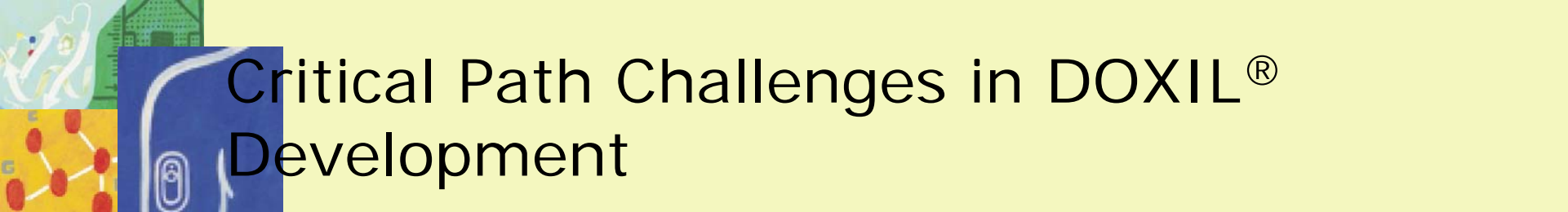
Personal Communication, Dr. Ananth Annapragada, U of Texas, Houston



# Tumor Localization and anti-Tumor Effect of Doxorubicin STEALTH<sup>®</sup> Liposomes




Personal Communication, Dr. Ananth Annapragada, U of Texas, Houston



# Critical Path Challenges in DOXIL<sup>®</sup> Development


- Manufacturing (“Industrialization Process”)
- Mechanism of delivery renders *in-vitro* cell culture systems unsuitable for product characterization and development studies
- Great number of physico-chemical parameters that could potentially influence product performance:
  - Necessitated development of a large number of analytical methods to characterize and control product quality
  - Added challenges in process design and scale-up based on QbD (Quality by Design) principles
- Complex mechanism of action made IVIVC development difficult
- Potential interactions (physical and chemical) with filtration membranes added challenges in sterile filtration process development



# Physico-Chemical Parameters Potentially Relevant to Product Performance


- Potency
- Related substances
- % drug encapsulation
- Particle size
- Lipid composition
- Drug to lipid ratio
- Internal ammonium sulfate concentration
- Number of lamellae
- Internal drug crystal structure
- pH

Useful to develop analytical methodologies capable of measuring “distribution” (as oppose to the mean value)




# Potential Critical Path Research Opportunity 1

- Clearance mechanism of nanoparticles in human
  - Pre-clinical studies have related particle clearance efficiency with MPS (mononuclear phagocyte system) status
  - Can MPS status be used as a biomarker to identify patients that will have high exposure after nanoparticle administration?
    - Toxicity implications
    - Efficacy implications
    - Facilitate patient selection in enrichment clinical studies
- Approach:
  - Incorporate MPS status assessment procedures in clinical PK studies of systemic nanoparticle products during early development
  - Look for relationship between MPS status, PK and toxicity manifestation



# Potential Critical Path Research Opportunity 2

- Identify patients with tumors that have good EPR property
  - Enhanced particle-drug accumulation
  - Efficacy implication
  - Facilitate patient selection in enrichment clinical studies
- Approach:
  - Employ imaging techniques to identify tumors/patients with high particle accumulation efficiency
    - Examples of reagents: radio-labeled nanoparticles, nanoparticles containing a CT contrasting agent
  - Bioinformatics
    - Database on highly vascularized tumor types (histology, disease stage)?
  - Clinical responses or biomarker changes in highly vascularized versus poorly vascularized groups



# Other Potential Critical Path Research Opportunities

- Non-invasive methods to measure total, particle-associated and free drug levels in plasma and tissues
- Development of valid IVIVC methods for efficient evaluation of formulation and manufacturing process changes
- Development of bioequivalence methods for generic and reference products