The expanding role of liposome-based cancer nanomedicine : "Doxil" and beyond

Alberto A. Gabizon, M.D., Ph.D.

Center of Nano-oncology / Shaare Zedek MC.

Hebrew University-Faculty of Medicine,

Jerusalem, ISRAEL

Séance: Les Nanomédicaments: d'où vient-on et où allons nous?

Paris, 2019

PARIS 2010

Disclosures:

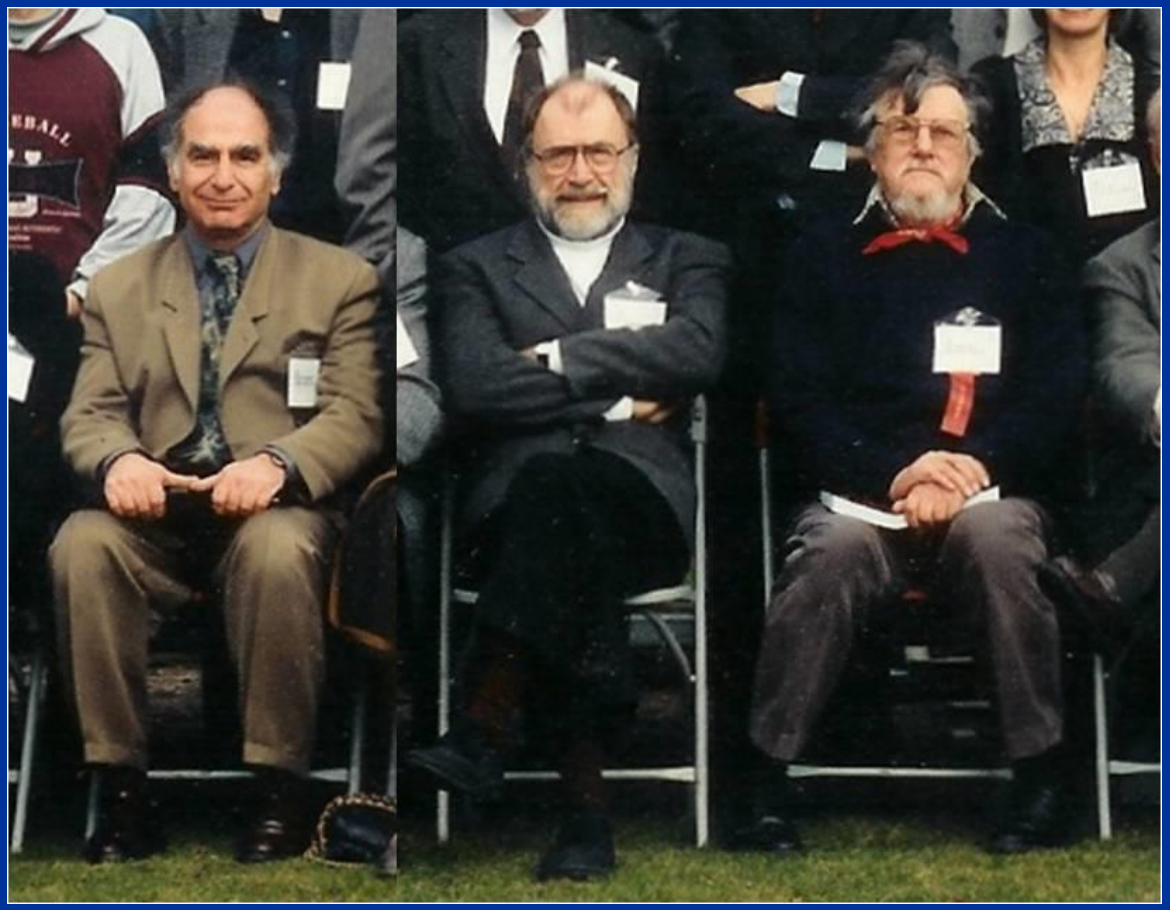
- Founder & Director of Lipomedix Pharm.
- Grant support form MERCK Co.
- Sci.Adv.Board member of Cristal Therap.

- **1964: Discovery of liposomes – Alec Bangham (Cambridge, UK)**

- **1971: First *in vivo* applications of liposomes for drug delivery**

- **1995: US-FDA approval of liposomal doxorubicin for cancer therapy**

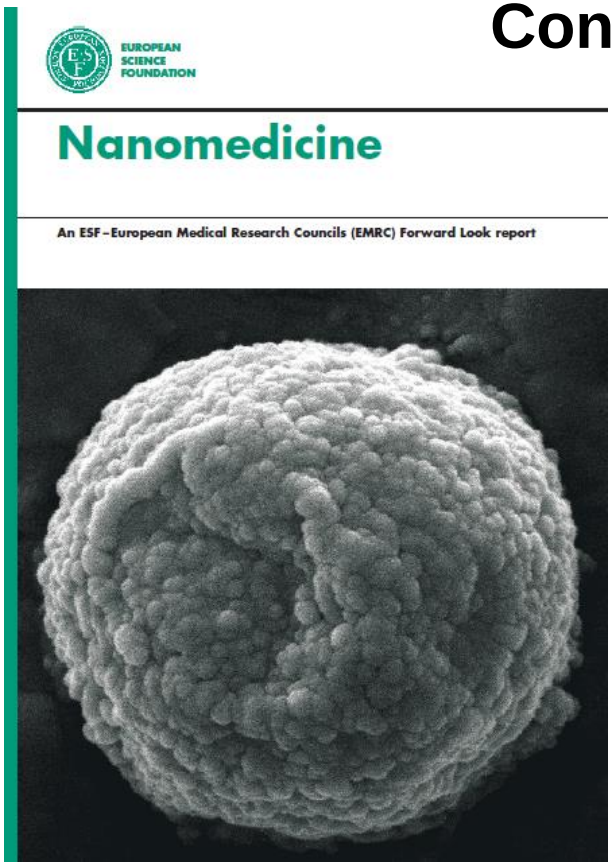
G. Gregoriadis, D. Papahadjopoulos, A. Bangham (2001)



Liposomal Anti-Cancer Agents Clinically Tested

Pegylated	Non-Pegylated	Regional therapy	Non-cytotoxic
DOXIL/Caelyx, Lipodox (PLD)	**Myocet (NPLD)**	**DepoCyt (intrathecal)**	**Lip. MTP-PE** (Mepact)
Nano- Irinotecan (Onivyde)	**DaunoXome**	Lip. NDDP (intrapleural)	Lip. ATRA
PROMITIL (MMC Prodrug)	Lip. Annamycin	Lip.Camptothecin (aerosol)	**BLP25 Lip. Vaccine**
Lip. Eribulin	**Marqibo (Lip. Vincristine)**	Lip. IL2 (aerosol)	Lip.Nucleotides
Lipoplatin SPI-077	Lurtotecan (OSI-211) TS inhib. (OSI-7904L)	Lip. E1A (intra-tumoral)	
S-CKD602	Lip. Paclitaxel		
FF- 10832 (Gemcitabine)	Dual Drug: **Lip.AraC-Dauno (Vyxeos)**		
Targeted: **αHer2-Doxil** MCC-465			

Consolidating Nanomedicine



Alliance for Nanotechnology in Cancer / National Cancer Institute, USA

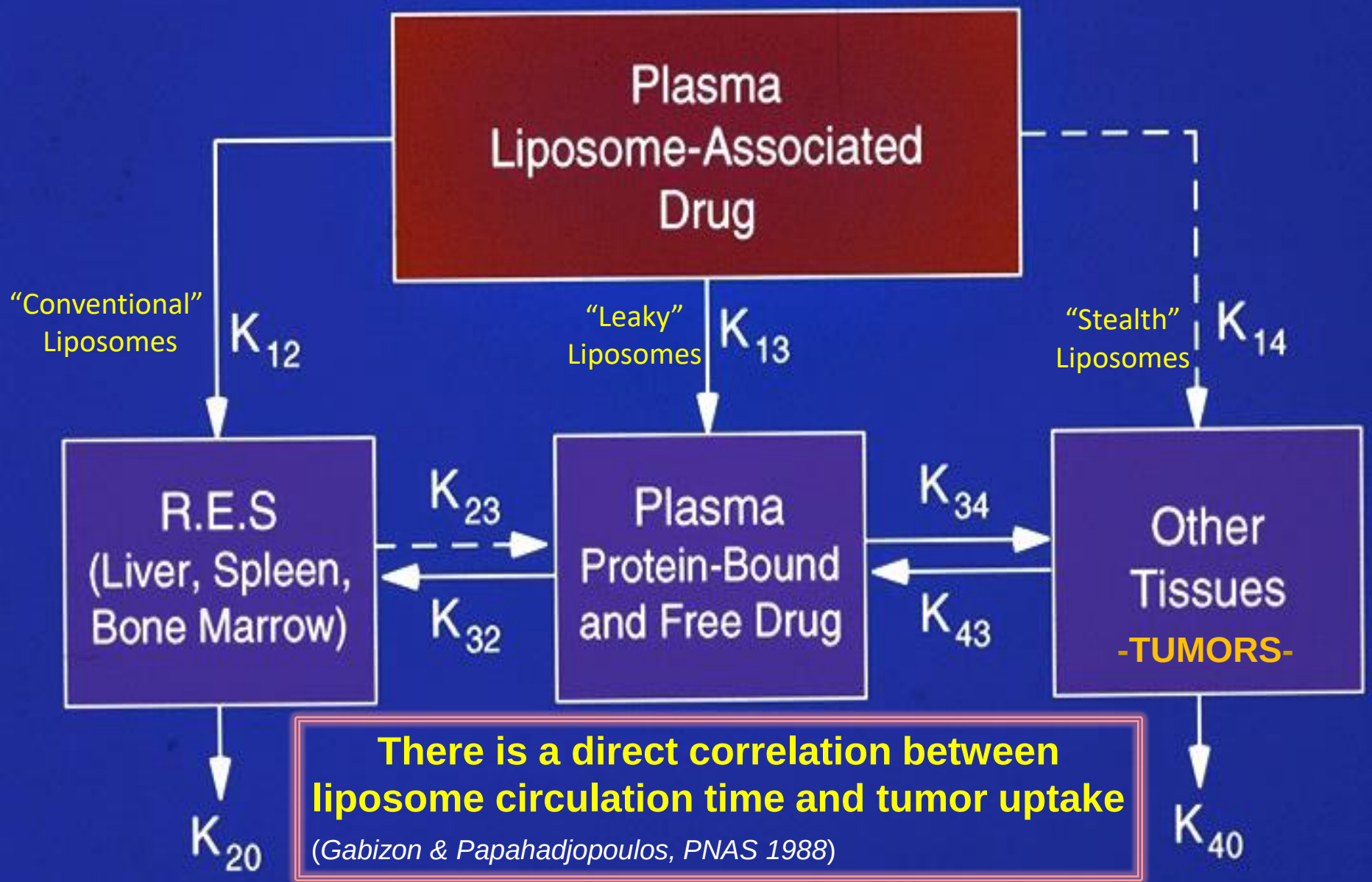
NCL: Nanotech Character. Lab

Challenges and Key Considerations of the EPR Effect for Nanomedicine in Oncology
Prabhakar U, et al., Cancer Research, 2013

Cancer nanomedicines - crossing the translational gap
Gabizon A, et al., **Lancet, 2014**

Integrating Nanotechnology into Cancer Care
Grodzinski P, et al., **ACS Nano, 2019**

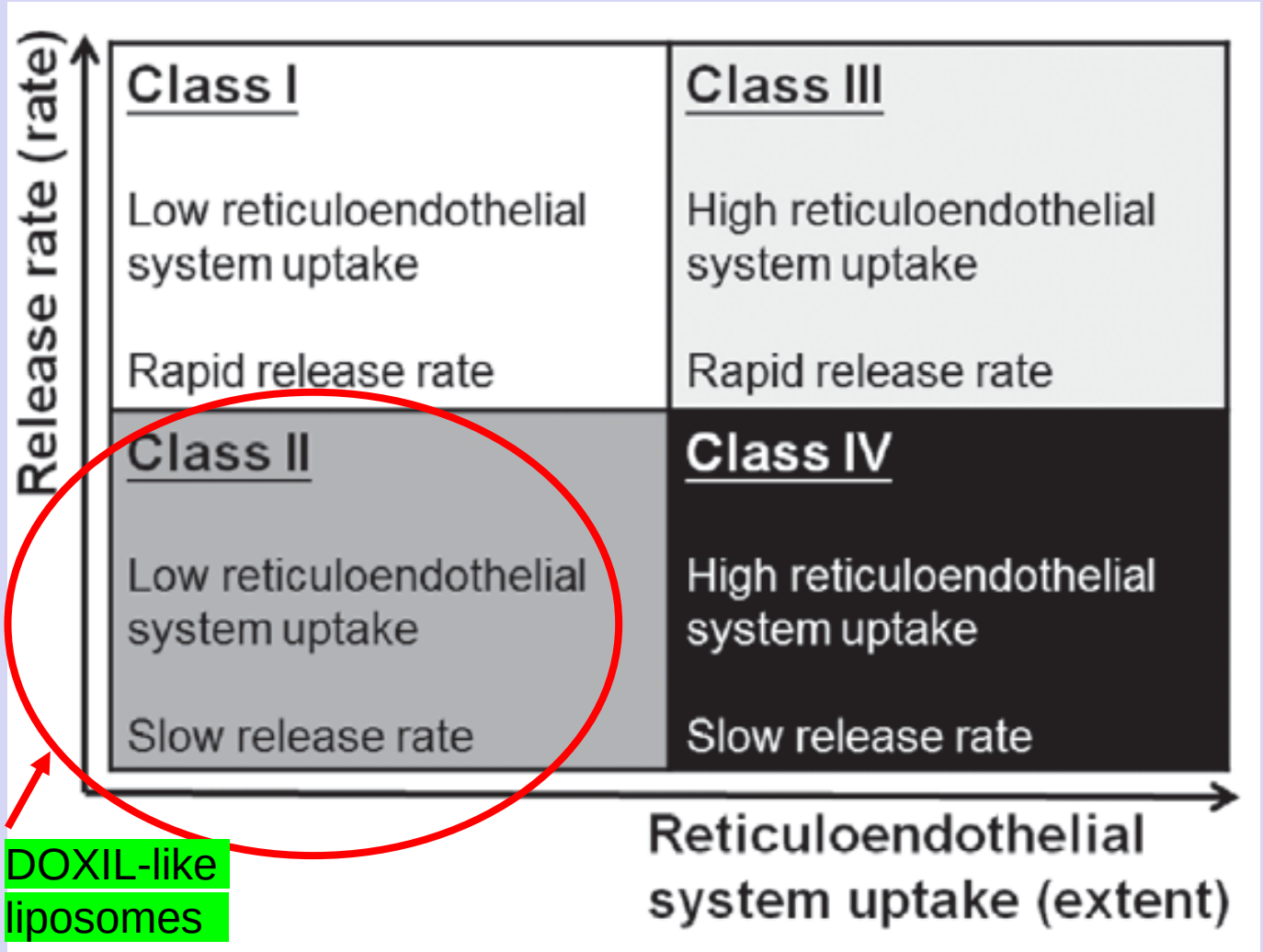
Pharmacokinetic Model



There is a direct correlation between liposome circulation time and tumor uptake

(*Gabizon & Papahadjopoulos, PNAS 1988*)

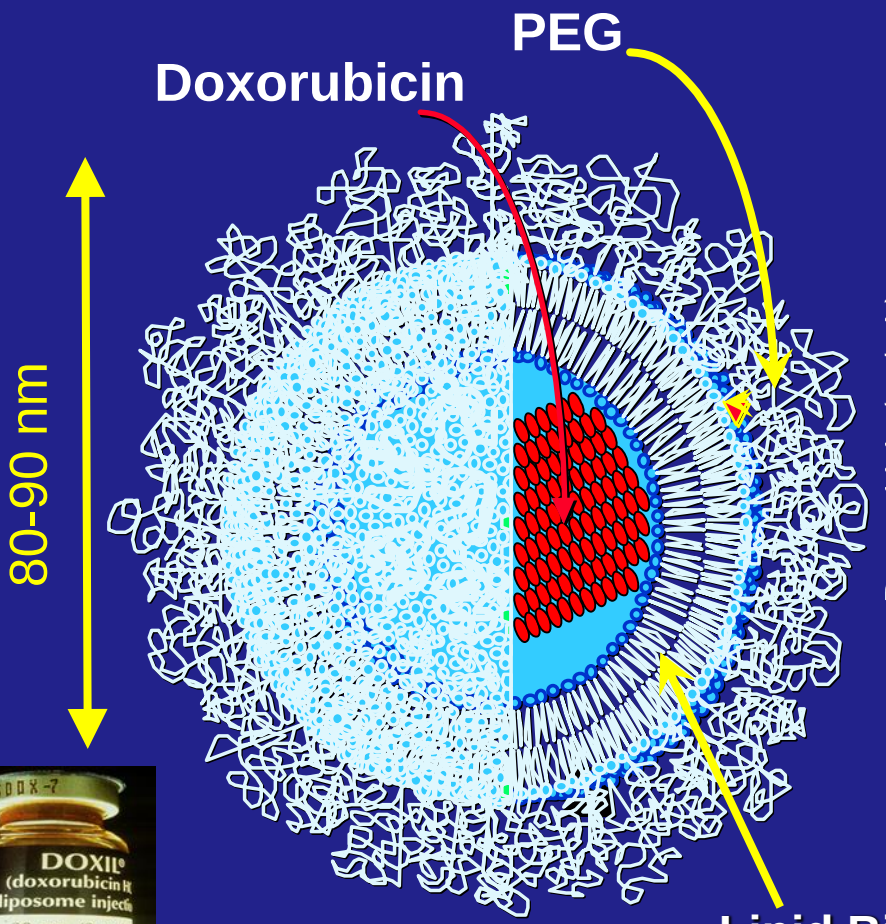
Nanoparticle/Liposome classification system for characterization of liposome drug products



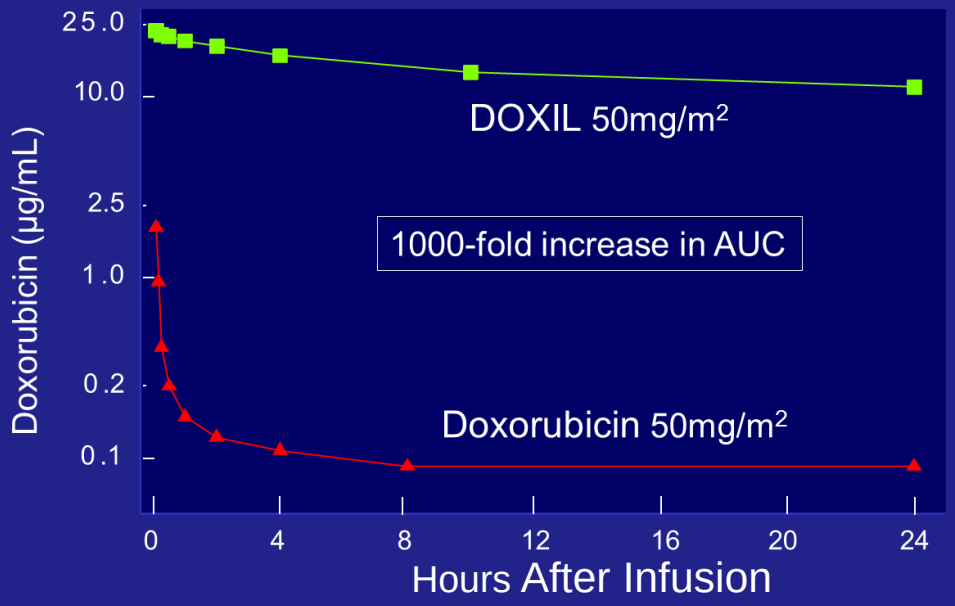
From: Hsu L and Huang J, *Int J Clin Pharm Therap*, 2014

Pegylated Liposomal Doxorubicin **(DOXIL, Caelyx)**

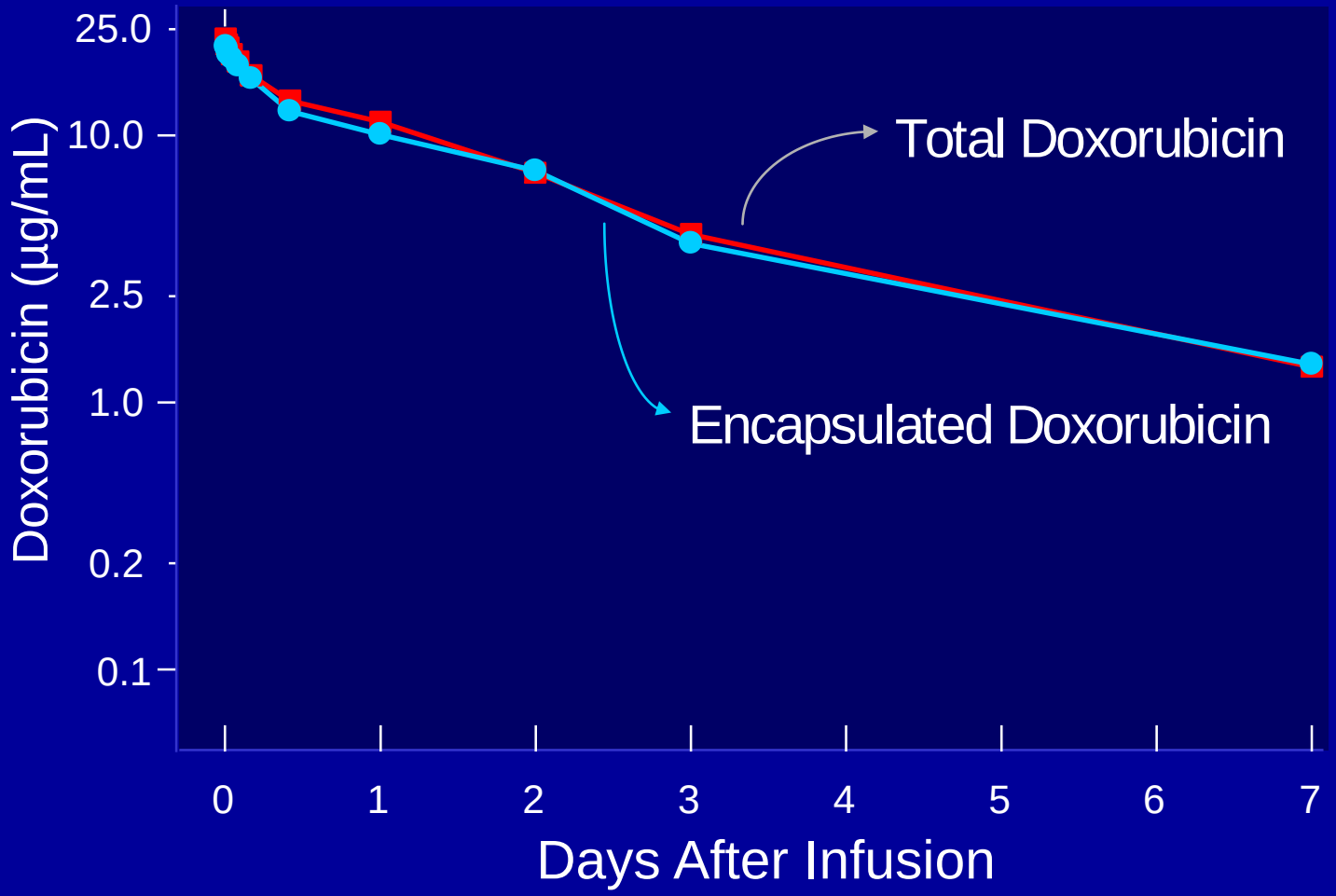
1. PEG Coating (Stealth Effect): ⇑ long circulation time
2. Ammonium sulfate drug loading gradient: ⇑ stability in circulation



Plasma Levels in Humans:
DOXIL vs. doxorubicin



DOXIL: doxorubicin remains in liposome-encapsulated form in circulation



Gabizon et al., Cancer Res. 1994

External medium

2x DOX-$NH_3^+Cl^-$

2x DOX-NH_2 + 2x H^+Cl^-

Neutral form of DOX crosses bilayer

Liposome aqueous phase

2x DOX-NH_2 + 2x H^+

2x DOX-NH_3^+ + $(NH_4)^2SO_4^{-2}$

$(DOX–NH_3^+)_2SO_4^{-2}$ + $(NH_4)^2$

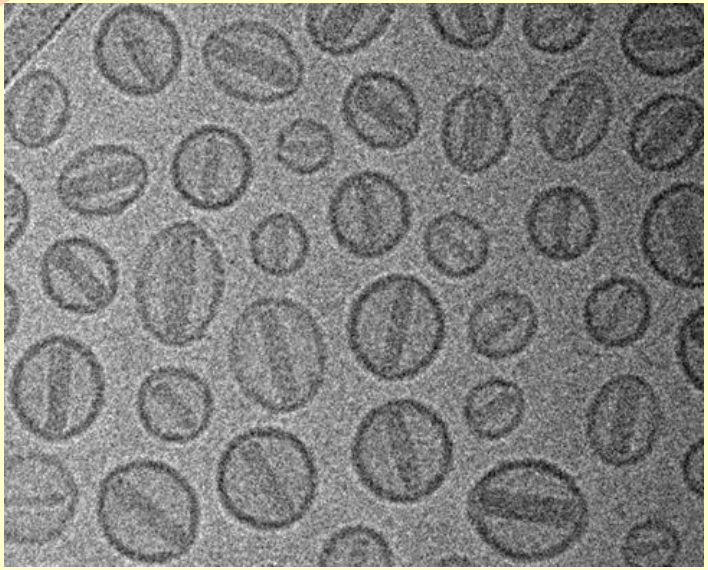
Rod-like precipitate

(reversible)

2x NH_3

2x NH_3 *Ammonia escapes*

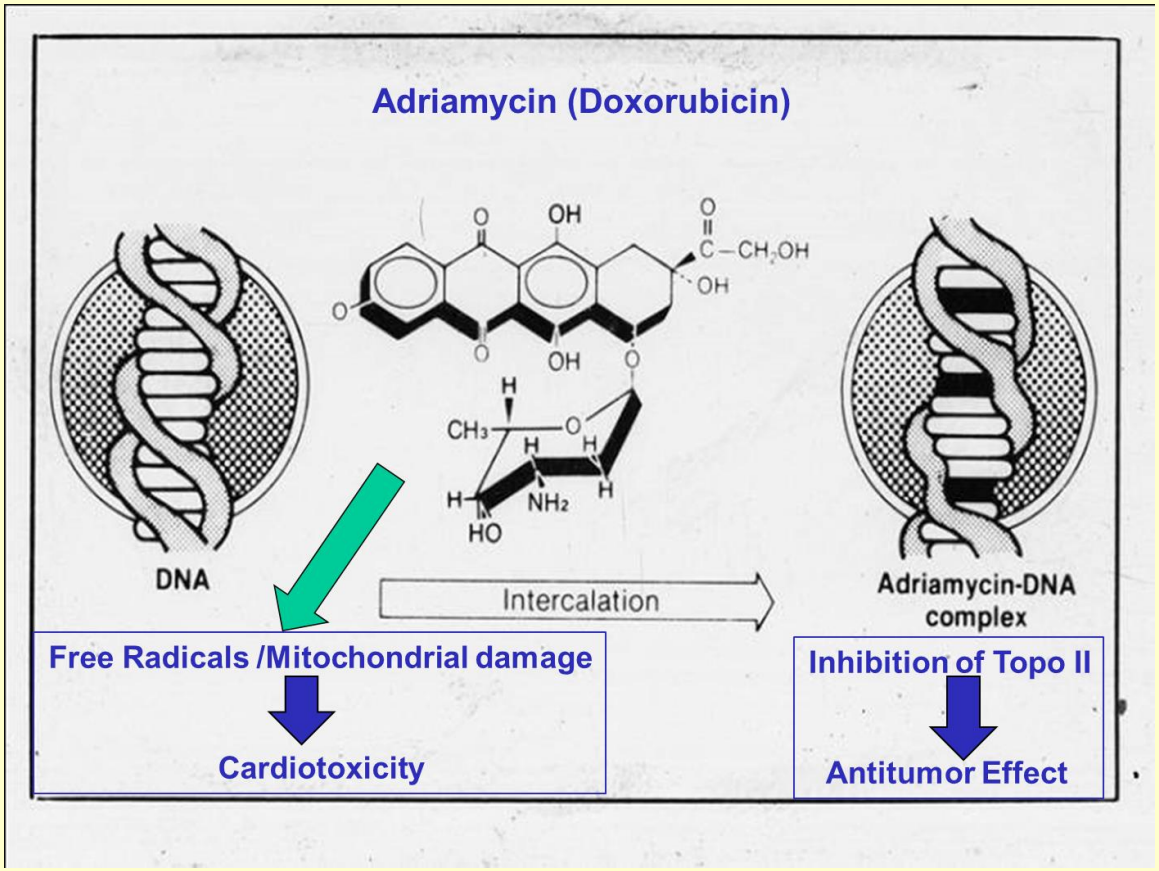
Cryo-TEM "coffee bean"



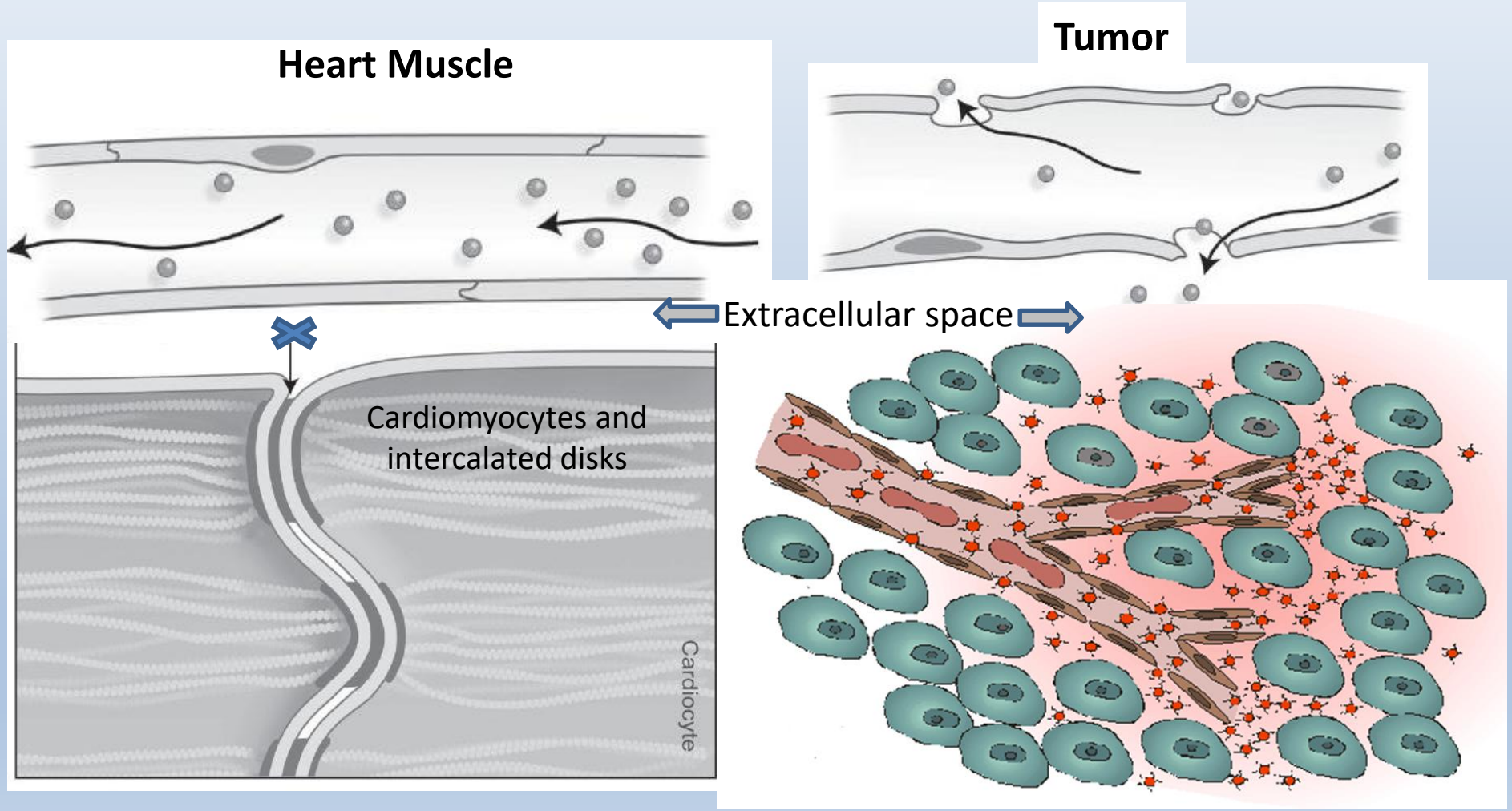
Remote Loading of Doxorubicin to form DOXIL Liposomes

Cardiac Toxicity of Doxorubicin (Adriamycin)

- Limits the cumulative dose leading to early treatment discontinuation
- Irreversible, crippling and life-threatening

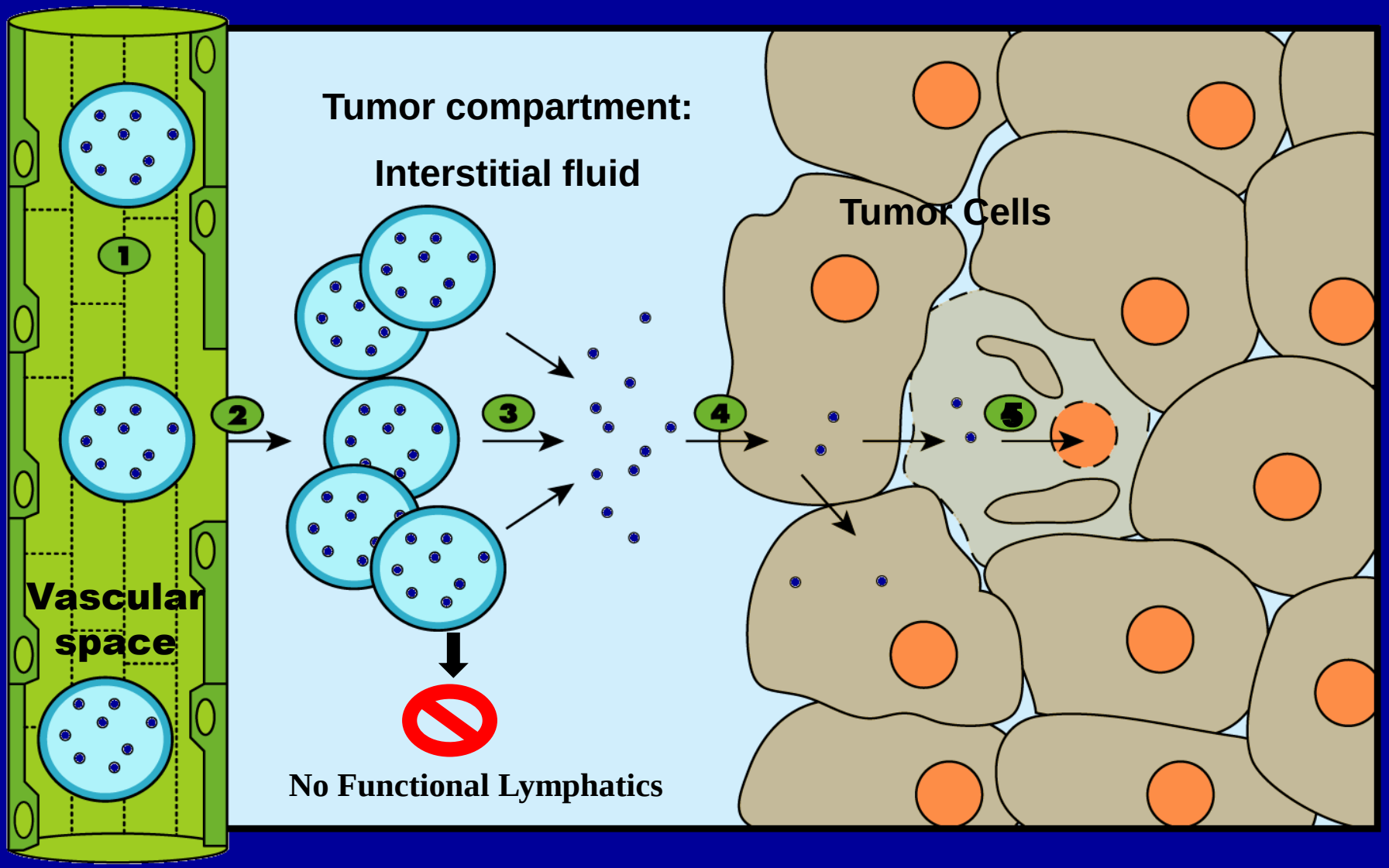


Rationale for DOXIL: Heart vs Tumor: Liposome Tissue Access is the Key!

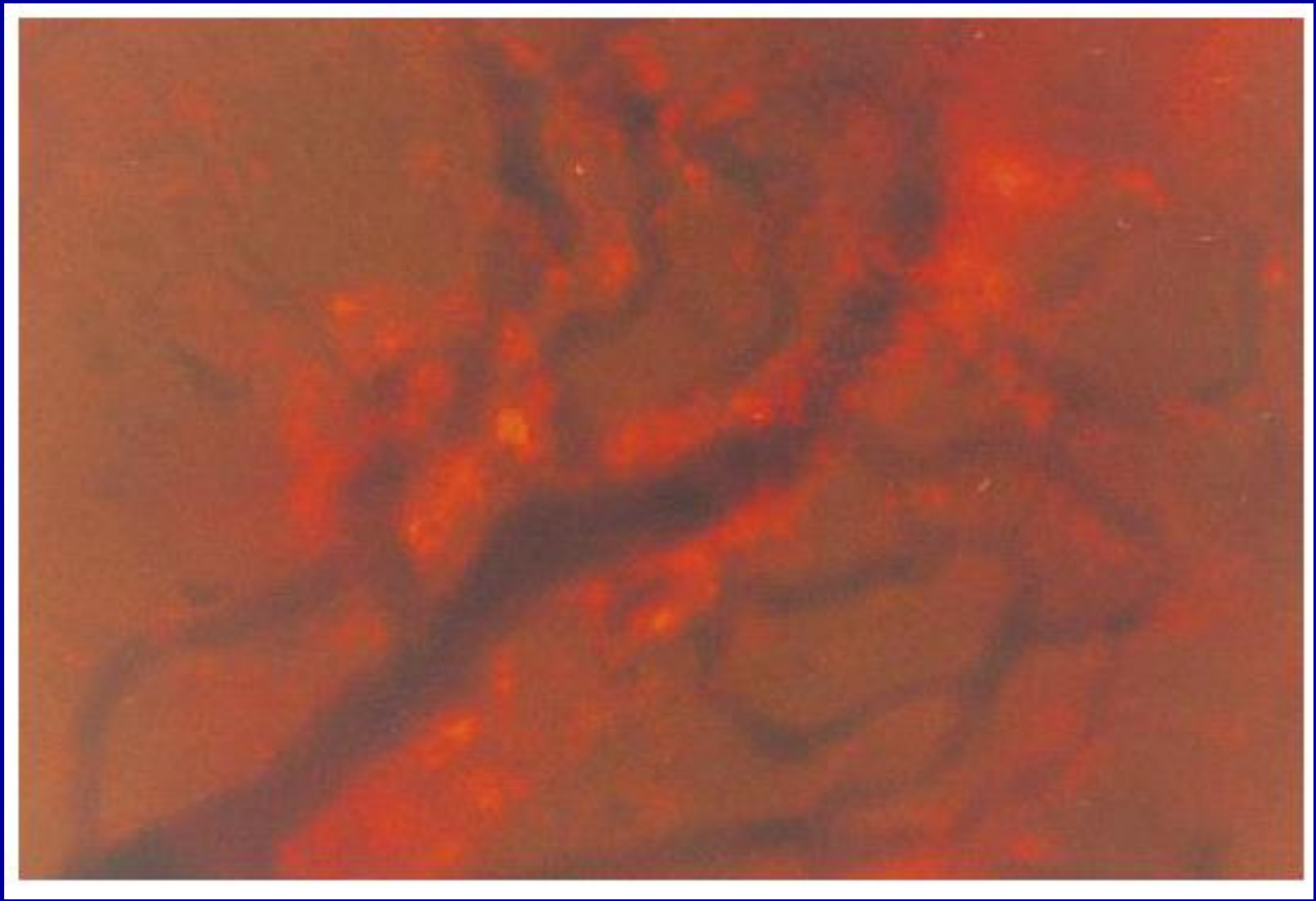


Enhanced Permeability and Retention (EPR)

Extravasation and Release of Liposomal Drug in Tumor Interstitial Fluid



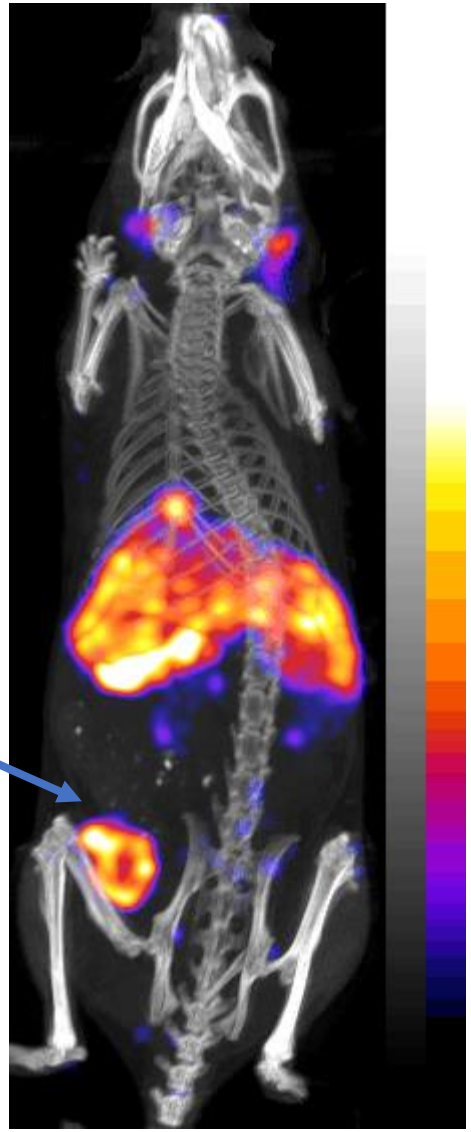
Blood Vessels: The Achyless Heel of Cancer



Extravasation of liposomes across tumor vessel:
Skin-fold window in vivo model (*R. Jain et al.*)

^{111}In-PLA

Mice bearing MDA-MB-231 triple-negative breast cancer tumors (10-90 mg) imaged with In^{111}–labeled Doxil-like Liposomes



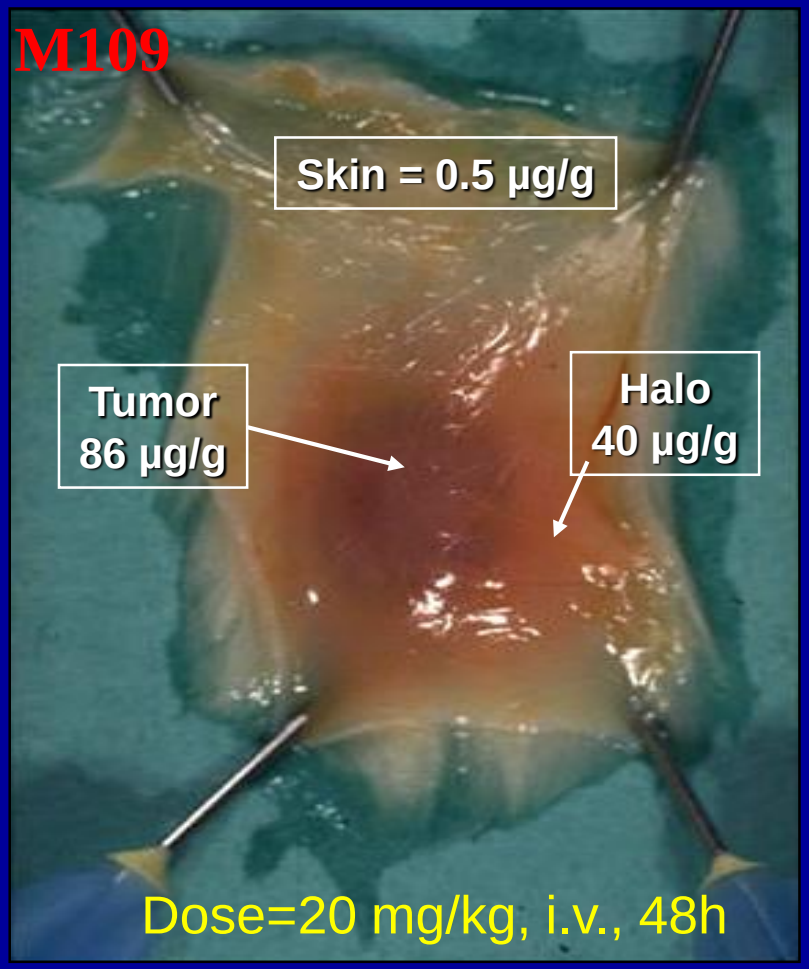
Median tumor uptake: ~25% ID/gram

Liposomes can be PET-imaged to select patients for therapy with good targeting to in tumors.

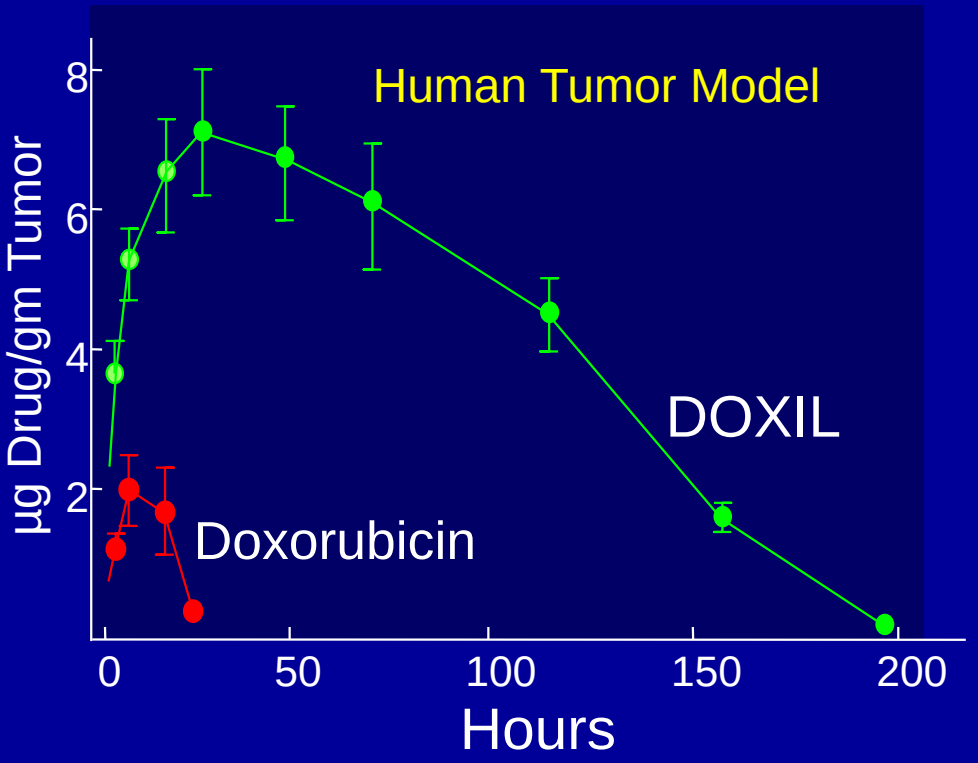
F. Man, A. Gabizon, R.TM de Rosales et al.

Marked Drug Deposition in Subcutaneous Tumor Implants After i.v. DOXIL

Dox concentration:
~22% ID/gram tumor

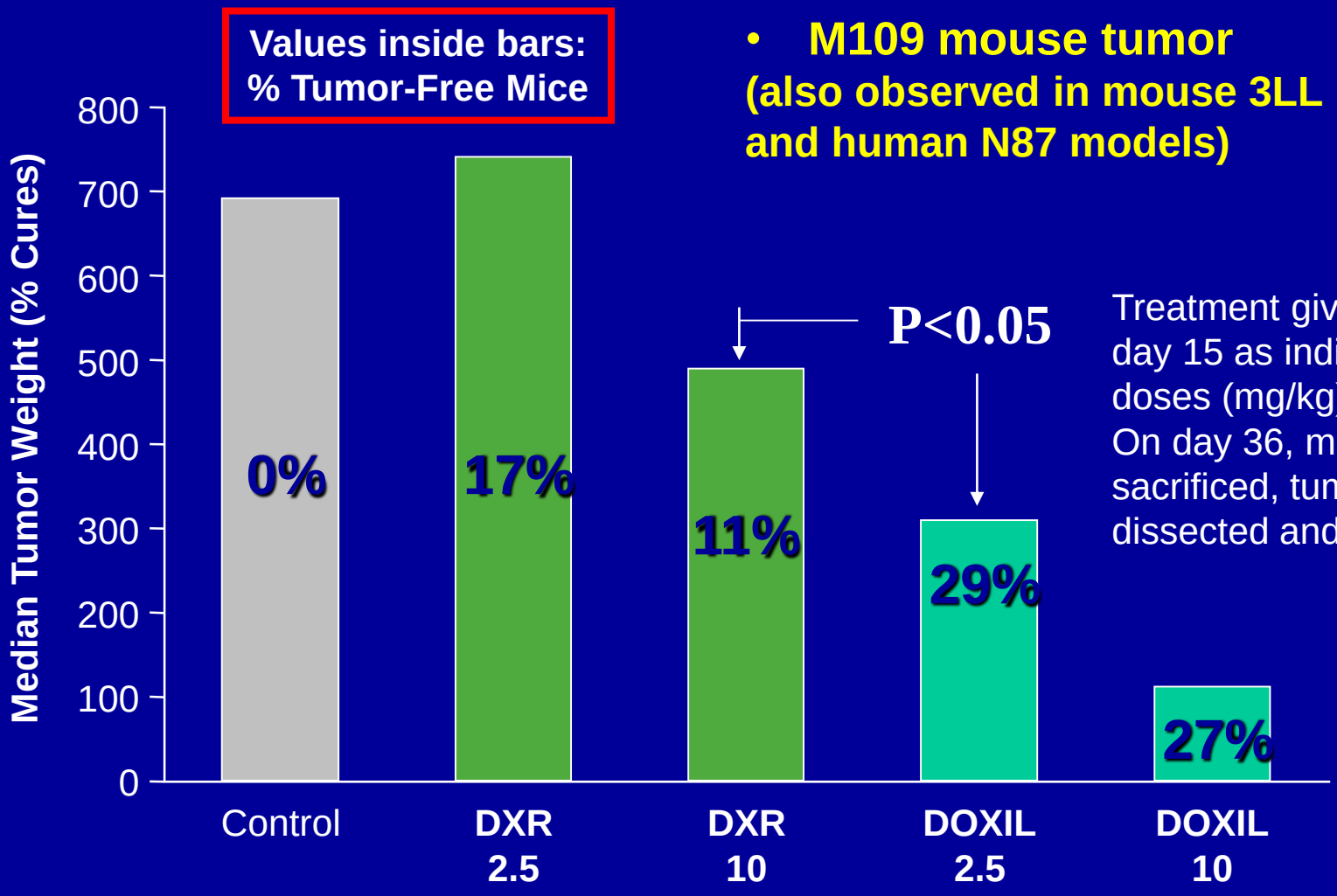


30- fold increase in AUC



Gabizon et al., Vaage et al.

Anti-Tumor Effect of DOXIL is >4-fold than doxorubicin (DXR)*



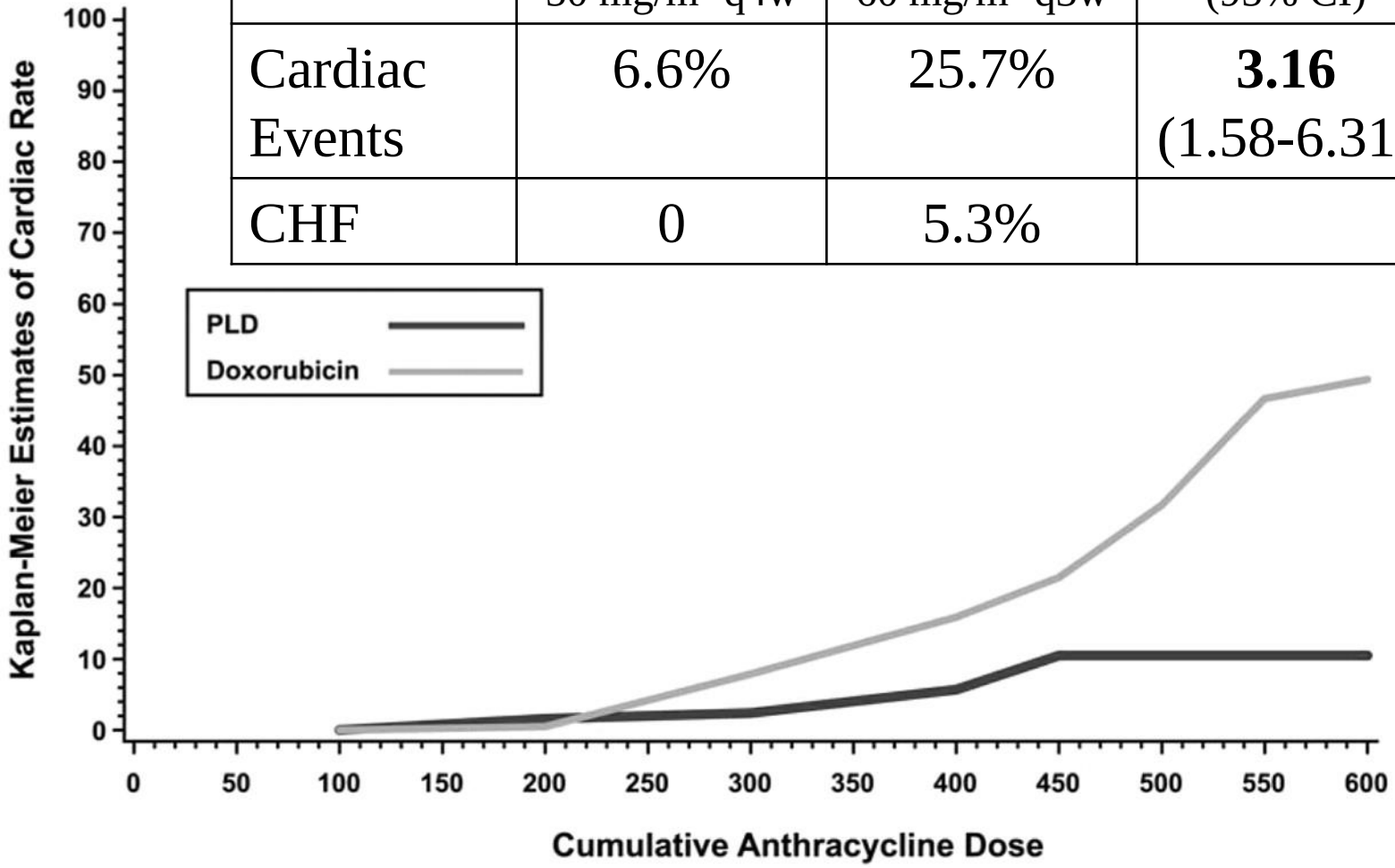
Gabizon et al, 2002

DOXIL Clinical Proofs of Added Value

- Cardiac function: Major reduction of cardiotoxicity as compared to free doxorubicin in all settings. (2000)
- AIDS-related Kaposi's Sarcoma: Superior efficacy over former conventional therapy (1995)
- Recurrent Ovarian Cancer: Superior efficacy and improved safety profile over comparator drug (topotecan) (1998)
- Metastatic Breast Cancer: Equivalent efficacy and reduced cardiotoxicity compared to free doxorubicin (2003)
- Multiple Myeloma: Equivalent efficacy and improved safety profile compared to free doxorubicin combo. Superior efficacy in combination with bortezomib over single agent bortezomib. (2007)

Reduced rate of cardiac events with DOXIL (vs doxorubicin)

	PLD 50 mg/m^2 q4w	Doxorubicin, 60 mg/m^2 q3w	Hazard Ratio (95% CI)
Cardiac Events	6.6%	25.7%	**3.16** (1.58-6.31)
CHF	0	5.3%	



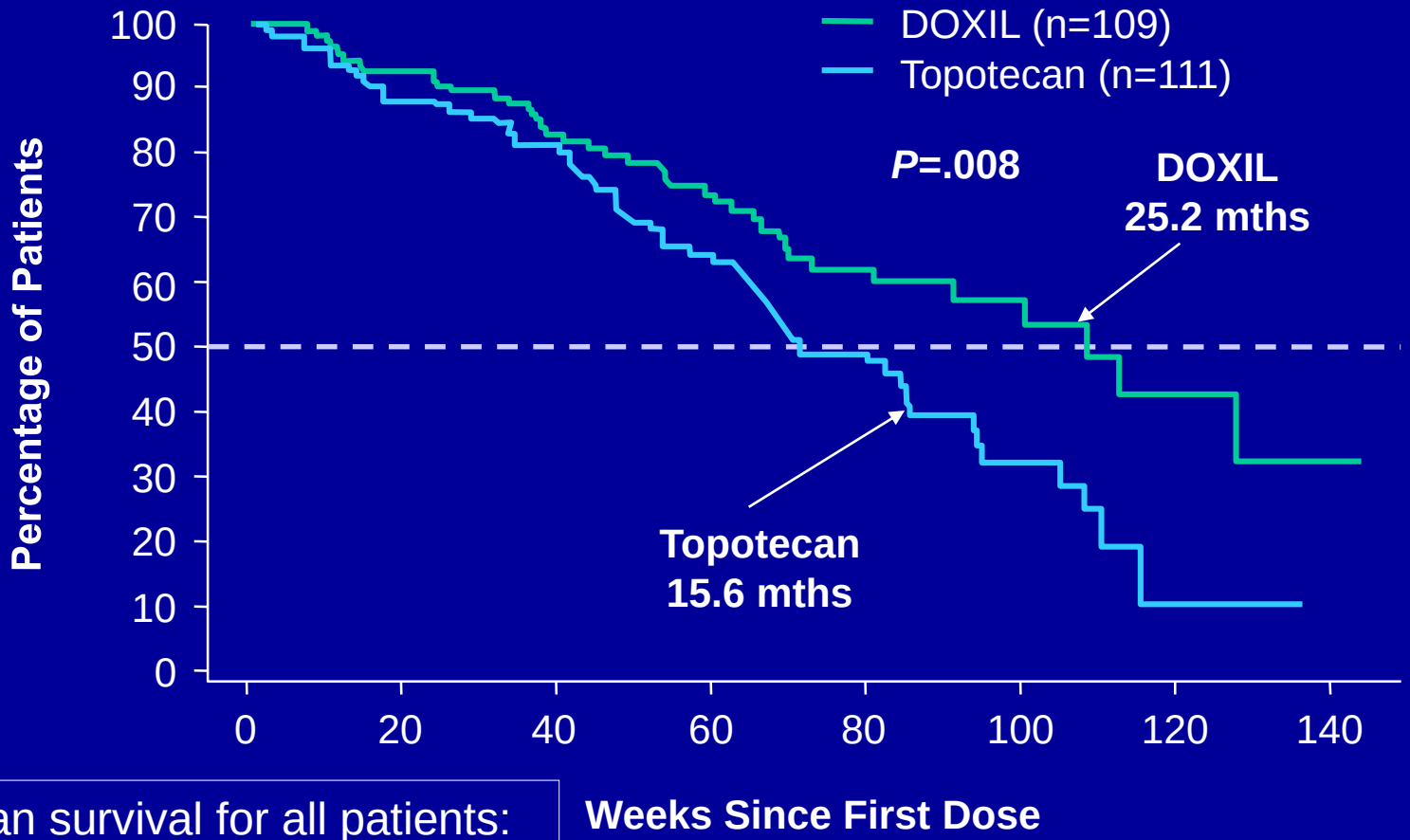
O'Brien M E R et al. Ann Oncol 2004;15:440-449

Annals of Oncology

DOXIL (PLD) vs Doxorubicin: Change of toxicity profile

Side Effect	Effect of liposome delivery
Vesicant effect	**Absent** (irritation only)
Nausea/Vomiting	**Reduced**
Myelosuppression	**Reduced** (no neutropenic fever)
Stomatitis/Mucositis	**Increased**
Hand-Foot (PPE)	**Increased**
Cardiotoxicity	**Reduced or Absent**
Alopecia	**Reduced or Absent**
Maximal Single Dose	**Slightly decreased** ($50mg/m^2$ q4w)
Maximal Cumulative Dose	**Greatly increased** (>$900mg/m^2$)

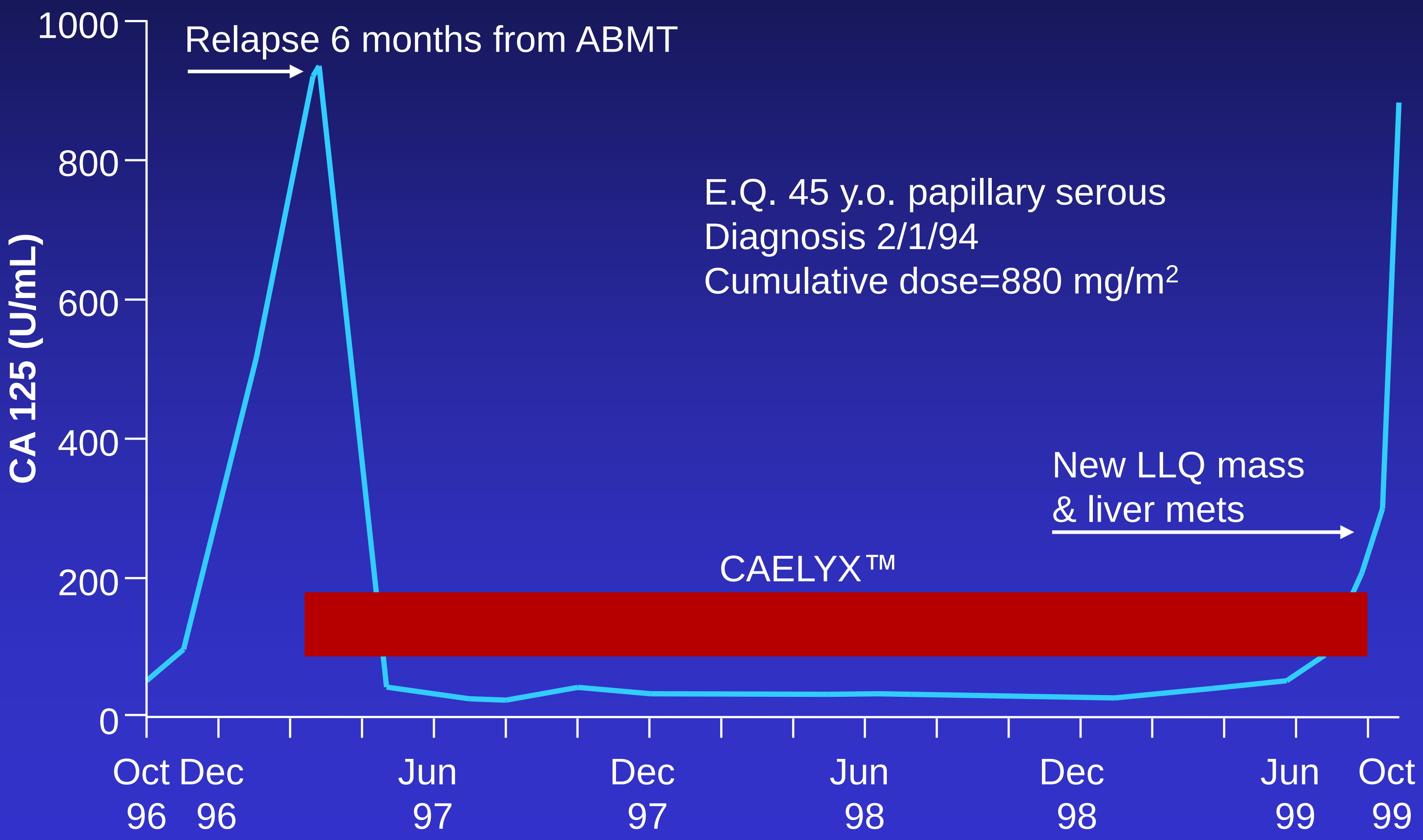
Doxil in Ovarian Ca: Major Improvement in Survival in “Pt-Sensitive” Patients*



* Median survival for all patients: DOXIL=15mth; Topotecan=13mth (p=0.025)

Gordon AN, et al. *J Clin Oncol.* 2001;19:3312-3322.

Example of Response and Prolonged Remission on CAELYX™ Maintenance



Long-term use of pegylated liposomal doxorubicin to a cumulative dose of 4600 mg/m^2 in recurrent ovarian cancer

Adam Pendlebury, Robert DeBernardo and Peter G. Rose

Pegylated liposomal doxorubicin (PLD) is used widely in gynecologic oncology and other oncology disciplines. Native doxorubicin use is associated with the potential for significant toxicity. Cardiac toxicity in particular limits lifetime dose. PLD has not been shown to be associated with clinical cardiac toxicity. We report on the long-term use of PLD in a patient with recurrent high-grade serous ovarian cancer to a lifetime dose of 4600 mg/m^2. This therapy was associated with long-term stable disease, good performance status, and minimal adverse effects.

Anti-Cancer Drugs 2017, 00:000–000

Keywords: cardiotoxicity, gynecologic malignancy, long-term chemotherapy, pegylated liposomal doxorubicin, safety

Cleveland Clinic Taussig Cancer Center, Cleveland, Ohio, USA

Correspondence to Adam Pendlebury, MBBS, BMedSci, Cleveland Clinic Taussig Cancer Center, 9500 Euclid Avenue, A81, Cleveland, OH 44195, USA
Tel: +1 216 444 1712; fax: +1 216 444 8551; e-mail: apendez@hotmail.com

Received 16 November 2016 Revised form accepted 2 May 2017

115 cycles of PLD (40mg/m^2) during 9 years with stable disease:

No Cardiac Toxicity!

This is >10 times more than the maximal recommended dose of free Doxorubicin

Caelyx in 1st Line Ovarian Cancer

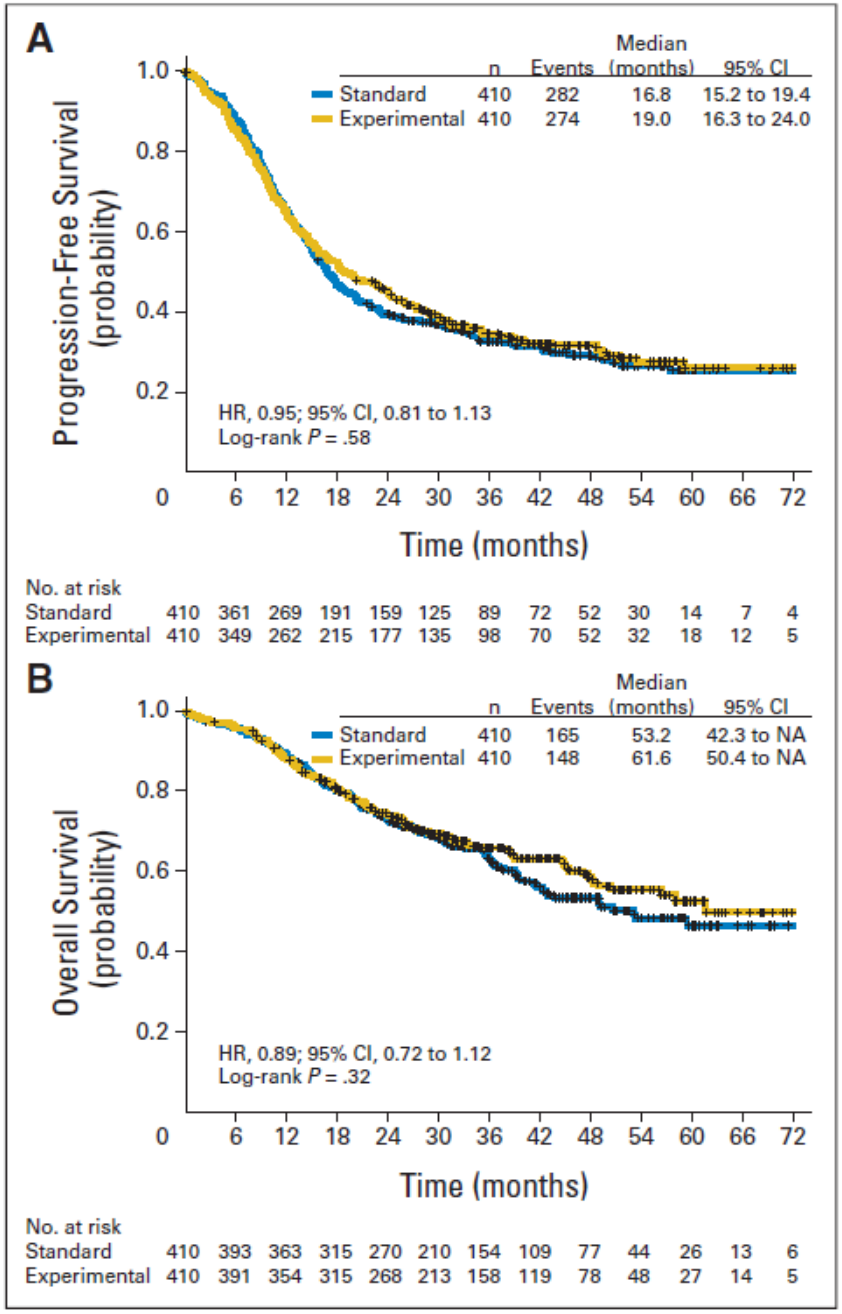
VOLUME 29 · NUMBER 27 · SEPTEMBER 20 2011

JOURNAL OF CLINICAL ONCOLOGY

Carboplatin Plus Paclitaxel Versus Carboplatin Plus Pegylated Liposomal Doxorubicin As First-Line Treatment for Patients With Ovarian Cancer: The MITO-2 Randomized Phase III Trial

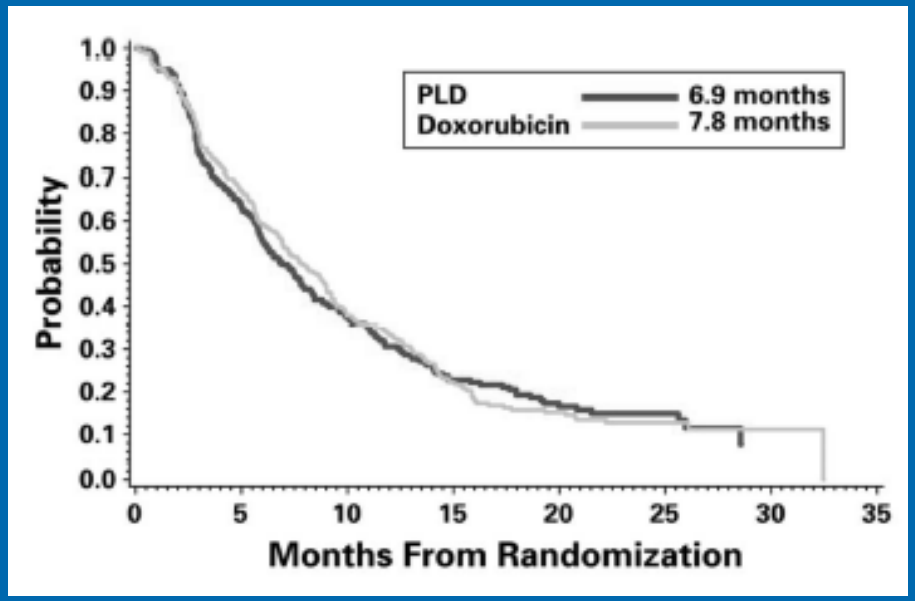
Sandro Pignata, Giovanni Scambia, Gabriella Ferrandina, Antonella Savarese, Roberto Sorio, Enrico Breda, Vittorio Gebbia, Pietro Musso, Luigi Frigerio, Pietro Del Medico, Alessandra Vernaglia Lombardi, Antonio Febbraro, Paolo Scollo, Antonella Ferro, Stefano Tamberi, Alba Brandes, Alberto Ravaioli, Maria Rosaria Valerio, Enrico Aitini, Donato Natale, Laura Scaltriti, Stefano Greggi, Carmela Pisano, Domenica Lorusso, Vanda Salutari, Francesco Legge, Massimo Di Maio, Alessandro Morabito, Ciro Gallo, and Francesco Perrone

No significant advantage of Doxil-based combo over Paclitaxel based combo!

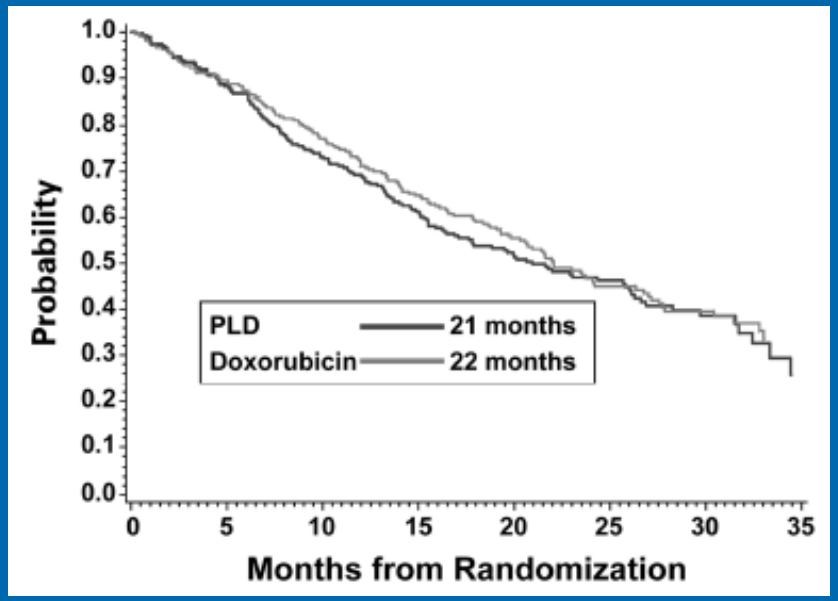


Doxil® (PLD) vs free doxorubicin (1st line) in Metastatic Breast Cancer

No sig efficacy advantage of Doxil over free doxorubicin!



Progression-free Survival



Overall Survival

Reduced cardiotoxicity and comparable efficacy in a phase III trial of pegylated liposomal doxorubicin HCl (CAELYX™/Doxil®) versus conventional doxorubicin for first-line treatment of metastatic breast cancer

O'Brien et al., Ann Oncol 2004

The DOXIL-Nanomedicine Efficacy Gap:

Preclinical Results → 

Clinical results → 

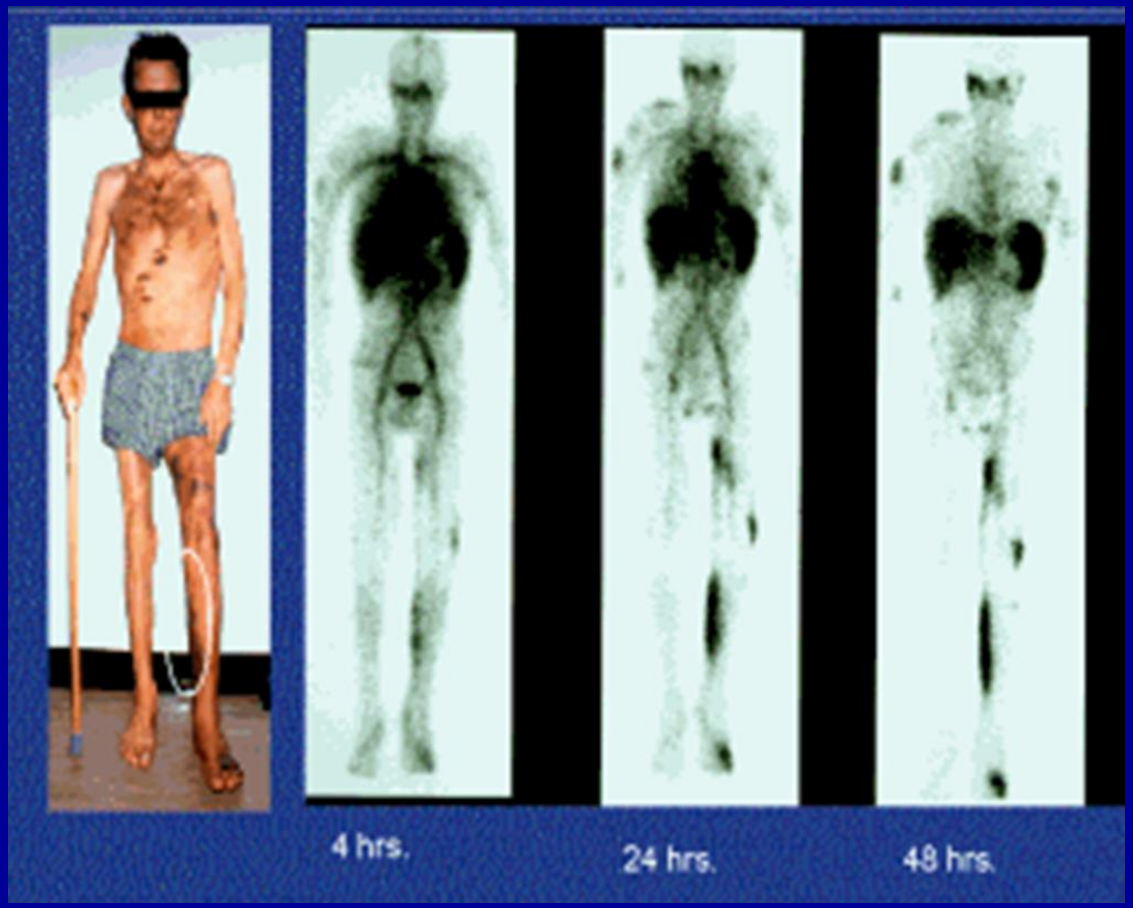
- EPR in human cancer limited or even insignificant.
- Dose translation from animals to humans may differ for nanomeds.
- Liposomes activate macrophages to enhance tumor growth and blunting the therapeutic advantage in humans.
- Drug release rate from liposomes in the tumor bed is suboptimal in humans.
- Poor clinical study design.

Despite its great safety, DOXIL has not been approved for 1st Line or Adjuvant/Curative Settings

EPR in human cancer: present but variable

iGamma Scintigraphy after Injection of [DTPA-In^{111}] Stealth Liposomes

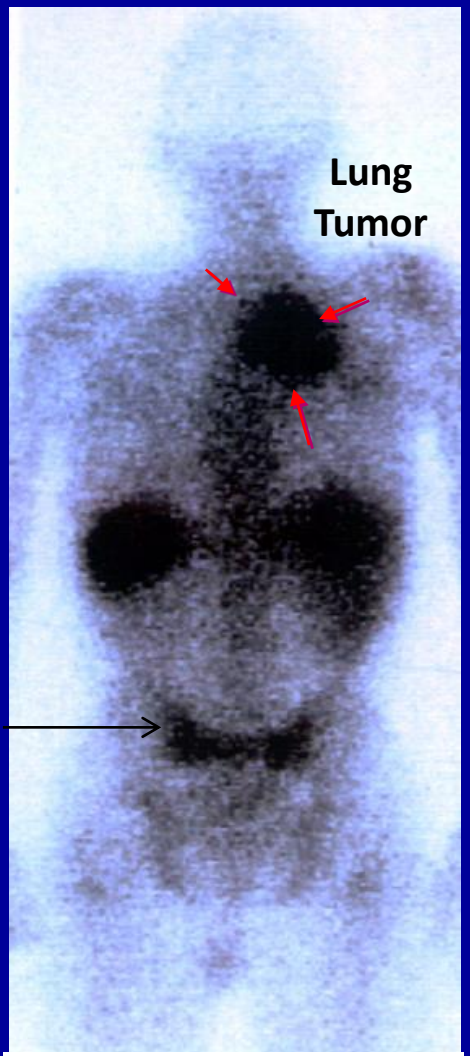
Kaposi Sarcoma



Harrington et al., Clin. Cancer Res. 2001

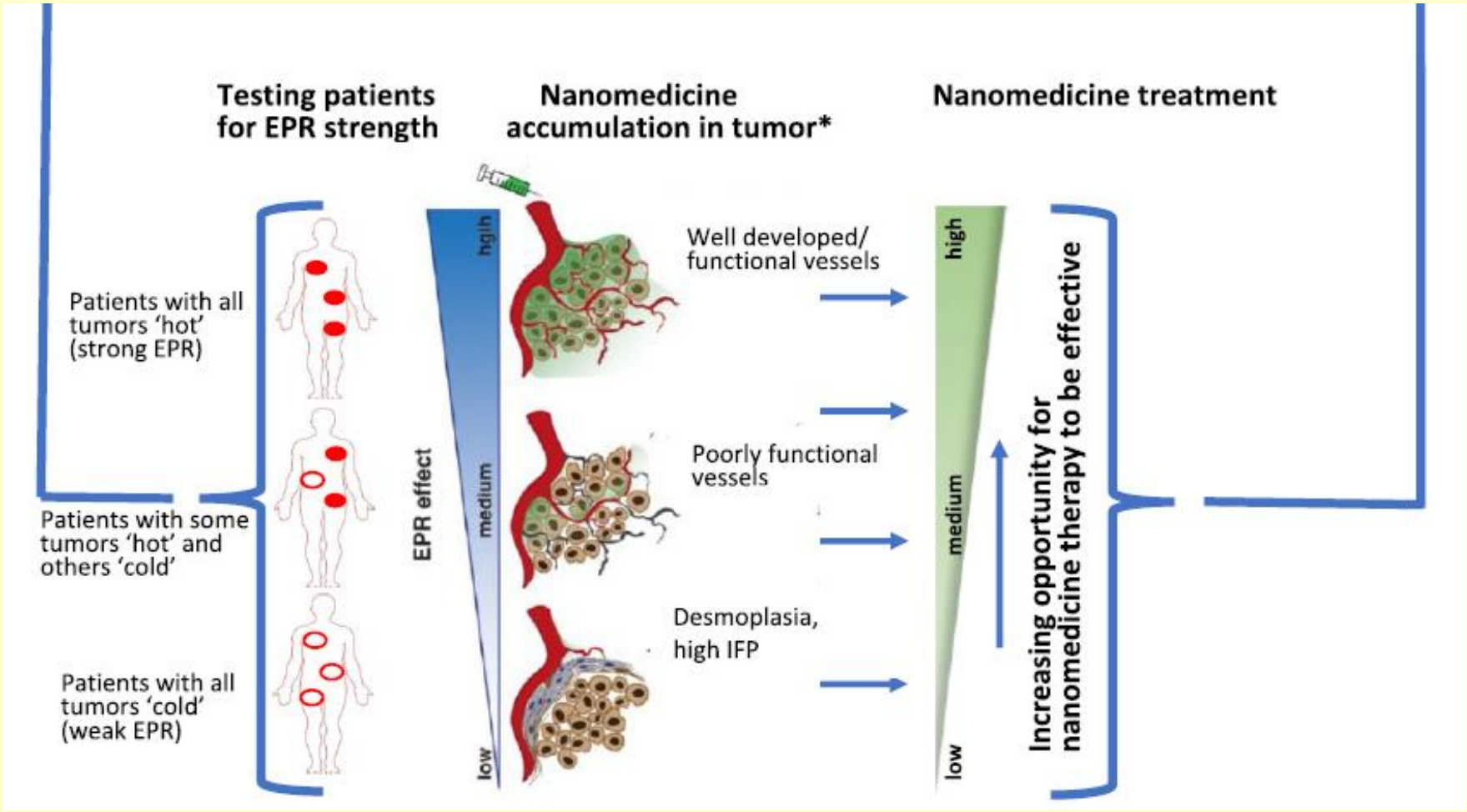
Liver,
Spleen

Bone
Marrow



Posterior view 48h

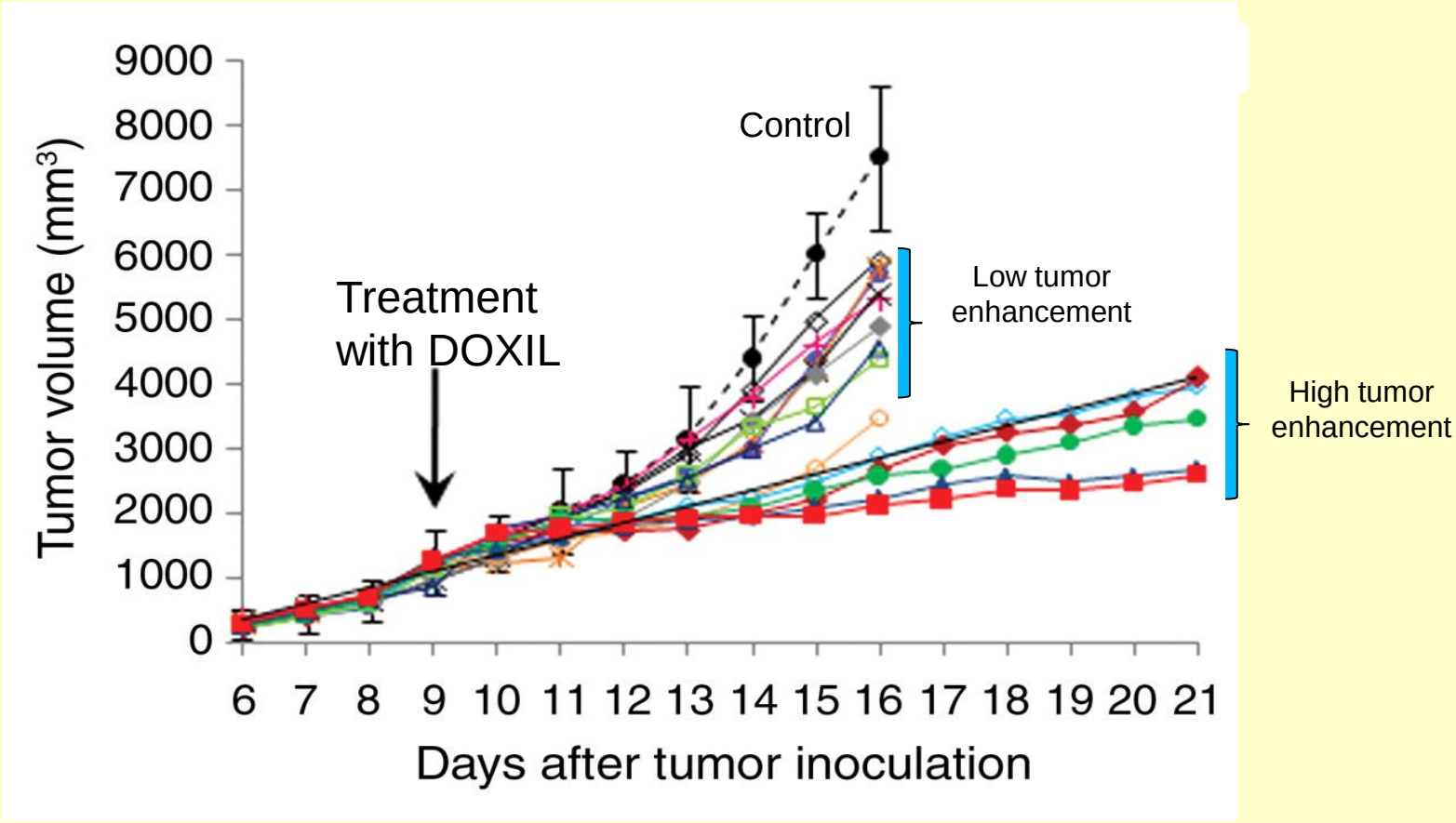
Controlling for the variability of EPR effect in Human Cancer: Real time imaging for Patient Selection



Grozinski et al., ACS Nano 2019

Imaging of EPR to predict Efficacy

(X-ray enhancement = measure of EPR)



Karathanasis et al., Radiology 2009

EPR imaging in pancreas tumor patients
Tumor MRI signal and liposomal drug activity correlate!

Author Manuscript Published OnlineFirst on February 3, 2017; DOI: 10.1158/1078-0432.CCR-16-1990
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

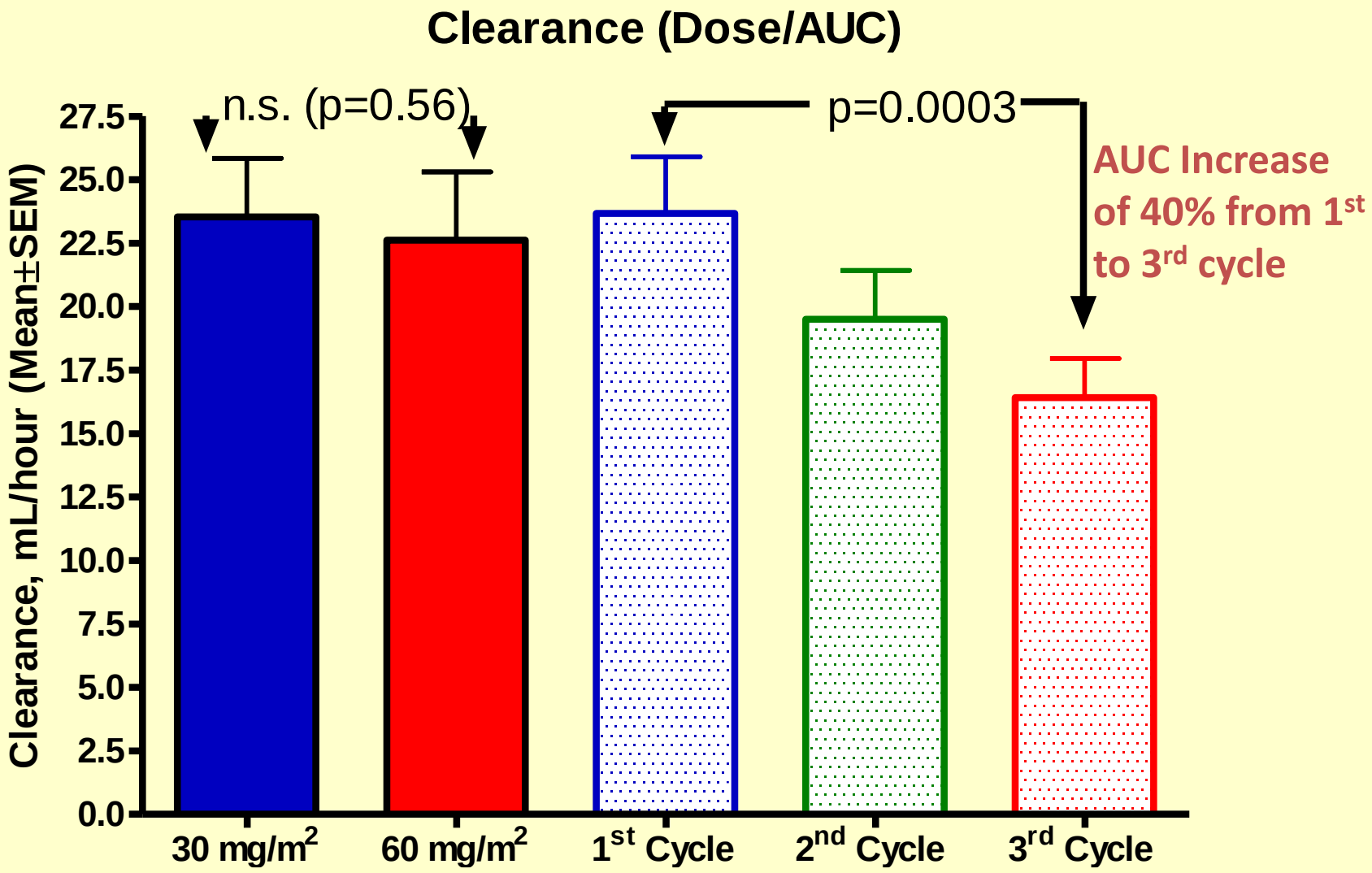
Correlation Between Ferumoxytol Uptake in Tumor Lesions by MRI and Response to Nanoliposomal Irinotecan in Patients With Advanced Solid Tumors: A Pilot Study

Patient EPR effect stratification possible:
Higher PET/CT signal corresponds with more favorable treatment outcome.

Author Manuscript Published OnlineFirst on March 15, 2017; DOI: 10.1158/1078-0432.CCR-16-3193
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

^{64}Cu-MM-302 Positron Emission Tomography Quantifies Variability of Enhanced Permeability and Retention of Nanoparticles in Relation to Treatment Response in Patients with Metastatic Breast Cancer

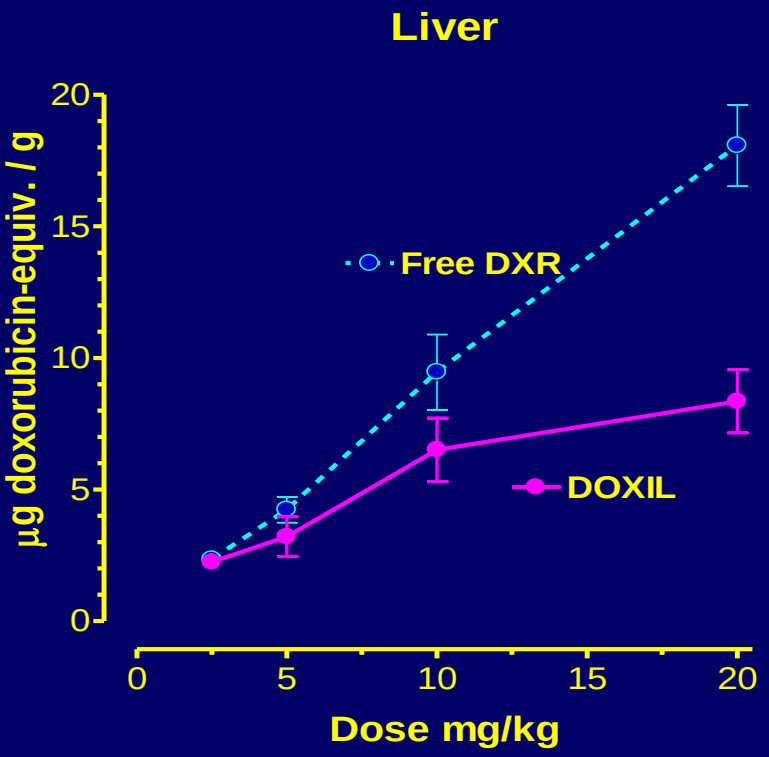
Muco-cutaneous toxicity of DOXIL is the result of slower clearance upon retreatment with DOXIL

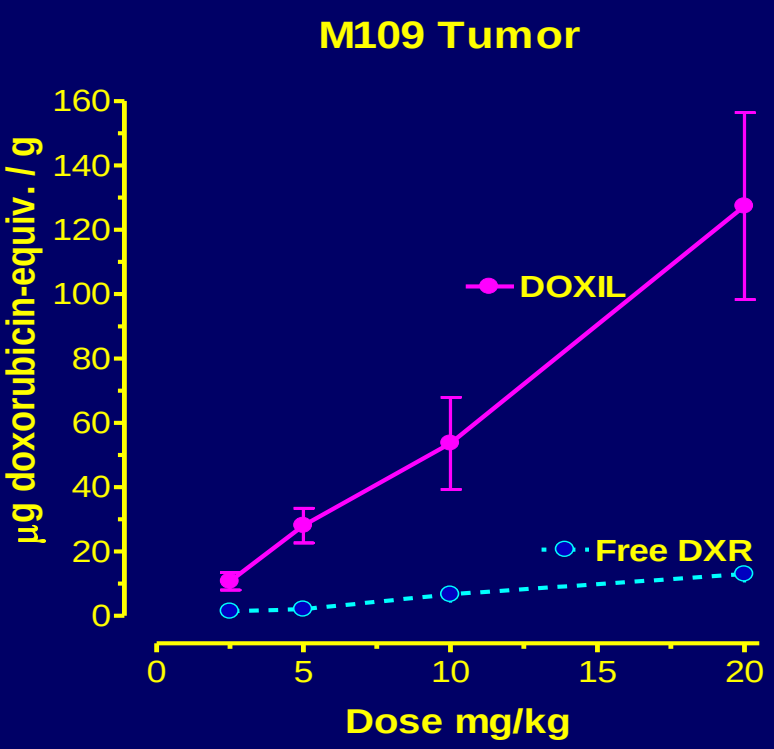


Gabizon et al., CCP 2007

Grenader and Gabizon, J Clin Oncol 2010

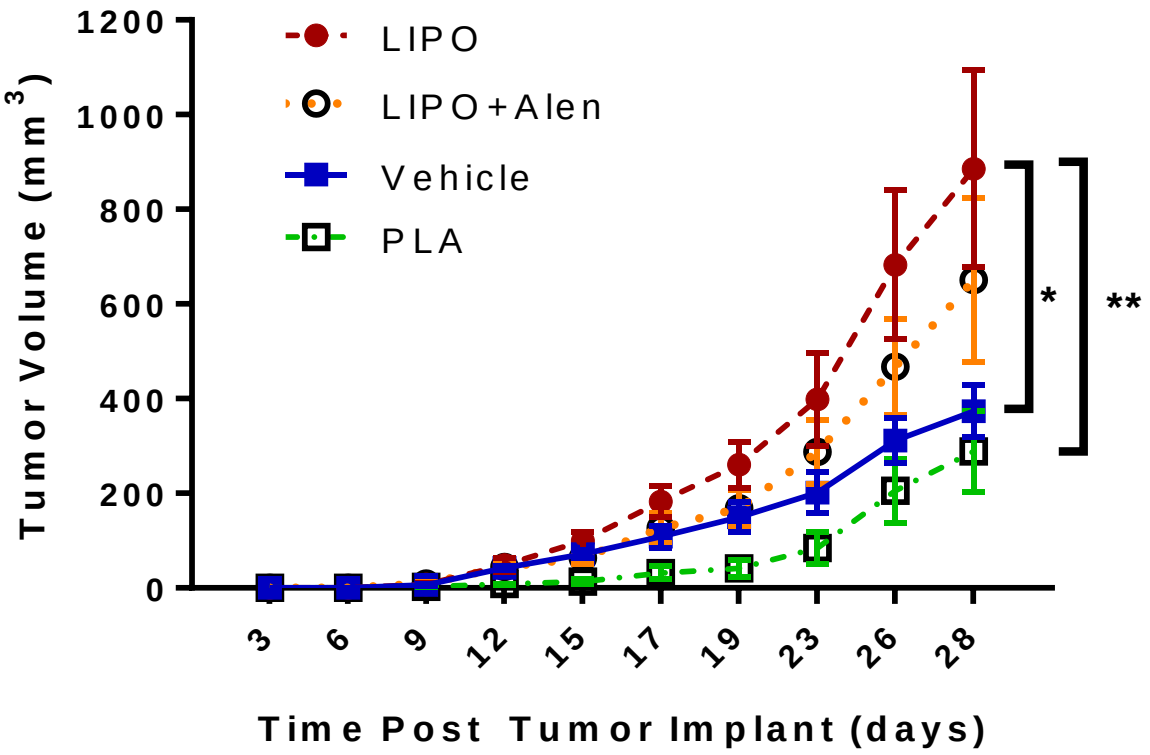
Effect of Dose: Increase in tumor accumulation of Doxil and saturation of liver uptake

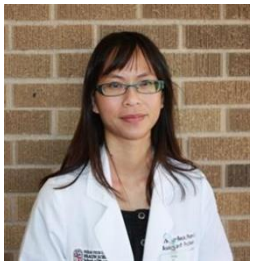




Hypothesis: Giving a high initial loading dose followed by a lower maintenance dose will increase efficacy without increase of toxicity

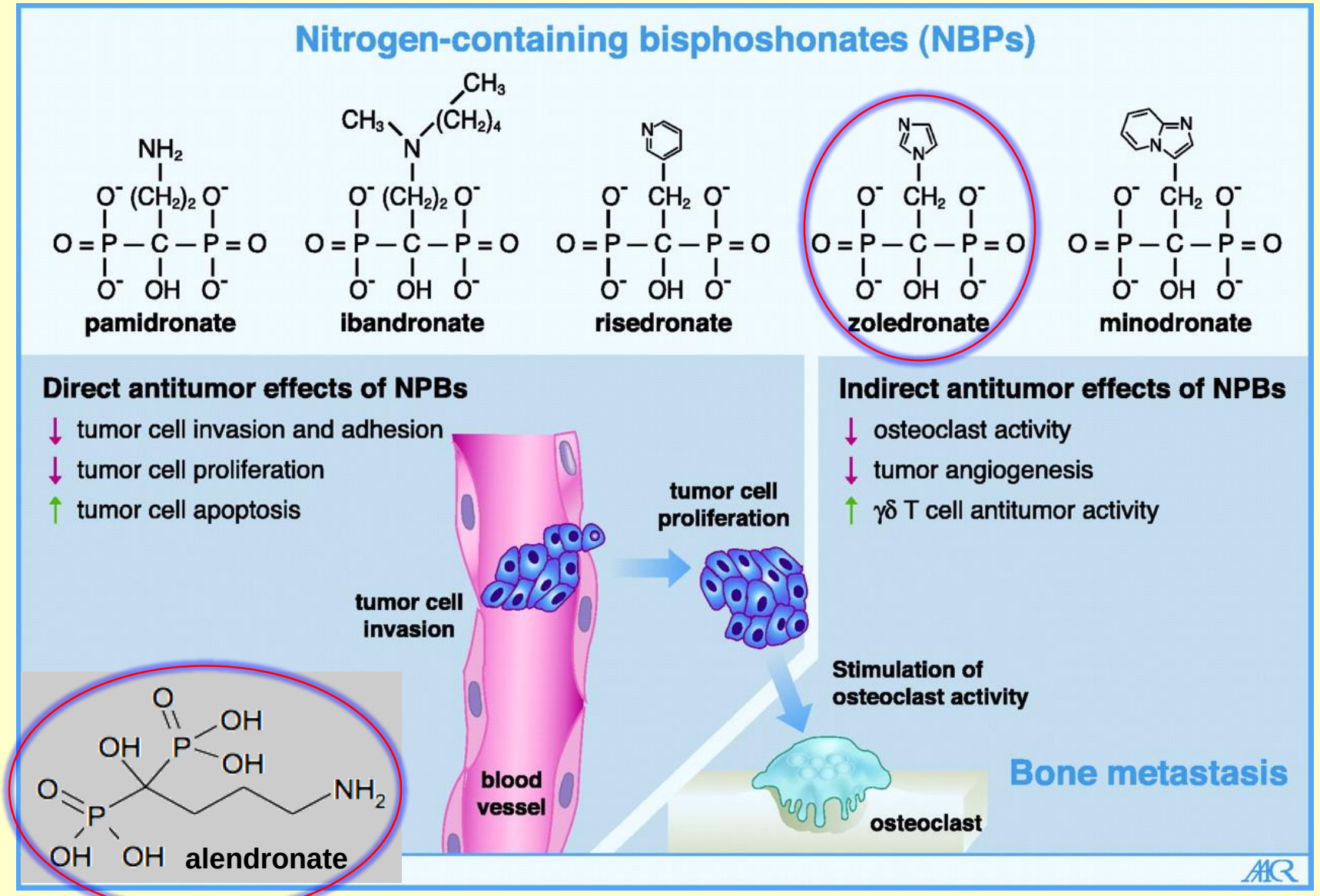
Liposome-induced tumor growth enhancement is macrophage-dependent and can be abrogated by liposomal Alendronate (PLA) but not by free Alen.





La-Beck NM, Rajan R, Wood LM, Gabizon AA, et al. (*manuscript under revision*)
Dept. of Immunotherapeutics & Biotechnology, School of Pharmacy, Texas Tech University Health Sciences Center

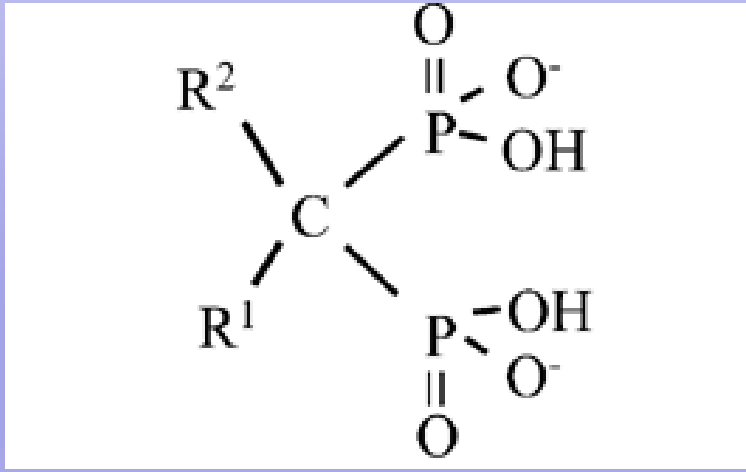
Nitrogen-containing Bisphosphonates



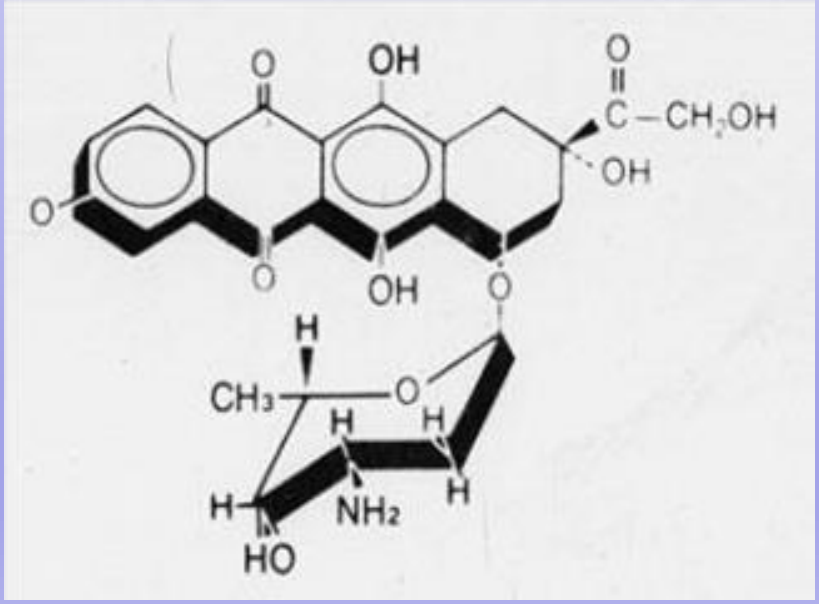
Clezardin, P. et al. Cancer Res 2005;65:4971-4974

Rationale for combining bisphosphonates with doxorubicin in the same liposome

- Double attack on tumor cells and tumor-infiltrating macrophages
- Different MoA's, Non-overlapping toxicities
- Immunological anti-tumor effects by gamma-delta T lymphocytes
- Co-delivery ensures timely co-exposure to both agents
- Bisphosphonates ammonium slats enable remote Dox Loading



Bisphosphonate

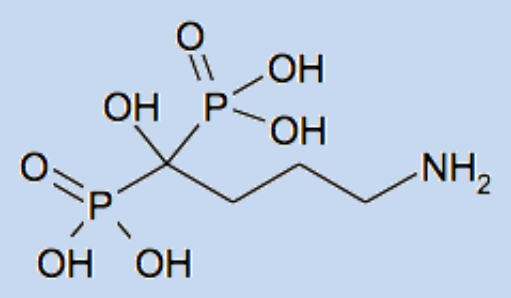


Doxorubicin

PLAD (*Pegylated liposomal alendronate of doxorubicin*)

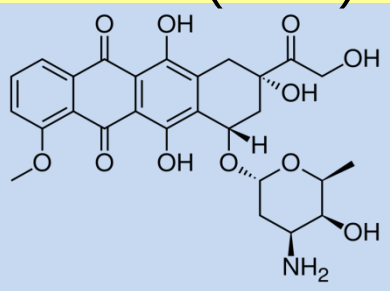
I. Passive Encapsulation: Ammonium ALD

Alendronic acid (ALD)



II. Active Loading: DOX

Doxorubicin (DOX) HCl



$DOX\text{-}NH_3Cl \leftrightarrow DOX\text{-}NH_3^+ + Cl^-$

$DOX\text{-}NH_3^+ \leftrightarrow DOX\text{-}NH_2 + H^+$

Liposome aqueous phase

$NH_4OH + ALD\cdot H \leftrightarrow (NH_4)\,ALD + H_2O$

pH 6.0

HSPC: cholesterol: PEG^{2000} DSPE
55:40:5 mole ratio

External medium

Liposome aqueous phase

$DOX\text{-}NH_2$

$DOX\text{-}NH_3^+ + (NH_4)ALD$

$DOX\text{-}NH_3\text{–}ALD$ (Precipitate)

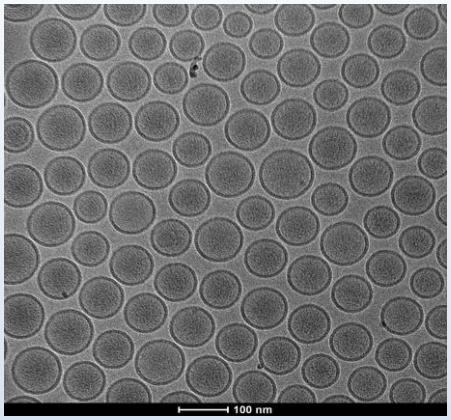
$NH_3 + H^+$

NH_3

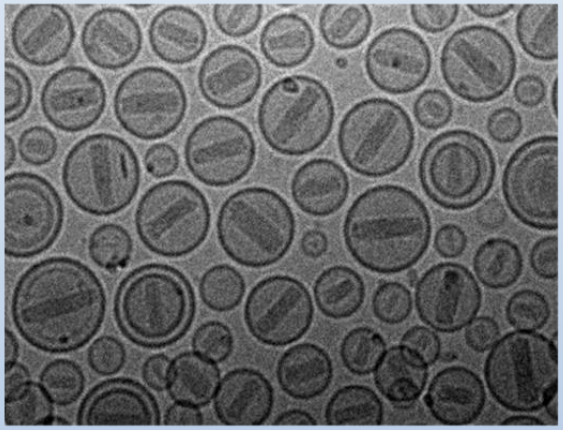
Unencapsulated (NH_4)ALD removed by resin/dialysis

Shmeeda et al., J Drug Targ 2016

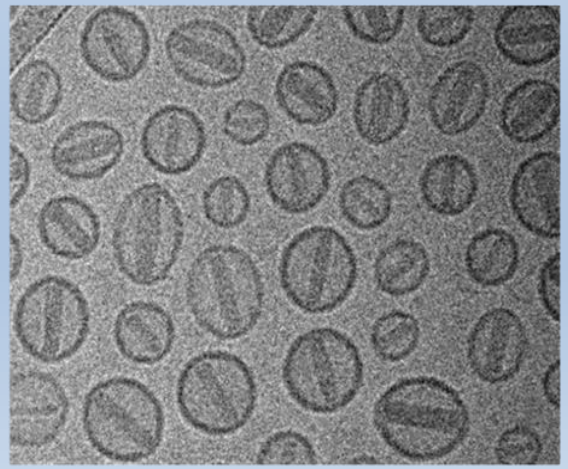
PLAD vs DOXIL



Liposomal Alendronate



PLAD



DOXIL

- More potent activator of the inflammasome pathway than PLD, leading to 40-fold greater secretion of IL-1b, and other cytokines
- Similar PK and comparable tumor drug levels
- Greater therapeutic efficacy in immuno-competent mouse tumor model

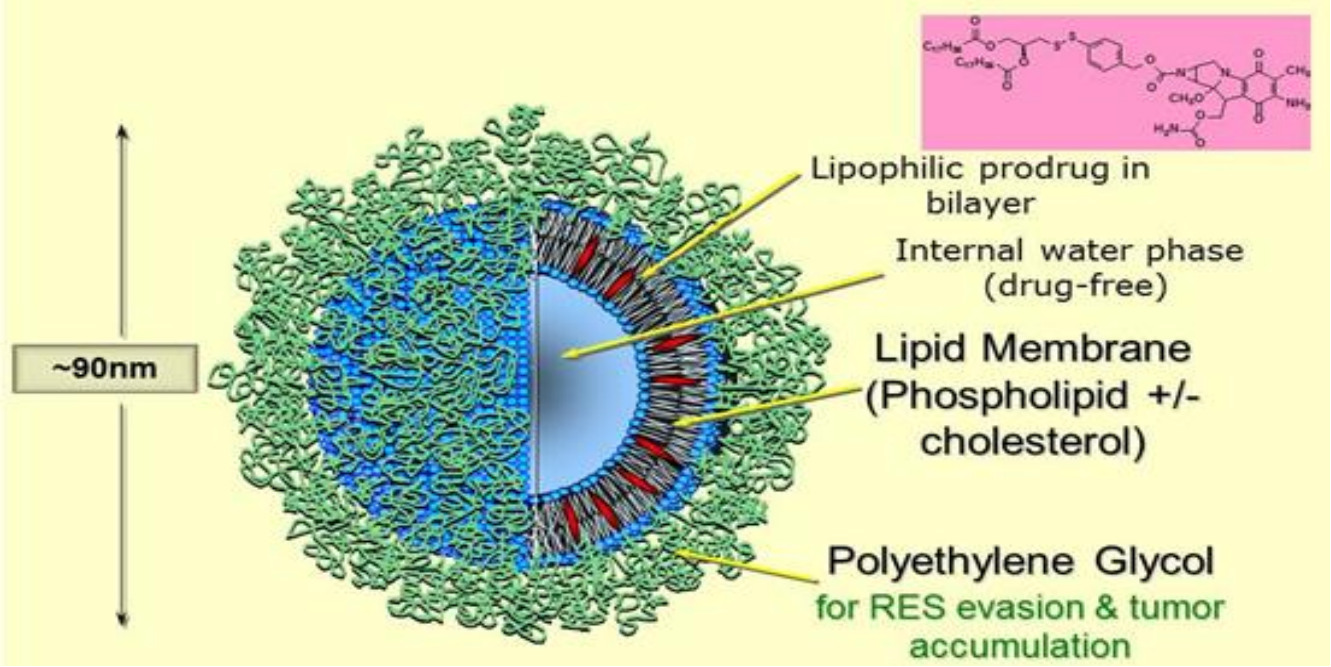
PLAD appears to be a synergistic dual drug liposome formulation for chemo-immunotherapy of cancer

Shmeeda et al., J. Drug Targ. 2016

NCL Nanotechnology Characterization Laboratory

Promitil®, a “smart” nanomedicine for cancer chemotherapy and chemo-radiotherapy

- **Delivers Mitomycin-C Lipidic Prodrug (MLP)**



Promitil® has a long circulation time and exploits the abnormal blood vessels of tumors for selective delivery of MLP to tumors by the EPR effect. Under clinical testing for gastro-intestinal cancer

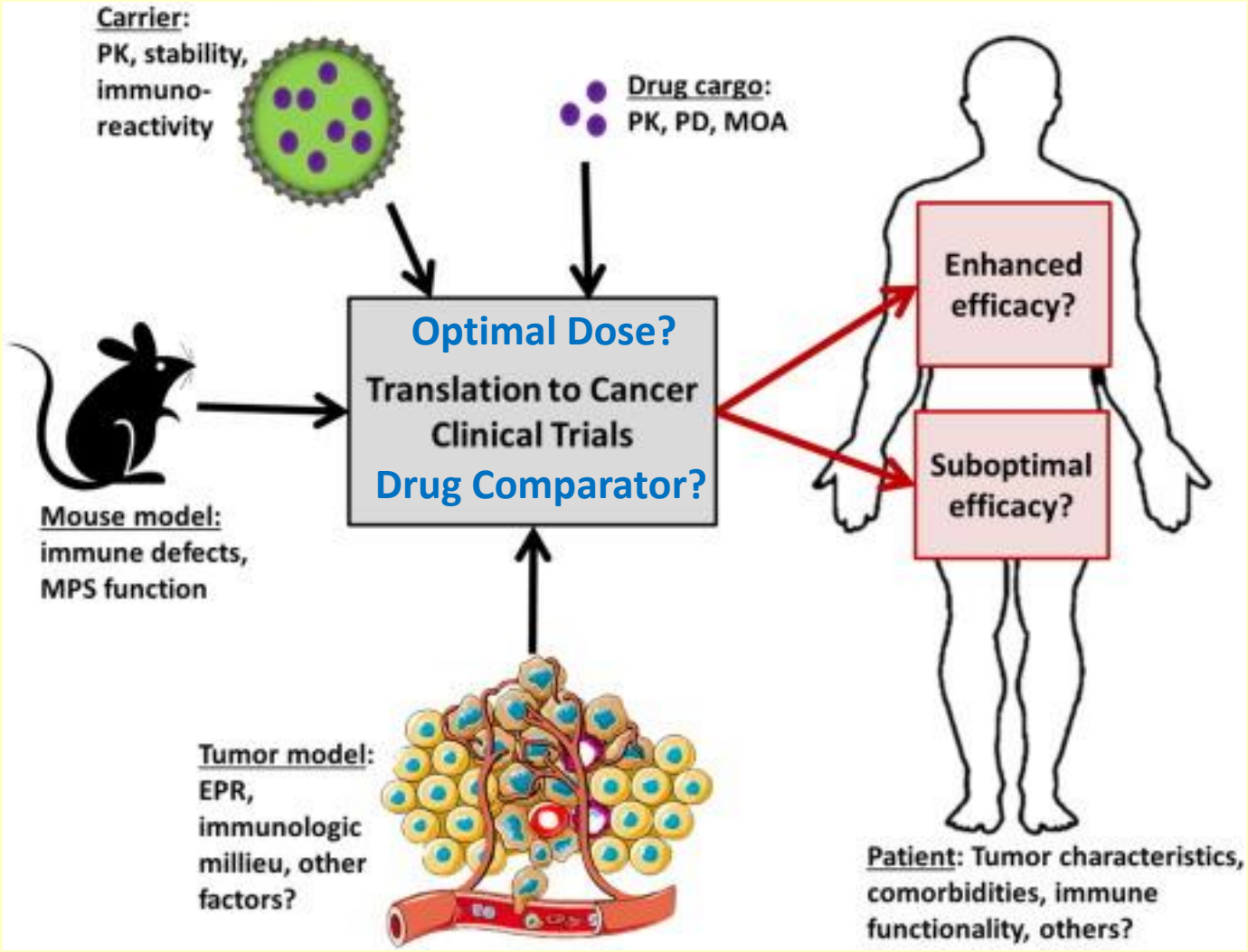
Promitil
Clinical Development

- **Phase 1A/1B completd (88 patients) demonstrating Stealth PK, lower toxicity than MMC, and anti-tumor activity**

- **Randomized Phase 2B-3 study: Promitil vs. Regorafenib or other in metastatic Colo-rectal Ca to obtain proof of value**

- **Phase 2A study: Explore the clinical activity of Promitil in combination with radiotherapy, as radiosensitizer for multiple cancer types (ongoing).**

Challenges associated with translating the anticancer efficacy of carrier-mediated drugs from preclinical studies to clinical trials



Acknowledgments:

- Nano-oncology SZMC Lab:
  - ✓ Hilary Shmeeda PhD
  - ✓ Yasmine Amitay PhD
  - ✓ Yogita Patil, PhD
  - ✓ Jenny Gorin, M.Sc.
  - ✓ Lidia Mak
  - ✓ Dina Tzemach
- Lipomedix: Patricia Ohana, PhD

Collaborators:

- Irene La-Beck, PhD (Abilene, TX)
- Andrew Wang, MD (UNC)
- Samuel Zalipsky, PhD (San Francisco)
- Franco Muggia, MD (NYU)
- Nano Charact. Lab (NCI)
- John Maher MD, Ana Parente, PhD, Rafael TM Rosales, PhD (KCL, London)
- Thomas Andresen, PhD (DTU, DK)
- Yechezkel Barenholz PhD (HU, J-lem)


Ramon Crater (Israel)
THANK YOU