Nanotechnology applied to Cancer Chemotherapy: Focus on Liposomes

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8th International Symposium on Phospholipids in Pharmaceutical Research - Heidelberg
Thudichum Award Lecture 2024

Disclosures

- Lipomedix Pharmaceuticals: Founder, Consultant
- InnoMedica AG: Consultant
- Fujifim Pharma: Consultant

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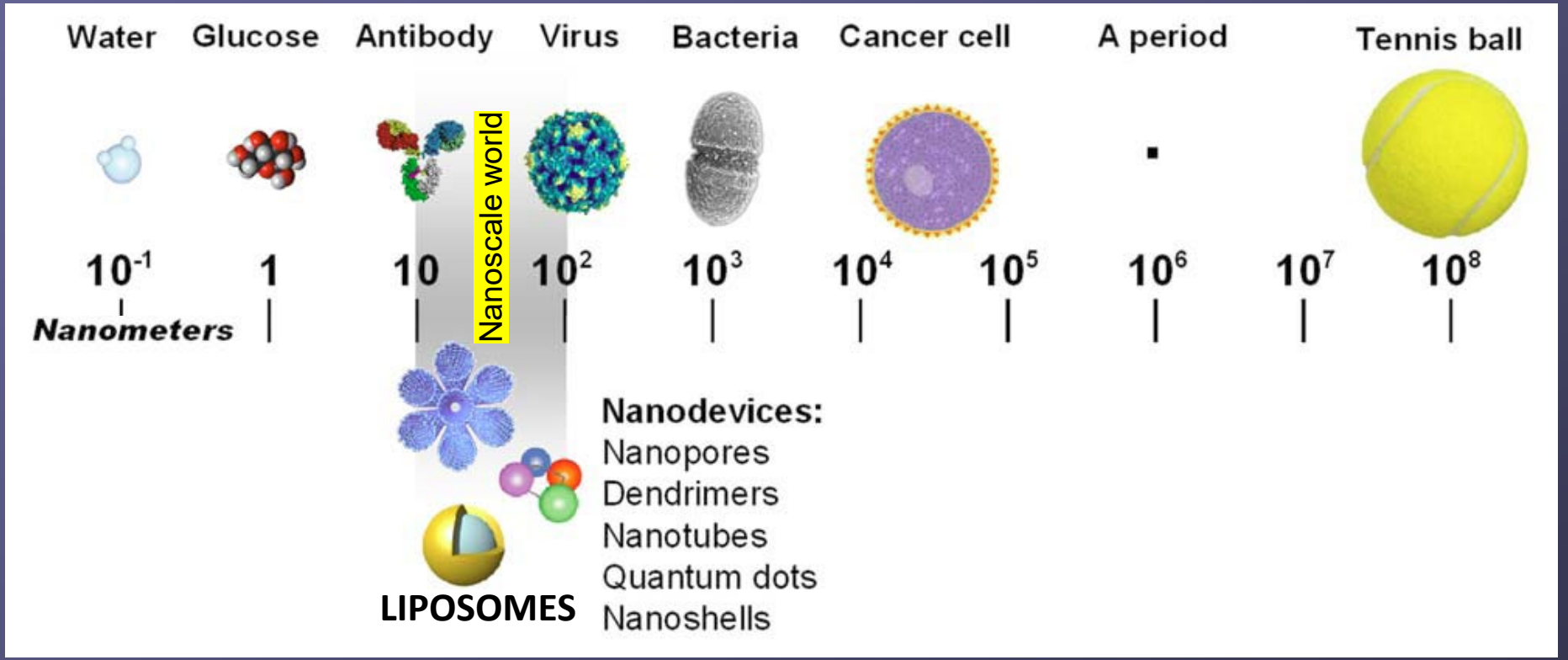
- MERCK Co.: Clinical Grant
- Lipomedix Pharmaceuticals: Research Grant

The Nanoscale world: Size matters!

Nanoparticles are one hundred to one thousand times smaller than human cells.



Richard Feynman, the "father" of Nanotechnology:
"There is plenty of room at the bottom" (Dec 1959)

Cancer drug therapy today

- **Cytotoxic agents (Chemotherapy):** DNA damage, microtubule poisons, antimetbolites
- **Agents interfering with hormone signals (Hormonal therapy):** hormone synthesis/release blockers, hormone receptor antagonists
- **Agents modifying biological response (Biological therapy):** anti-growth factor receptors, signal transduction inhibitors
- **Agents triggering the immune response (Immunotherapy):** immune checkpoint inhibitors, immunomodulators)

The beginning: the concept of chemotherapy and the experimental approach to drug development

PAUL EHRLICH 1854-1915

- German-Jewish physician-scientist, drug discoverer and pioneer in many fields of medicine
- He introduced and named the field of "CHEMOTHERAPY" to refer to drugs against infectious diseases and cancer
- He coined the term MAGIC BULLET (*Zauberkugel*) to describe drugs that act selectively against the disease site without harming other body systems

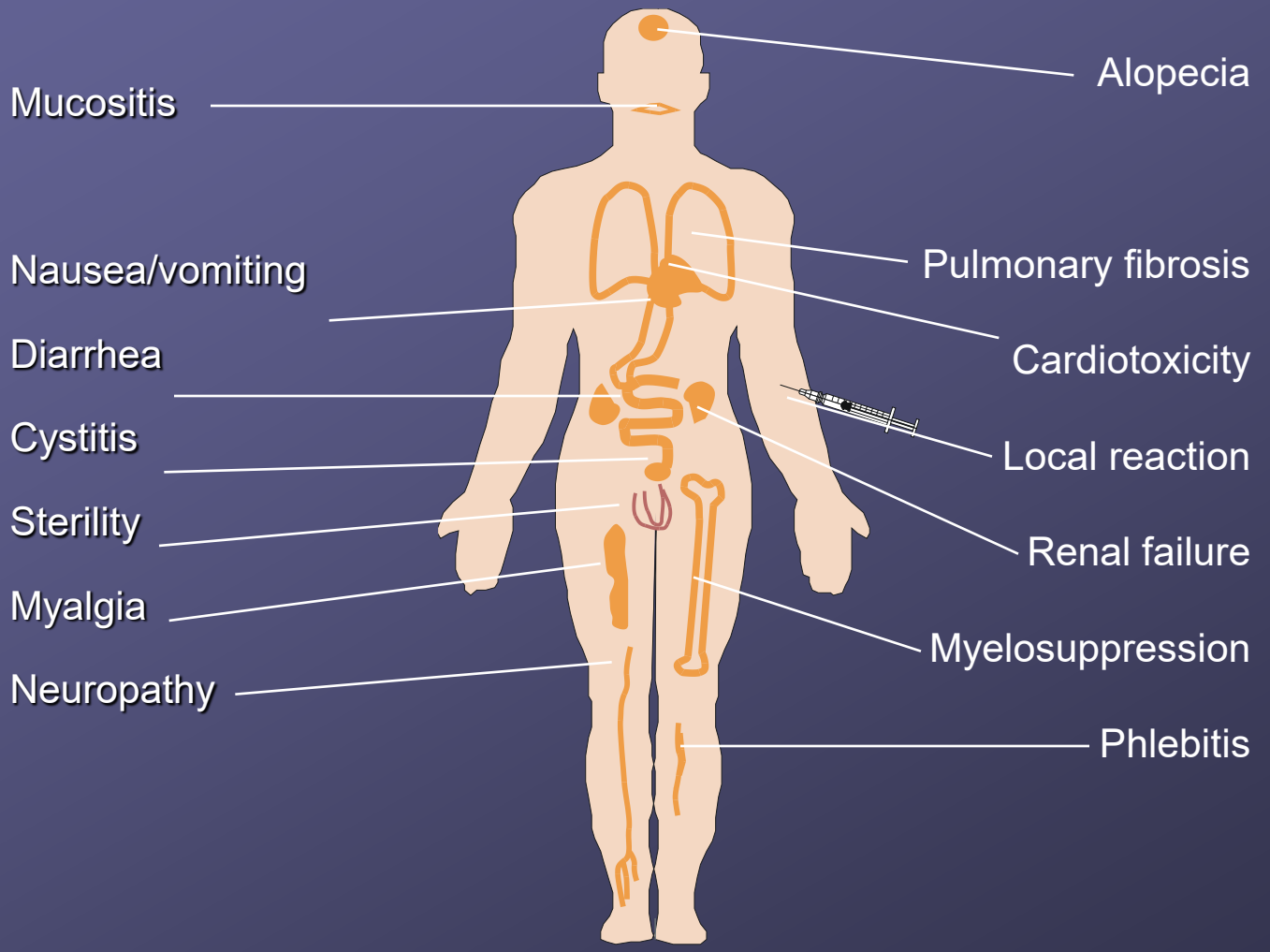
The first breakthrough in cancer chemotherapy

Bari, Italy: Dec 2, 1943

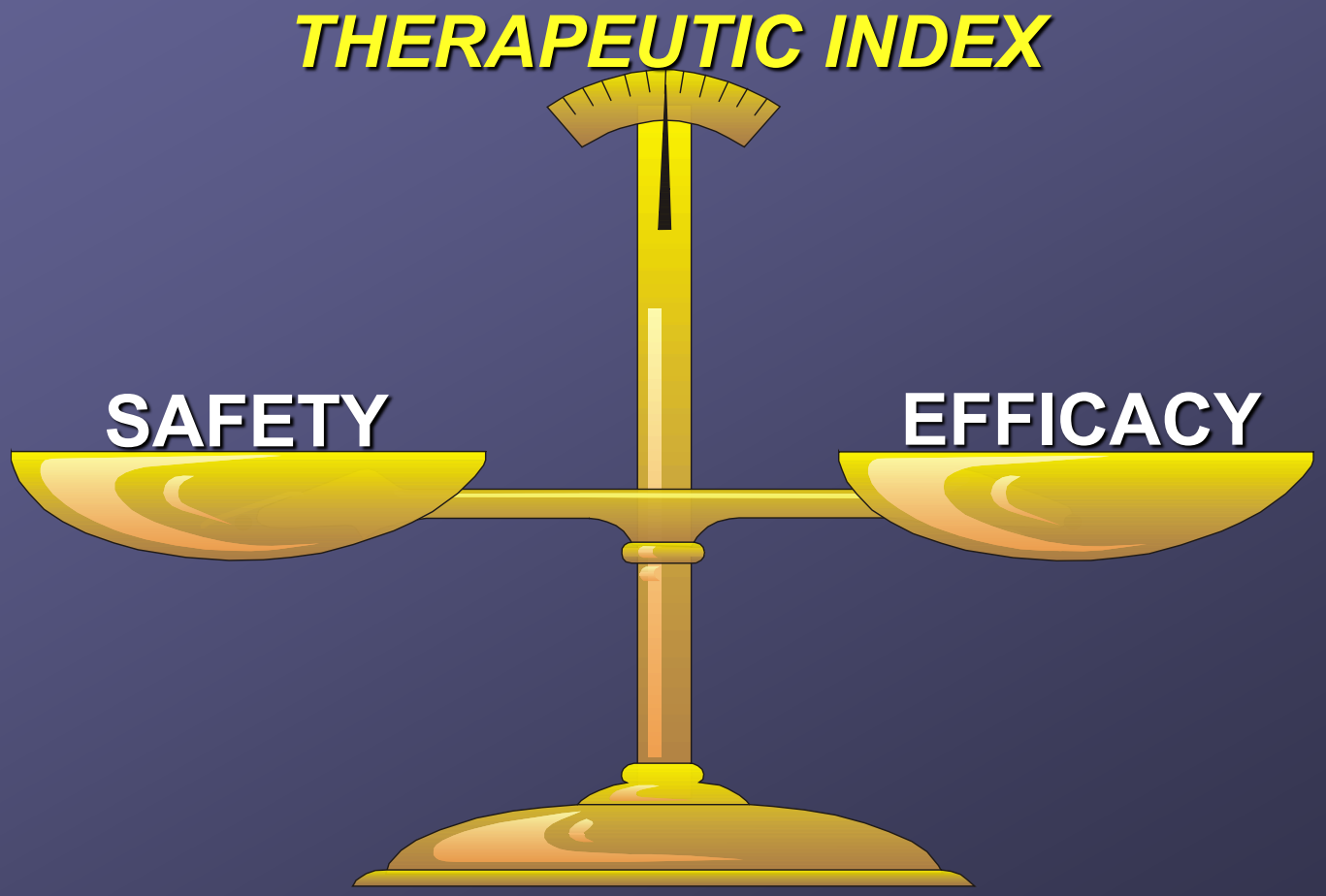
- In the bombing of an American ship loaded with 2,000 chemical weapons (sulfur mustard), soldiers were exposed to the toxic agent and developed burns and suppression of the bone marrow and lymphatic system.
- Goodman and Gilman (Yale, USA) tested a similar chemical agent (nitrogen mustard) in a patient with Lymphoma in critical condition and saw an impressive tumor regression.
- For 3 years, the US government banned the publication of the findings classified as military secret.
- In 1946, the observations were published, and extensive chemical work began, which led to many new drugs operating with the same mechanism (DNA alkylation).

Chemotherapy – chemical warfare against cancer was inspired literally by war!

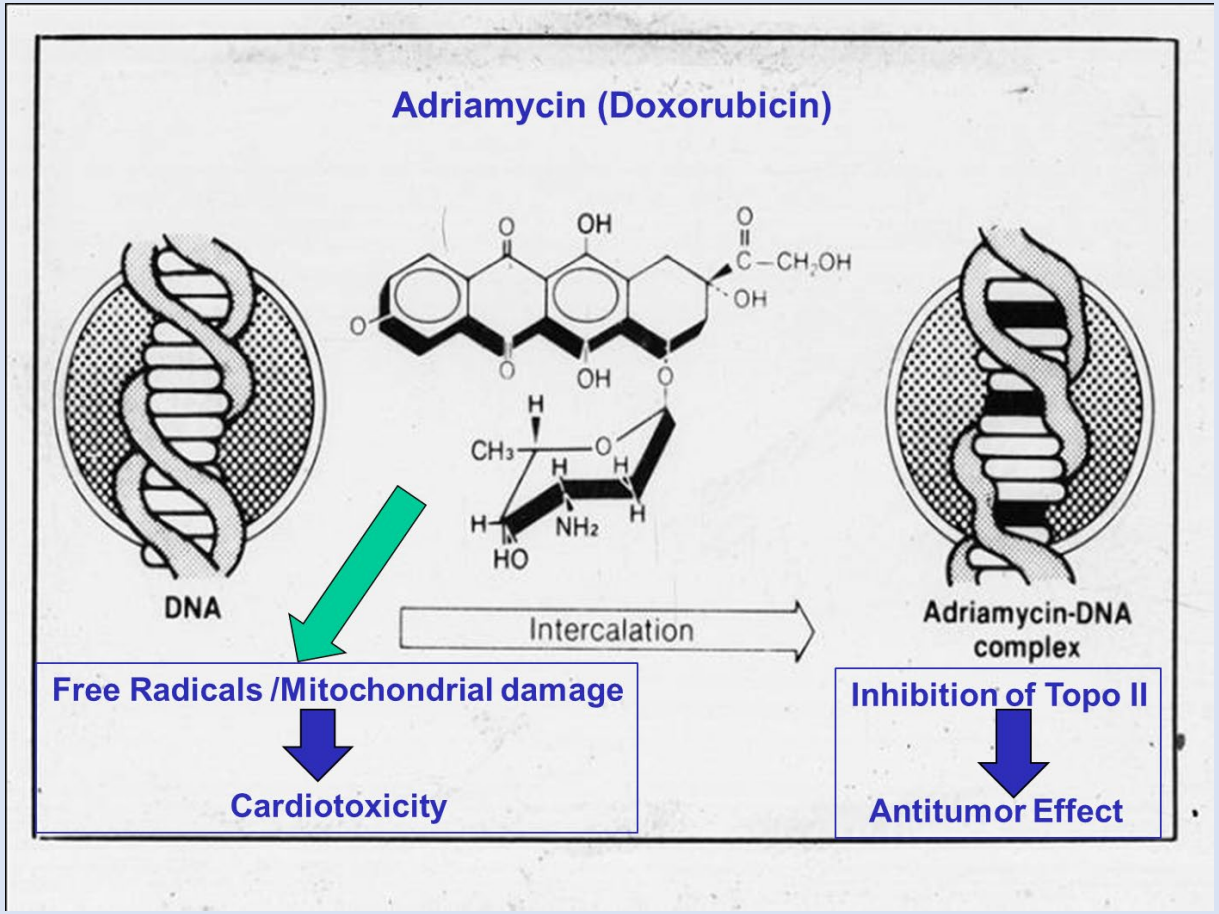
Side effects of chemotherapy



Chemotherapy has a narrow therapeutic window!



Cardiac Toxicity of Doxorubicin (Adriamycin)



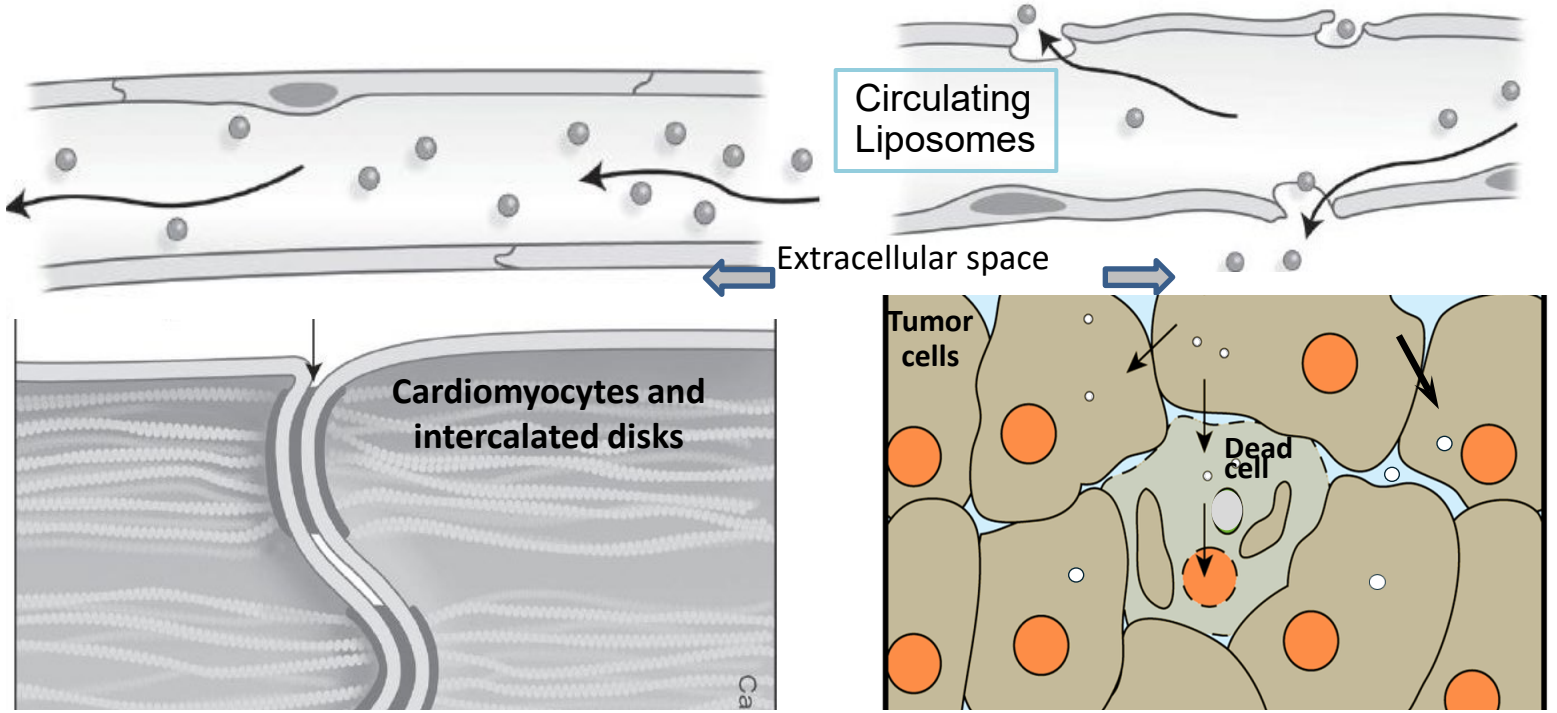
- Limits the cumulative dose
- Leads to early treatment discontinuation
- Irreversible, crippling and life-threatening

HOW CAN WE IMPROVE THE THERAPEUTIC INDEX OF DOXORUBICIN?

Blood Vessels and Nanodrug Delivery: The ACHILLES heel of cancer

Heart muscle: continuous vessel lining

Tumor: vessels with fenestrations



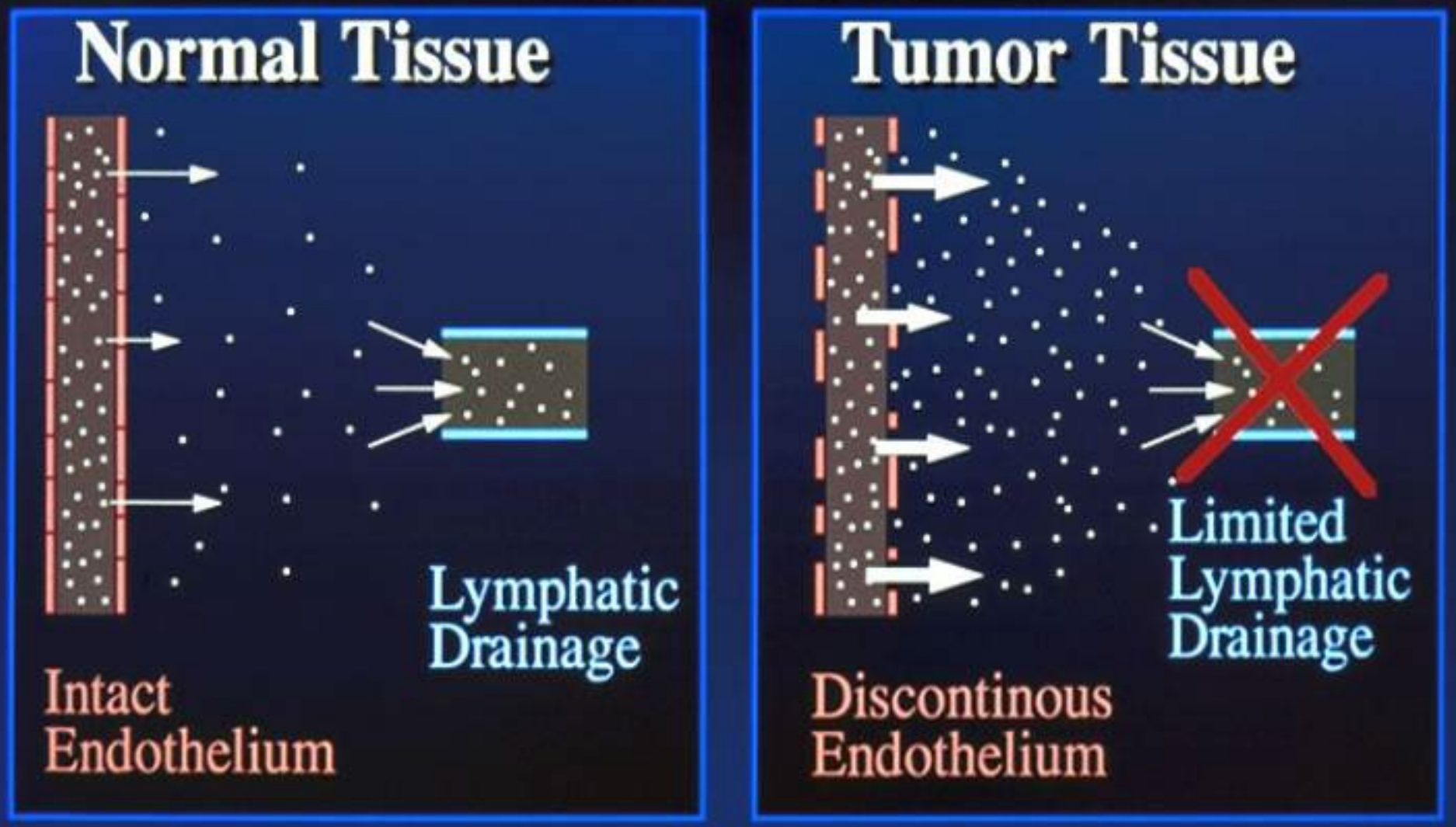
[CANCER RESEARCH 42, 4734–4739, November 1982]
0008-5472/82/0042-0000$02.00

Liposomes as *in Vivo* Carriers of Adriamycin: Reduced Cardiac Uptake and Preserved Antitumor Activity in Mice[1]

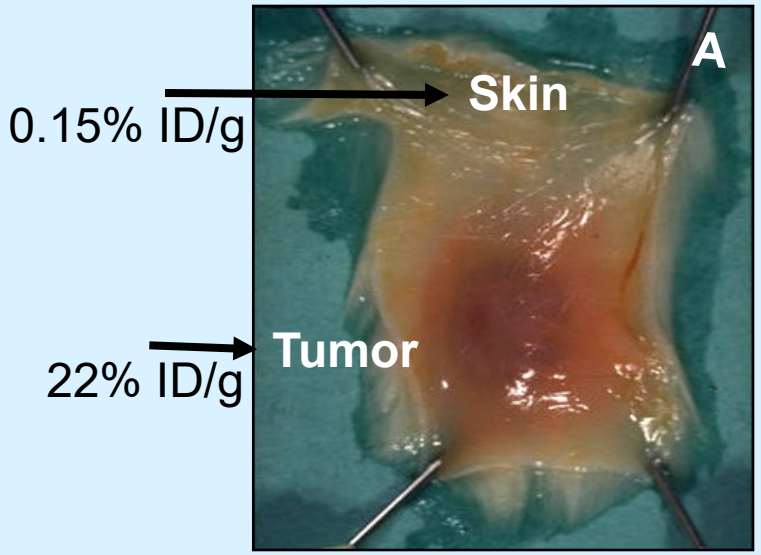
A. Gabizon,[2] A. Dagan, D. Goren, Y. Barenholz, and Z. Fuks

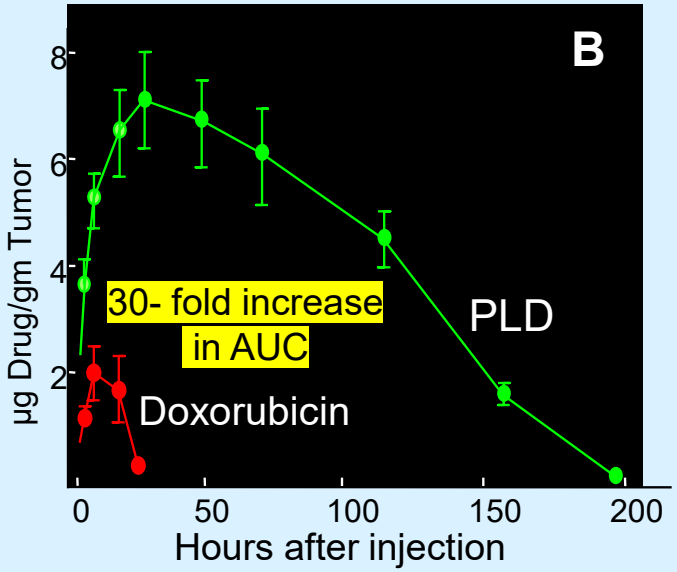
Department of Radiation and Clinical Oncology, Hadassah University Hospital [A. G., D. G., Z. F.], and Department of Biochemistry, Hebrew University-Hadassah Medical School [A. D., Y. B.], Jerusalem, Israel

Liposome localization in tumor tissue: the EPR effect

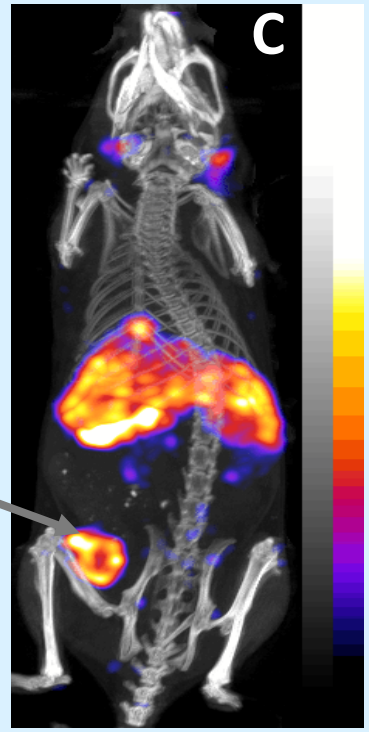


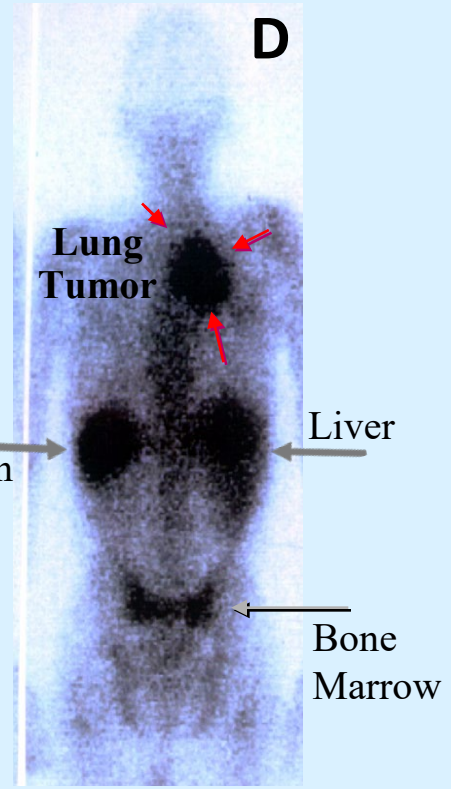
Adapted from: Maeda & Matsumura





Pharmacological Impact of EPR (Passive Targeting)

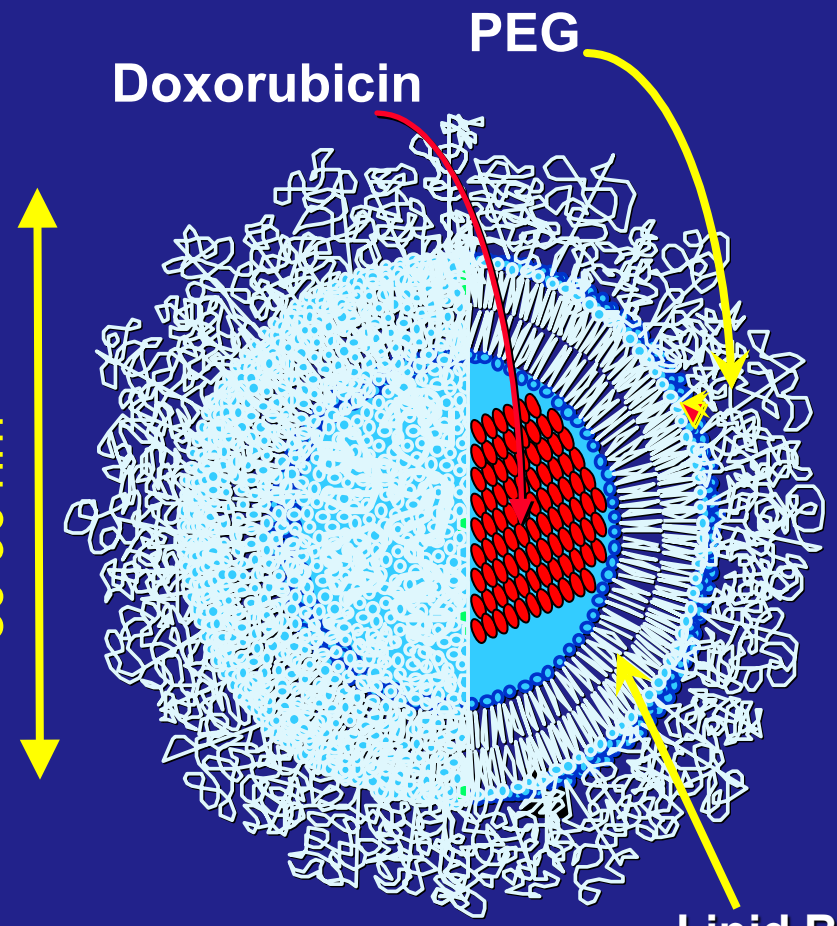




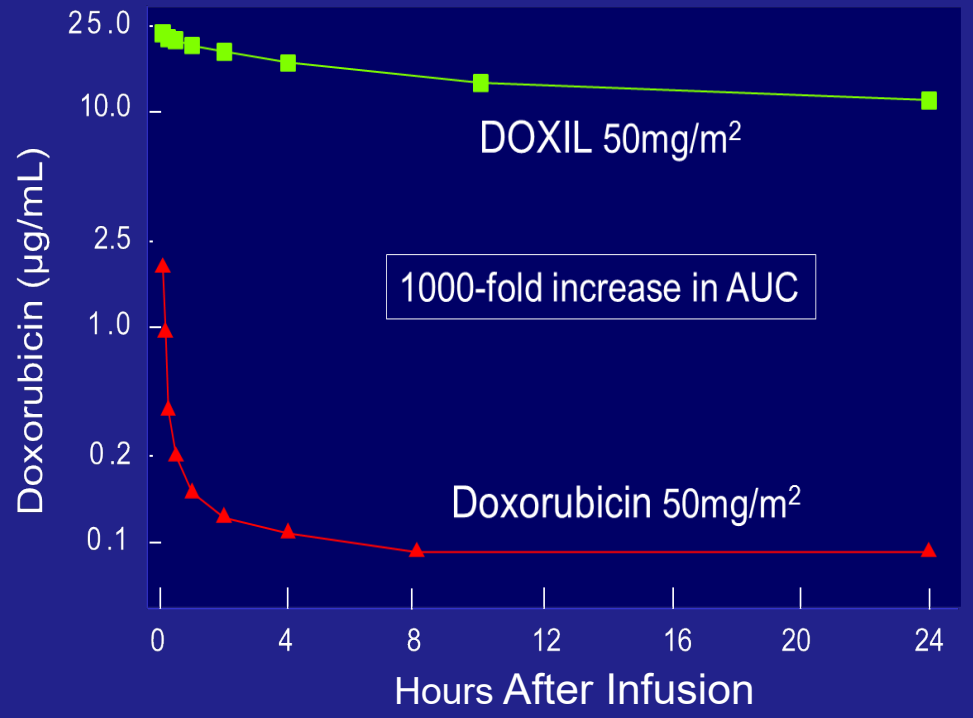
Solomon & Gabizon, Clinical Lymphoma & Myeloma (2008); Vaage et al., Cancer (1994); Harrington et al., Clin Cancer Res (2001); Man et al., Mol Ther (2019)

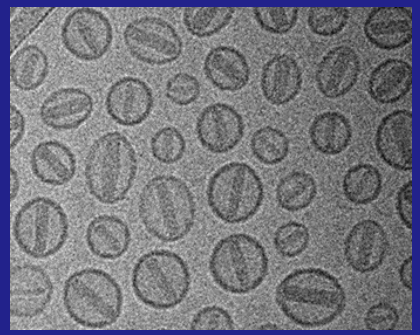
Pegylated Liposomal Doxorubicin **(DOXIL, Caelyx)**

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



Plasma Levels in Humans:
DOXIL vs. doxorubicin





[CANCER RESEARCH 54, 987–992, February 15, 1994]

Prolonged Circulation Time and Enhanced Accumulation in Malignant Exudates of Doxorubicin Encapsulated in Polyethylene-glycol Coated Liposomes[1]

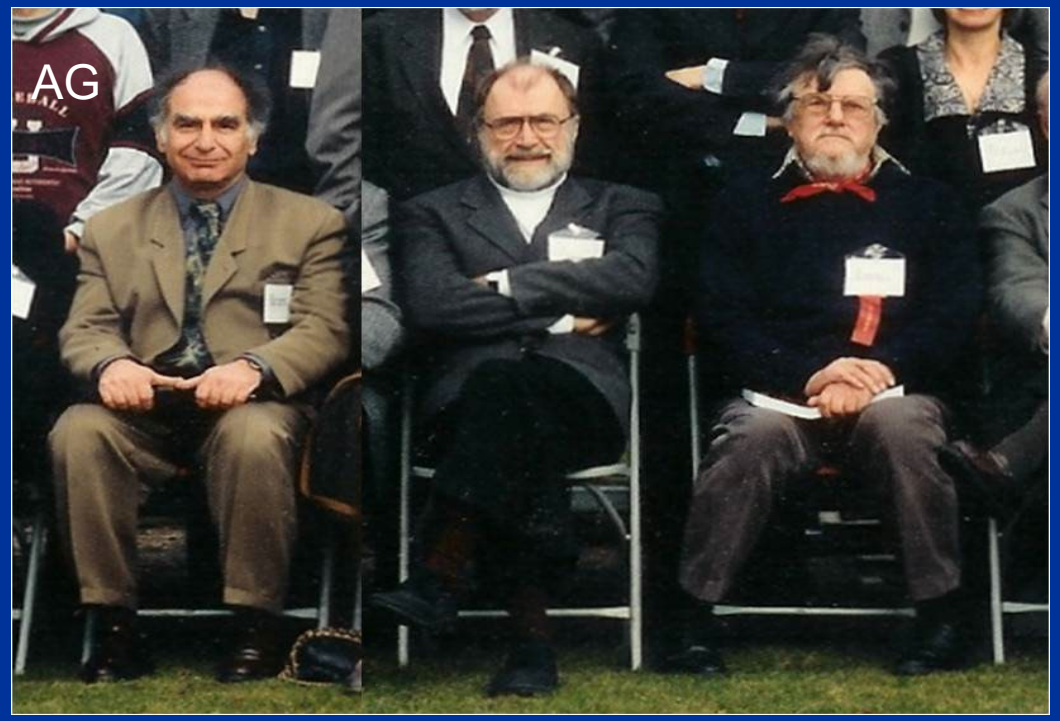
Alberto Gabizon,[2] Raphael Catane, Beatrice Uziely, Bela Kaufman, Tamar Safra, Rivka Cohen, Francis Martin, Anthony Huang, and Yechezkel Barenholz

Sharett Institute of Oncology [A. G., R. C., B. U., B. K., T. S.], Hadassah University Hospital, and Department of Membrane Biochemistry [R. C., Y. B.], Hebrew University-Hadassah Medical School, Jerusalem, Israel; and Liposome Technology, Inc., Menlo Park, California 94025 [F. M., A. H.]

The long-time scale of clinical translation

- **1964: Discovery of liposomes – Alec Bangham (Cambridge, UK)**
- **1971: First *in vivo* application of liposomes for drug delivery**
- **1982: Reduced cardiac uptake and toxicity of liposomal doxorubicin**
- **1995: US-FDA approval of liposomal doxorubicin for cancer therapy***
- **2003: Proof of reduced cardiac toxicity in humans in phase 3 study**

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



* Approved Indications:
- Kaposi Sarcoma
- Ovarian Cancer
- Breast Cancer
- Multiple Myeloma

O'Brien et al, Annals od Oncology, **2004: Reduced cardiotoxicity (H.R. 3.16) and comparable efficacy in a phase III trial of pegylated liposomal doxorubicin versus conventional doxorubicin for treatment of metastatic breast cancer.**

Annals of Oncology

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

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Received 16 November 2016 Revised form accepted 2 May 2017

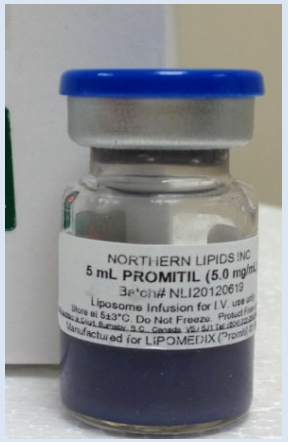
115 cycles of PLD (40mg/m^2) during 9 years with stable disease:

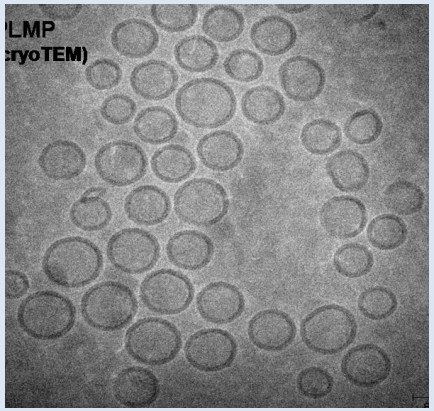
No Cardiac Toxicity!

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

Lipid-based Prodrugs and Liposomes: Promitil® (mitomycin c-lipidic prodrug)

- 3-fold less toxic than mitomycin-c
- Stable in plasma and long-circulating
- Active moiety released by thiolysis in tumor
- In phase 2 clinical testing for HRD+ pancreatic and ovarian cancer





Advanced Drug Delivery Reviews 154–155 (2020) 13–26

Contents lists available at ScienceDirect

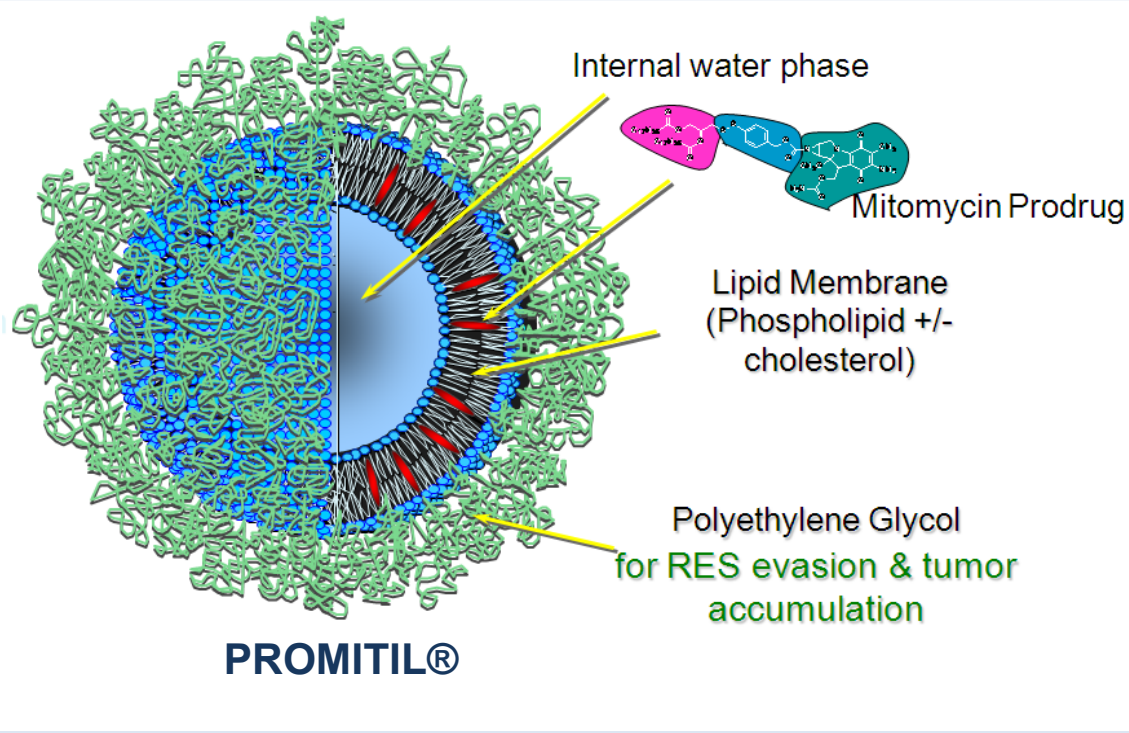
Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

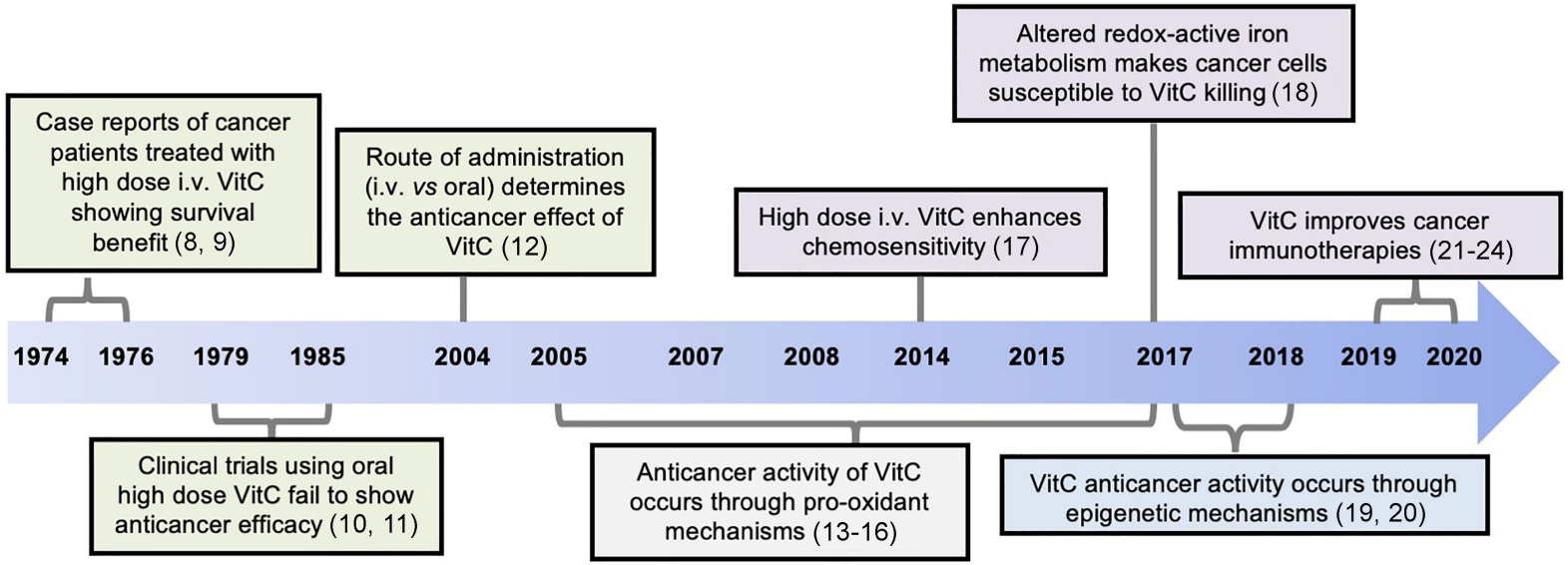
Development of Promitil®, a lipidic prodrug of mitomycin c in PEGylated liposomes: From bench to bedside

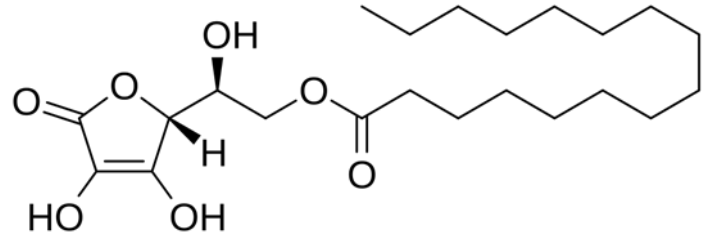
Alberto Gabizon [a,b,*], Hilary Shmeeda [a], Esther Tahover [a], Gleb Kornev [a], Yogita Patil [b], Yasmine Amitay [c], Patricia Ohana [c], Eli Sapir [d], Samuel Zalipsky [e]

[a] Oncology Institute and Nano-oncology Research Center, Shaare Zedek Medical Center, Jerusalem, Israel
[b] Hebrew University-Faculty of Medicine, Jerusalem, Israel
[c] Lipomedix Pharmaceuticals Ltd., Jerusalem, Israel
[d] Samson Assuta Ashdod University Hospital, Ashdod, Israel
[e] Independent Consultant, Redwood City, CA, USA



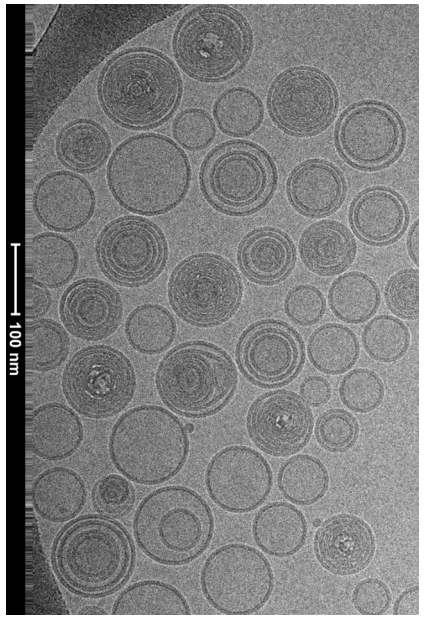
Lipid-based Prodrugs and Liposomes: Ascorbyl Palmitate (Vit. C Prodrug)



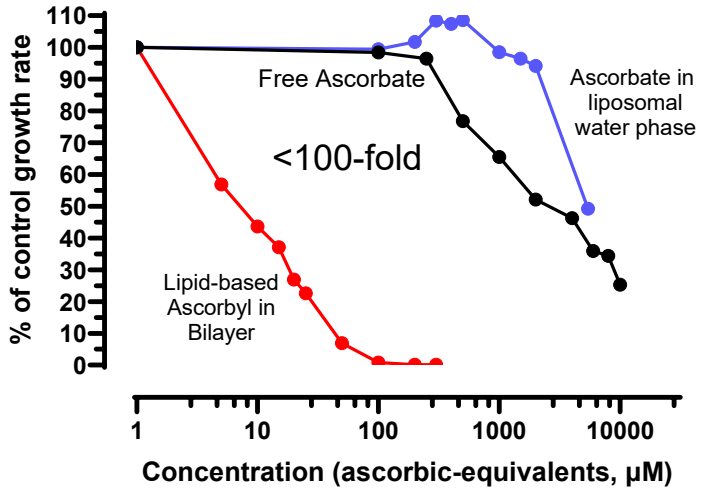


6-O-Palmitoylascorbic acid

- High loading efficiency; No free prodrug
- Enhanced Ascorbyl uptake by Tumor Cells via Vit. C receptors
- Enhanced In vitro Cytotoxicity
- In vivo antitumor activity in J6456 lymphoma and NB-49 carcinoma

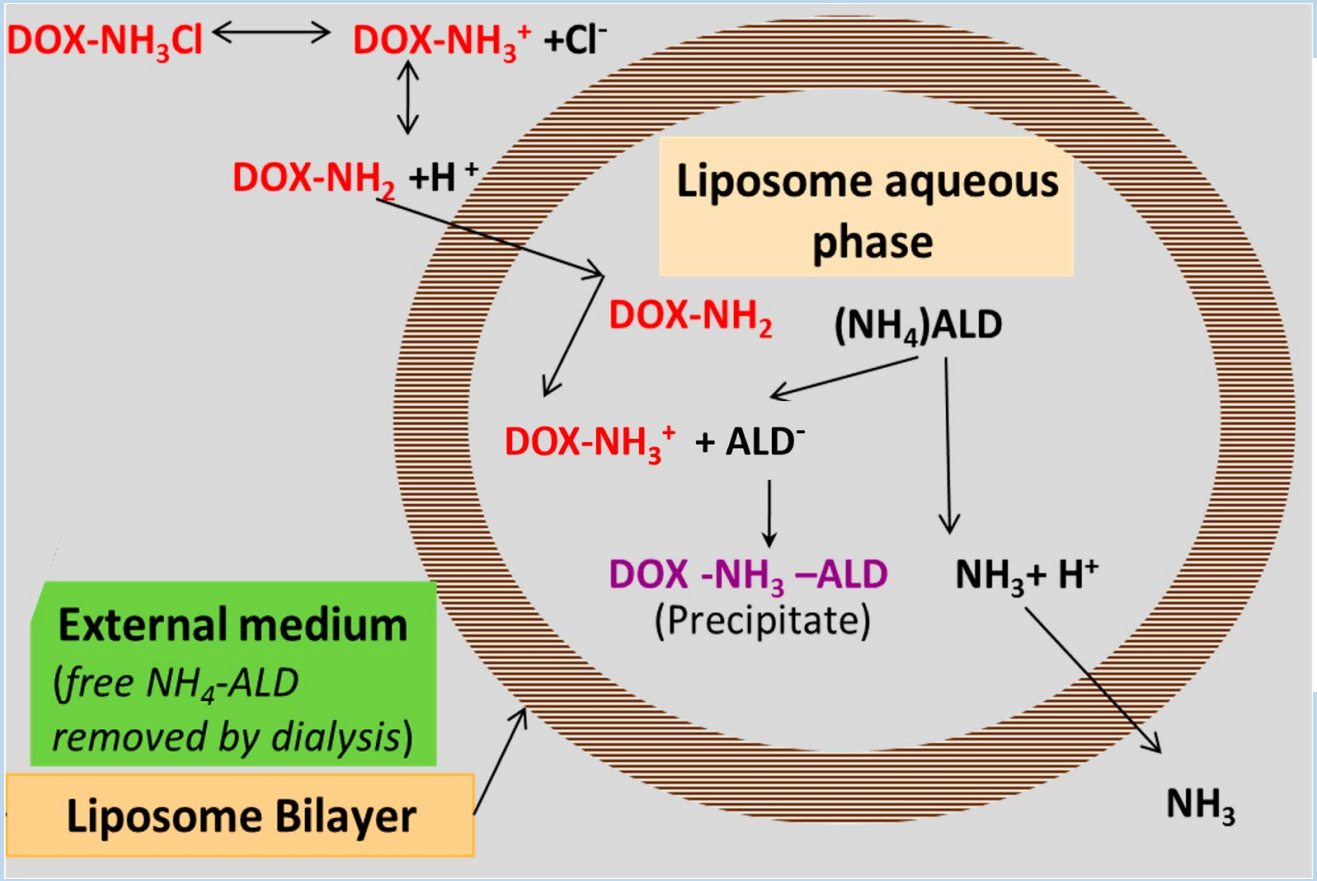


Enhanced *in vitro* cytotoxicity of Ascorbyl lipidic Prodrug in Liposomal Biayer



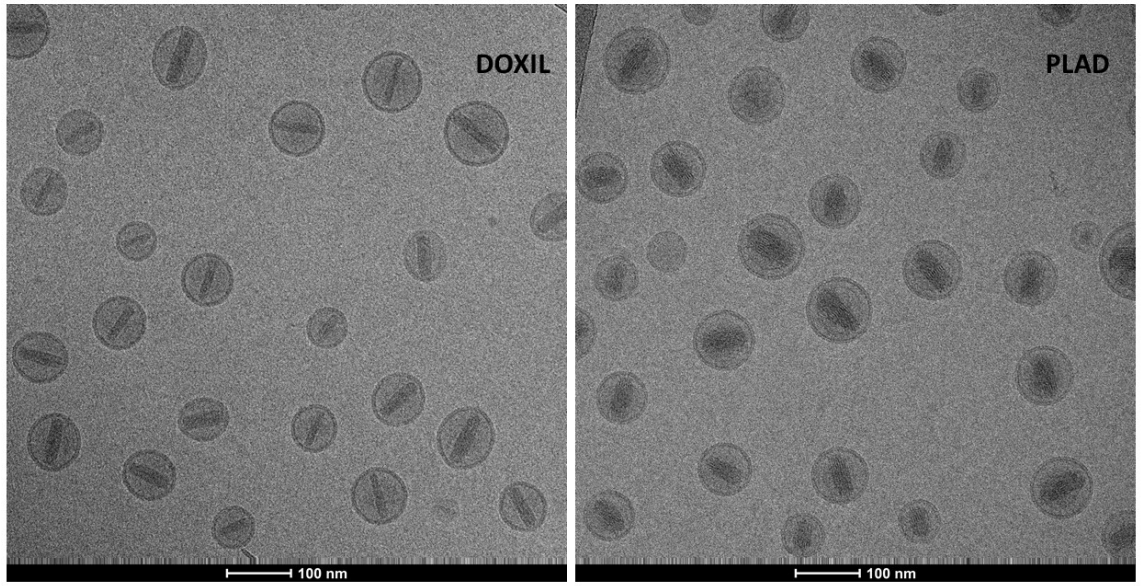
Co-Encapsulation: The PLAD example

(pegylated Liposomal alendronate-doxorubicin)



Dox loading using ammonium alendronate gradient

Doxil and PLAD liposomes by cryoTEM



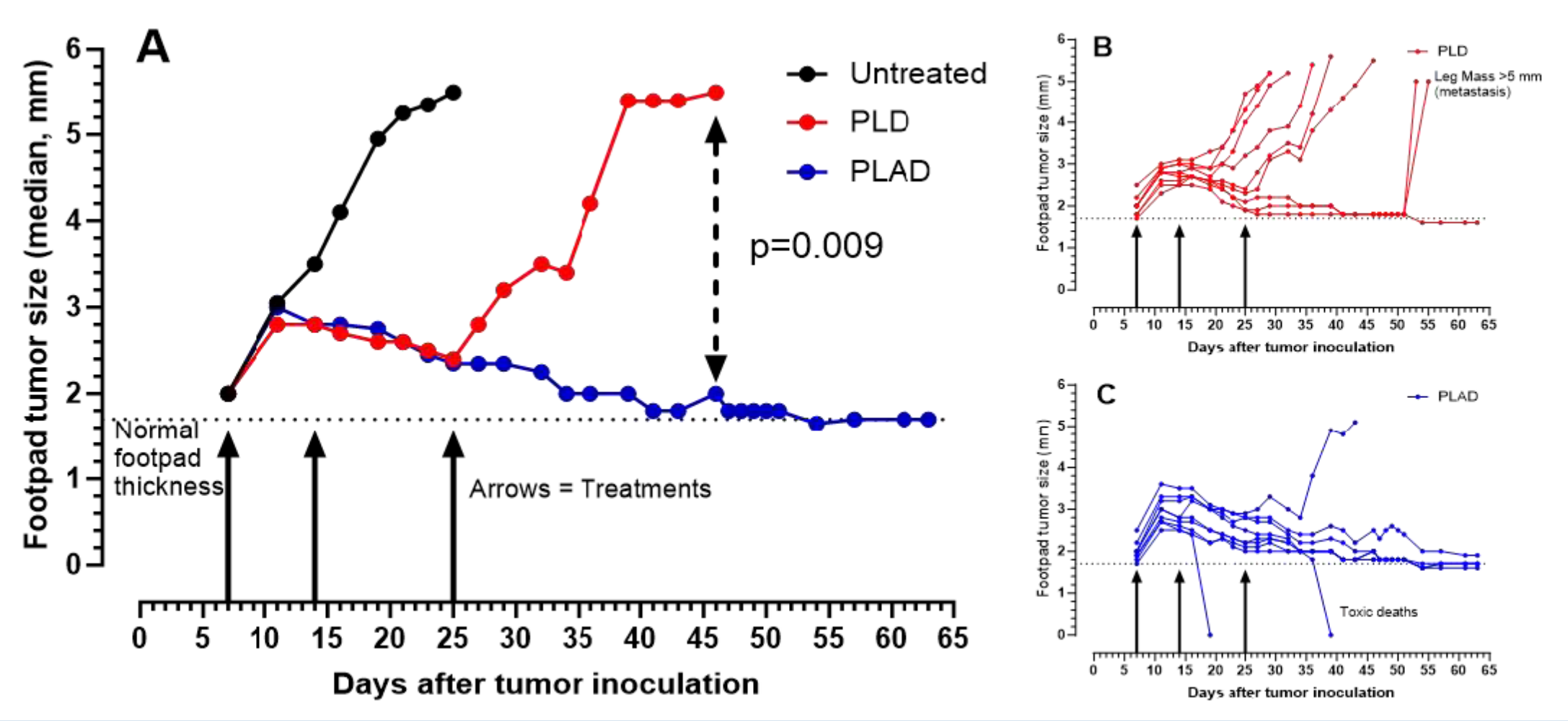
Doxil: thin, long rods

PLAD: thicker, shorter rods

Doxorubicin (cytotoxic) +
Alendronate (immuno-modulatory through interaction with TAM & activation of δ T Cells)

Gabizon et al, Pharmaceutics, 2023

Greater Therapeutic Efficacy of PLAD compared to DOXIL (PLD) in 4T1 triple negative breast cancer model

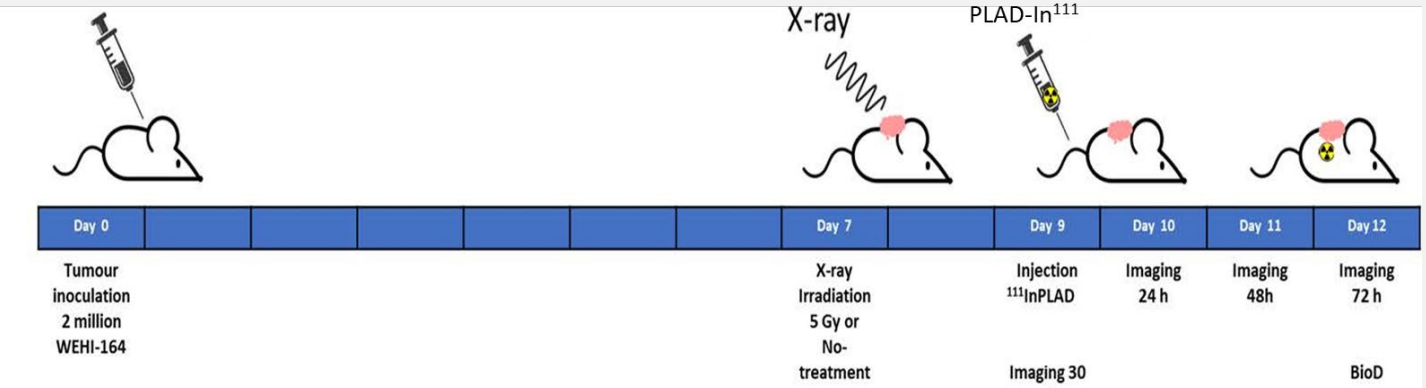


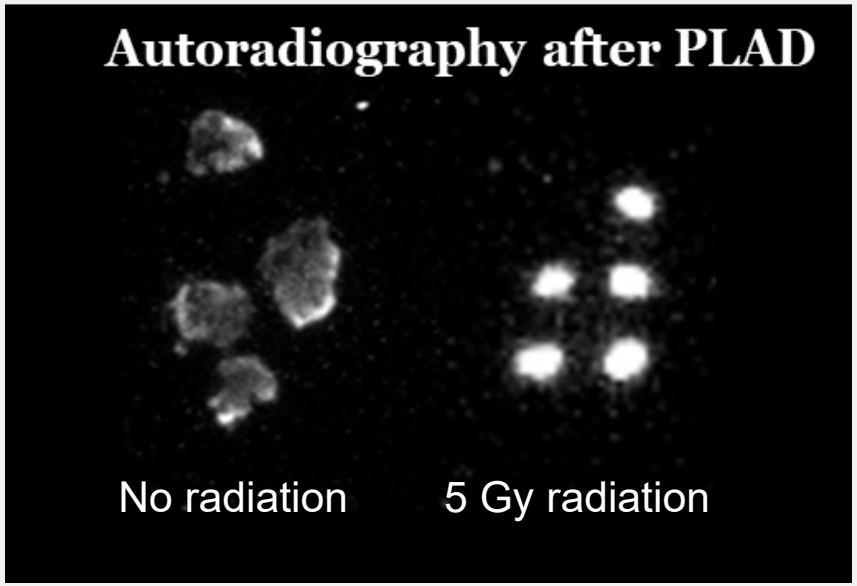
Gabizon et al., Pharmaceutics, 2023

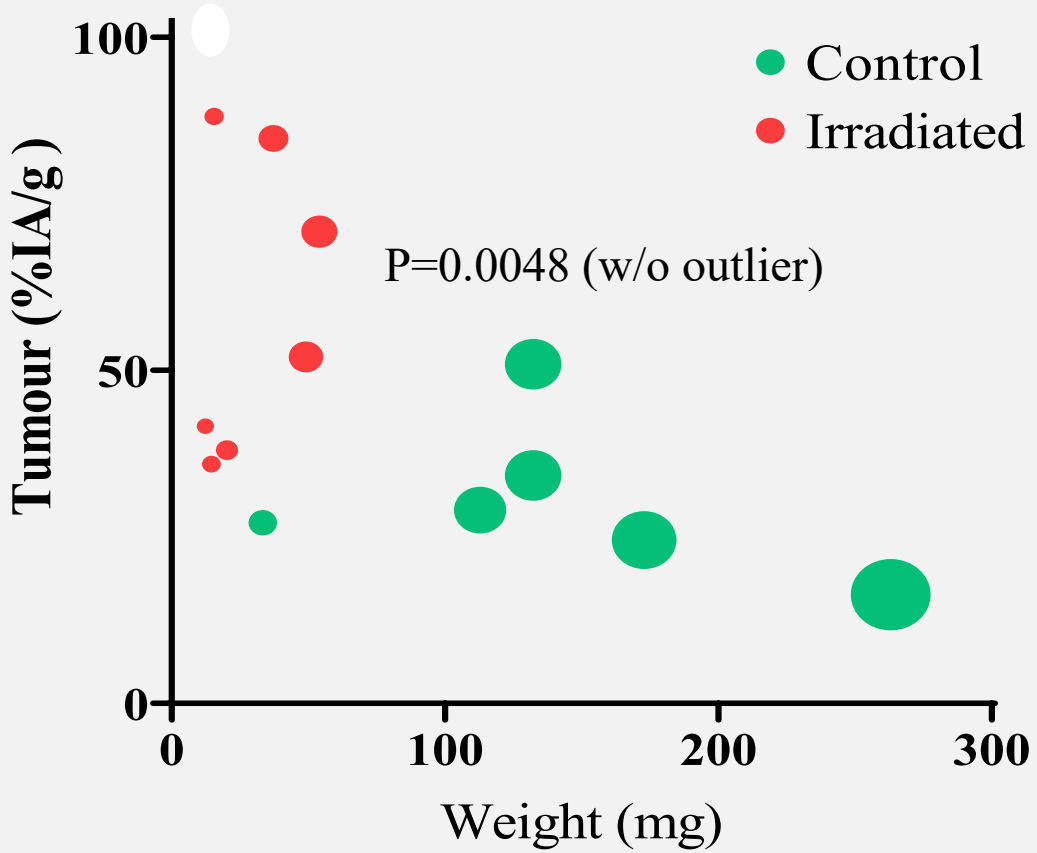
Multimodality Strategies in Nanomedicine

1. Nano-Theranostics: Combination of a nanodrug and an imaging agent for real time imaging and concomitant therapy
2. Nano-Radiotherapy: Combination of nanodrugs with radiotherapy (or other physical methods: HIFU, Hyperthermia, RFA, PDT) to enhance EPR and treatment efficacy
3. Nano-Immunotherapy: Combination of nanodrugs with immunotherapy.
4. Nano-Mechanotherapeutics: Combination of nanodrugs with mechano-modulators acting on tumor vessels/TME to enhance EPR and treatment efficacy

Radiotherapy increases tumor liposome uptake,

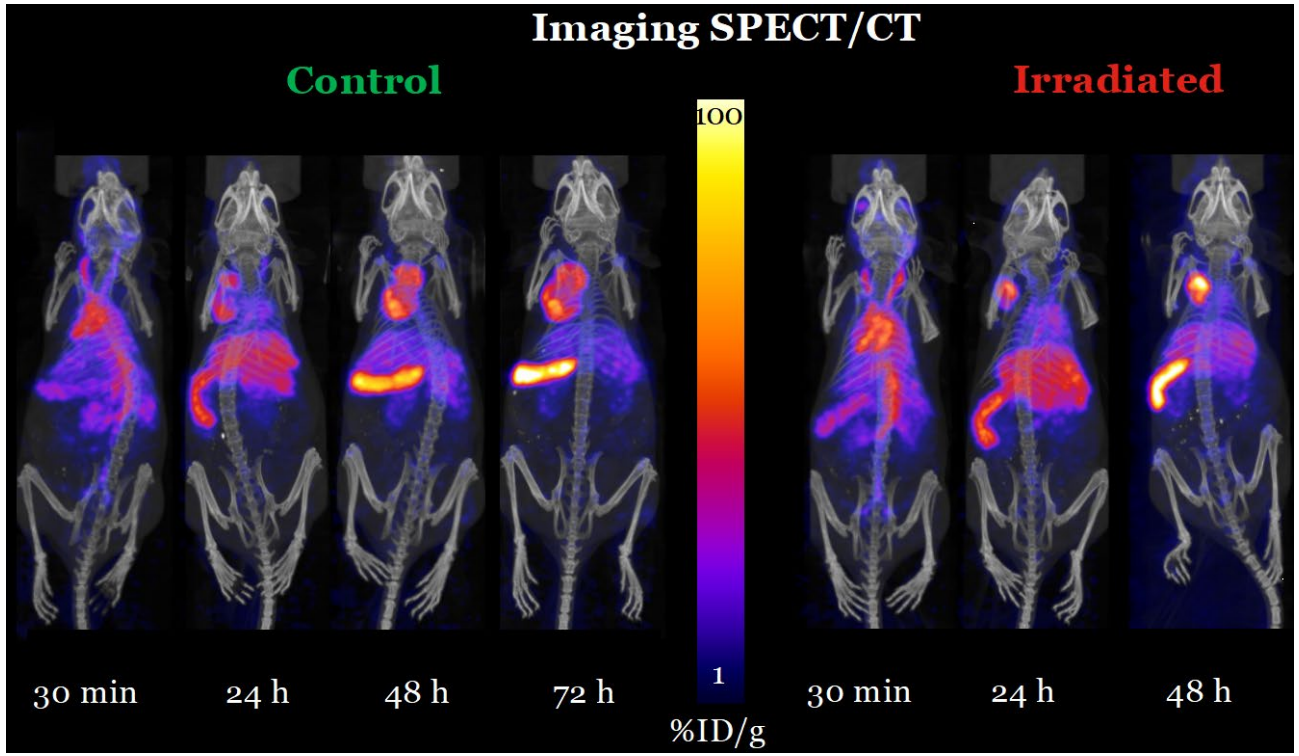






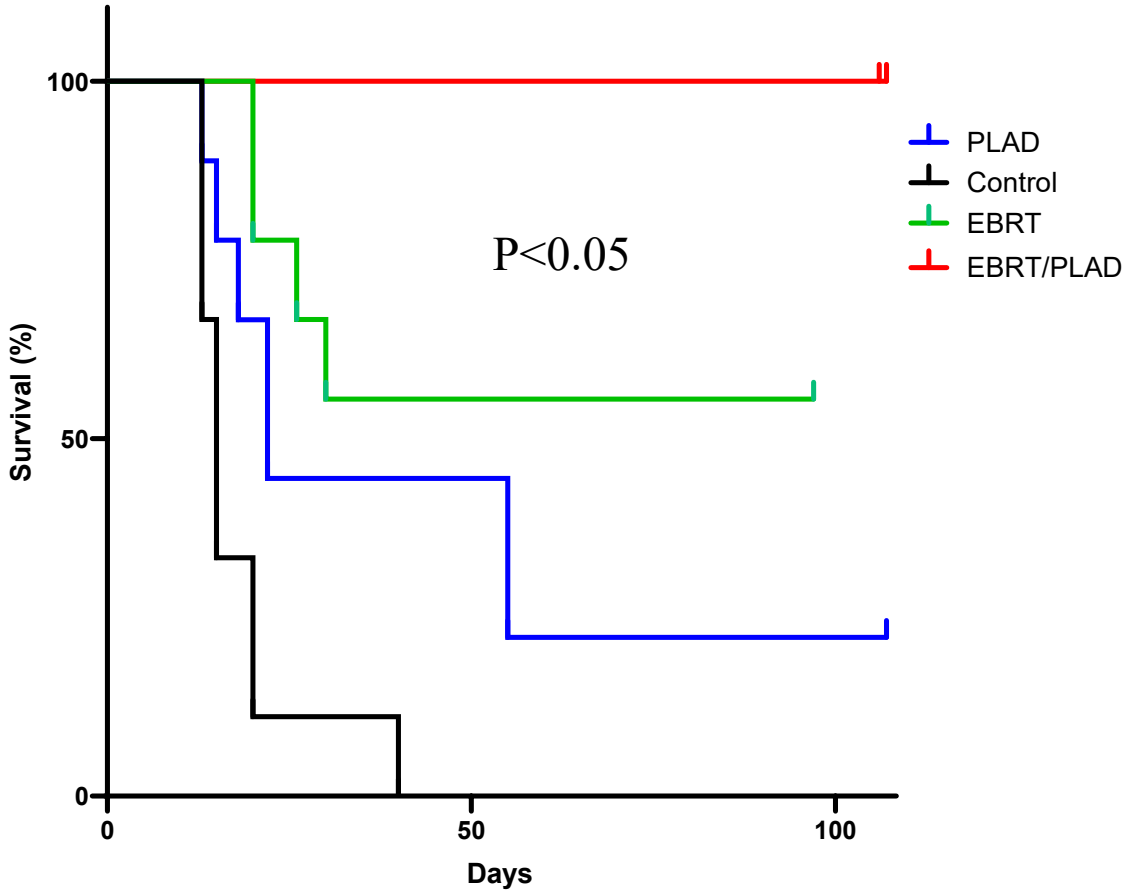
Collaboration with: RTM de Rosales, Kings College

Nonirradiated tumors are larger and show decrease uptake of radiolabel



All mice injected with low dose PLAD

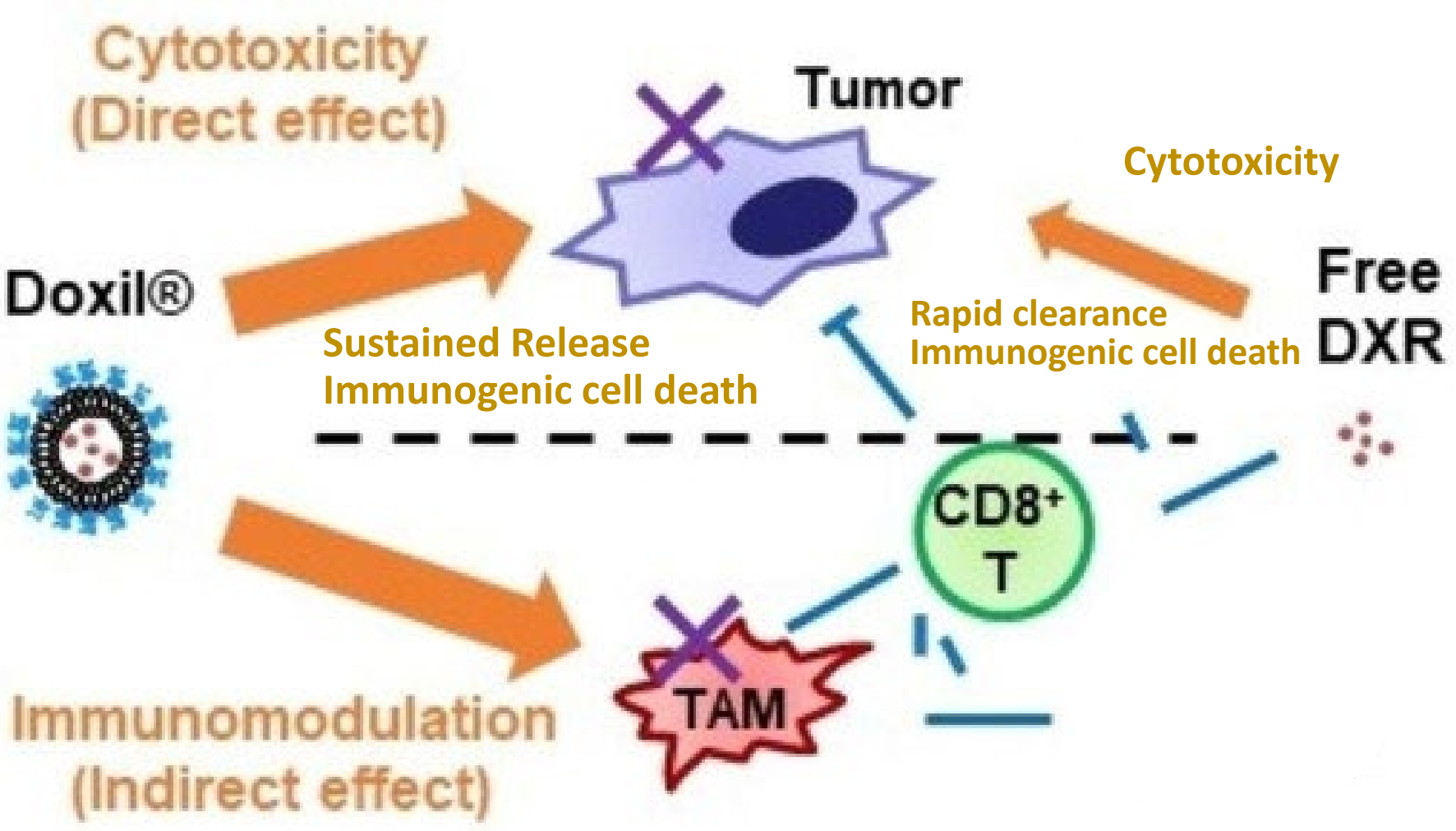
Increased Survival and Cure Rate with PLAD+RT



KING'S College LONDON

Amaia Carrascal-Miniño, Rafael TM de Rosales (unpublished results)

Rationale for PLD (Doxil) in Chemo-Immunotherapy with Anti-PD1: Sustained release and inhibition of tumor-associated macrophages (TAM)



Immunogenic Cell Death stimulates recruitment of Effector T cells which will destroy residual tumor cells as long as PD1 is blocked and inactive

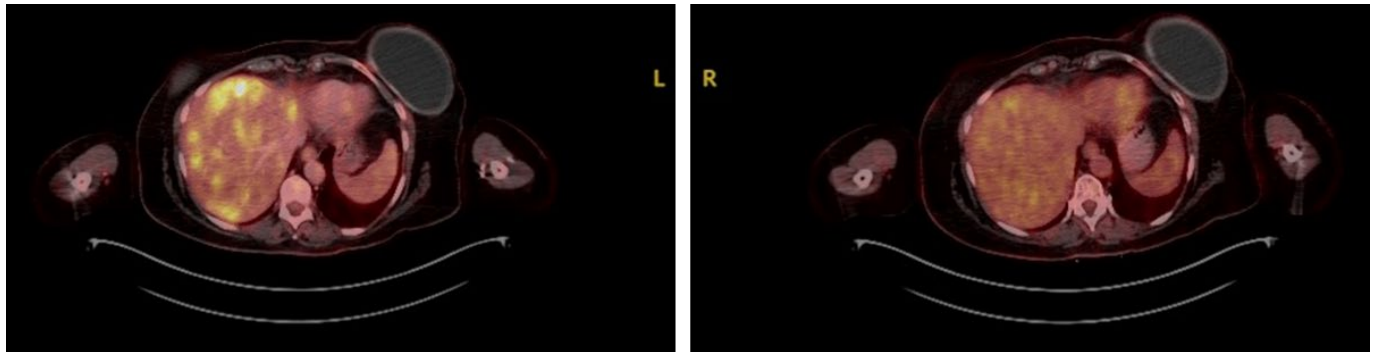
“Phase 1B study of chemo-immunotherapy with PLD (Doxil) and pembrolizumab in metastatic ER+/Her2- breast cancer”

PET/CT evaluation after 3 cycles

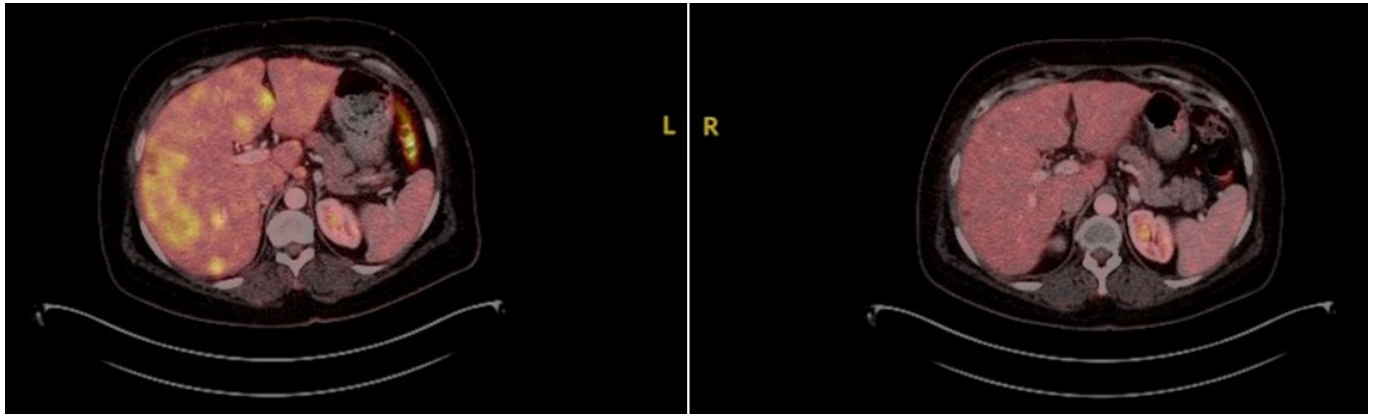
Response	N patients
PR/CR (6/8 responses in pts with liver metastases)	8 (6/2)
SD	5
PD	5

Response Summary	Evaluable, N=18
ORR	44%
CBR	72%

#15: Apr 21 → Sep 21

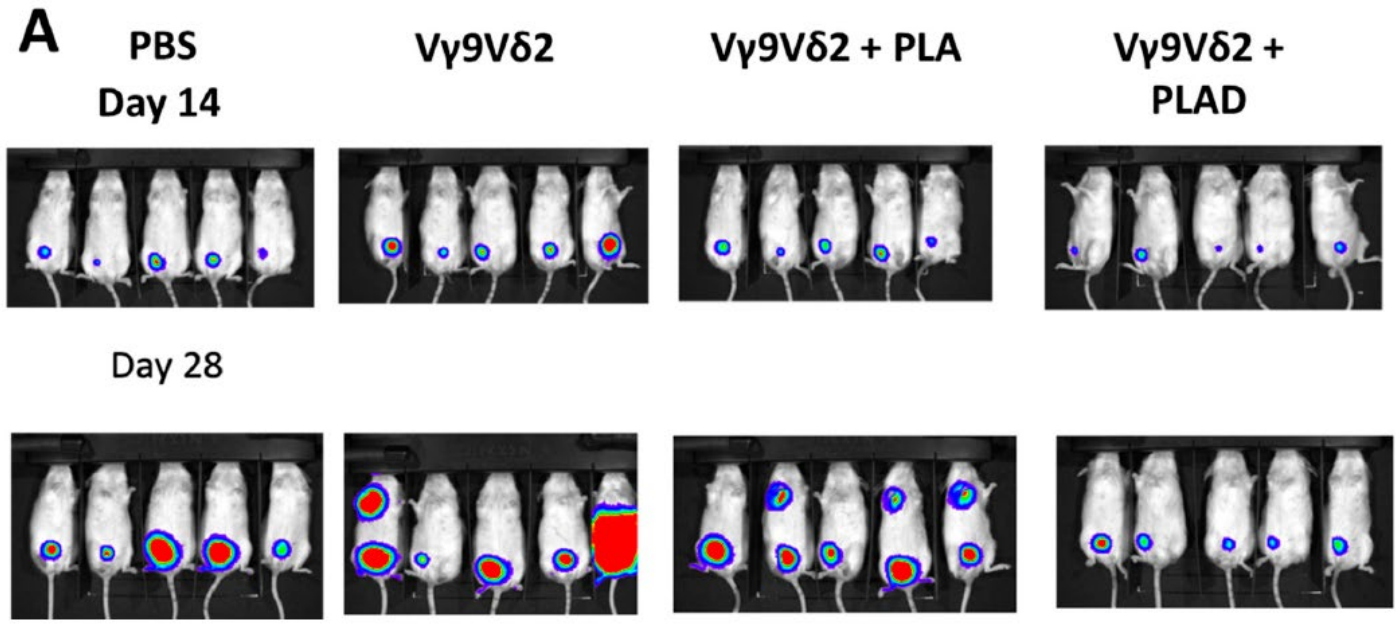


#21: Mar 22→-July 22

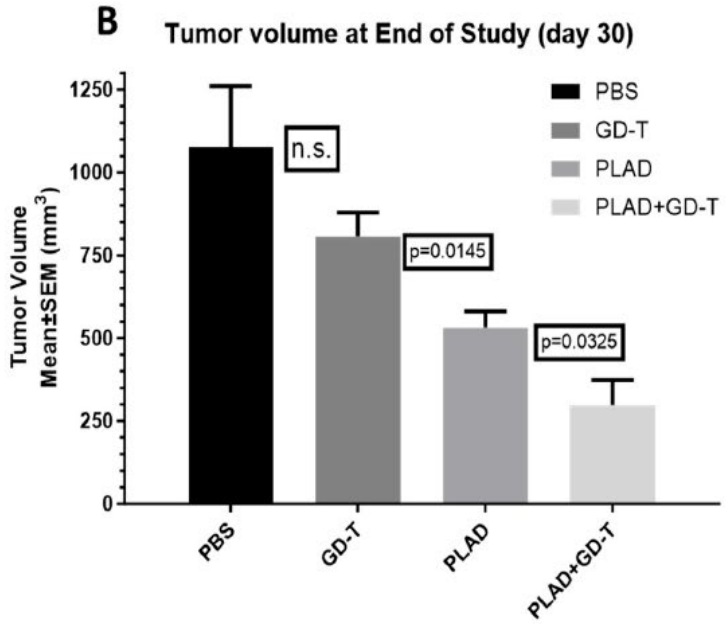


#10: July 20→July-22

PLAD and Immunotherapy



- Inject 1x10^5 MDA-MB-231 cells into mammary fat-pad
- Inject liposomes iv after 21 days
- Inject Vγ9Vδ2 T-cells iv 3 days later
- BLI weekly with luciferin



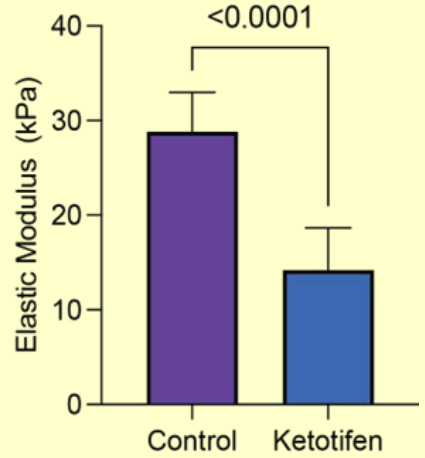
PLAD co-treatment improves the antitumor efficacy of gamma delta T cells

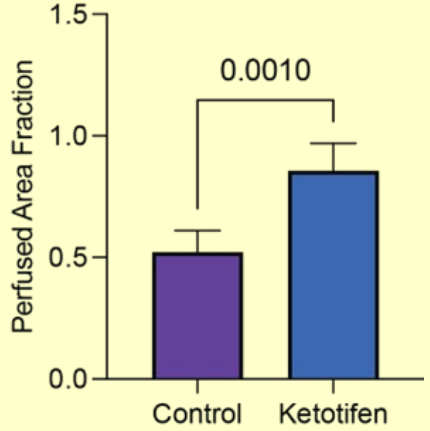
Gabizon et al., Pharmaceutics, 2023

Nanomedicines, Immunotherapy and Mechanotherapeutics

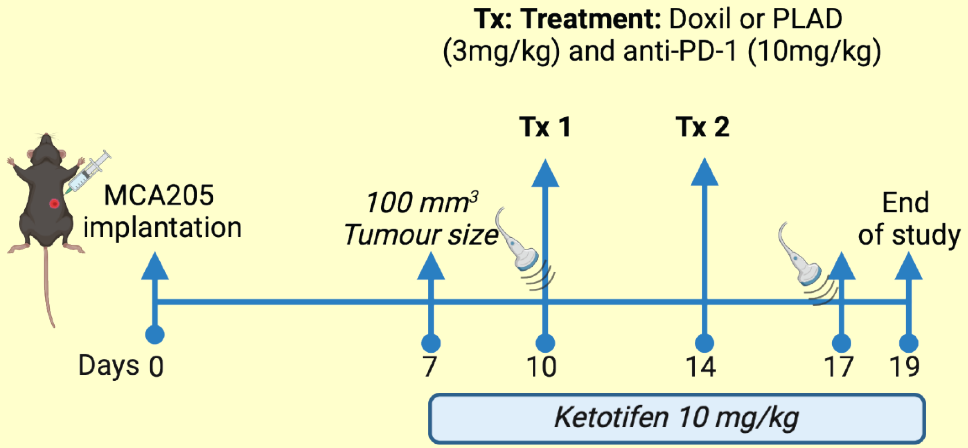
- Reducing the IFP and stiffness of tumors allows for better penetration of nanoparticles

- Ketotifen, H1-blocker and mast cell stabilizing agent, can change the mechanical properties of tumors and lead to better therapeutic outcomes of nano-based (PLAD and Doxil) and antibody-based (Anti-PD1) treatments



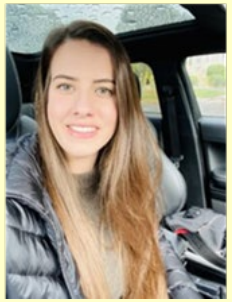


Experimental Design

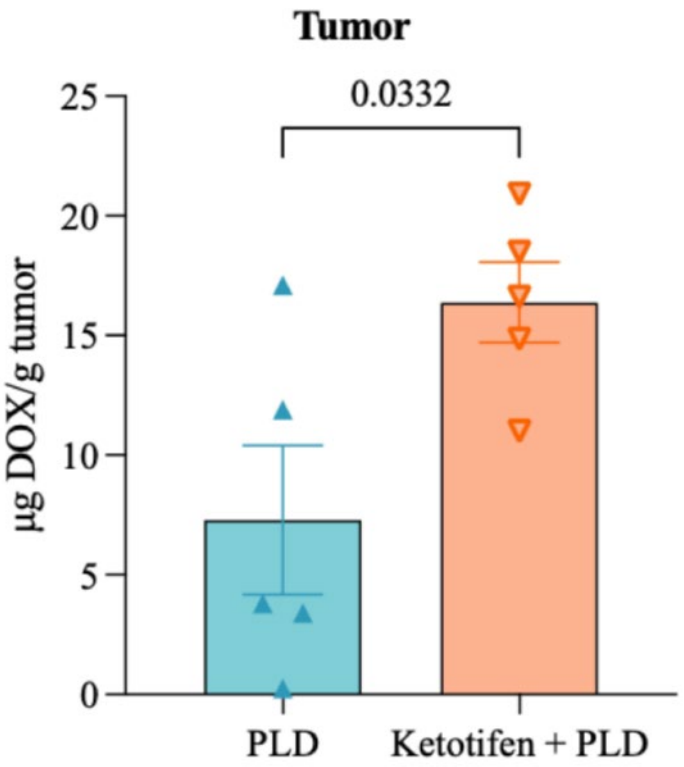


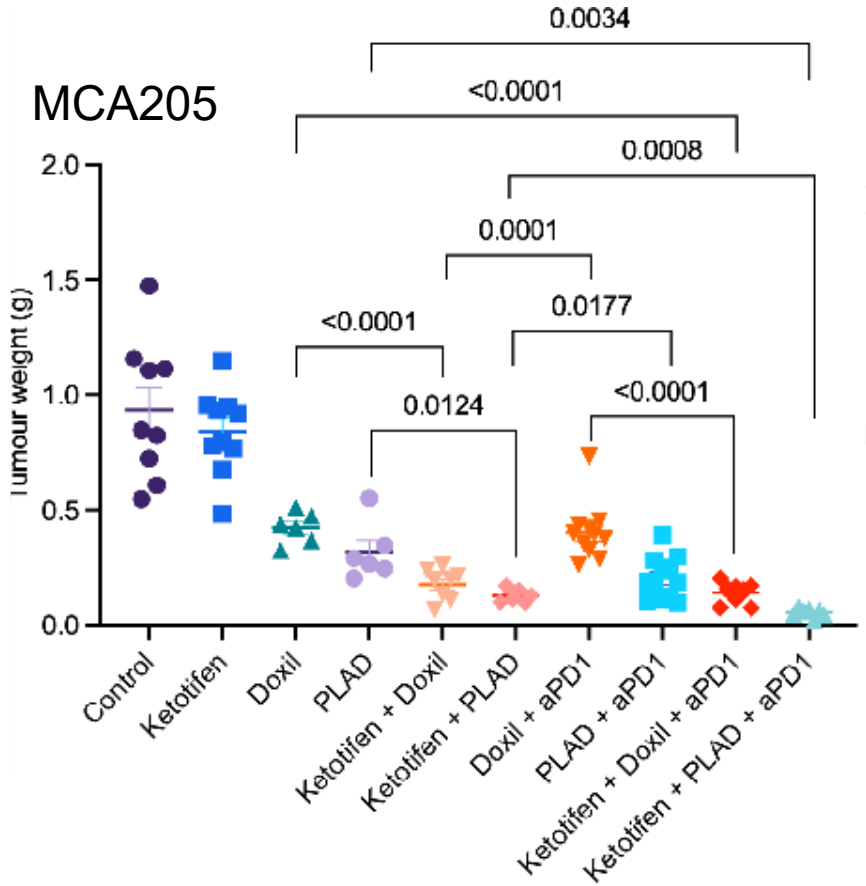
Triantafyllos Stylianopoulos

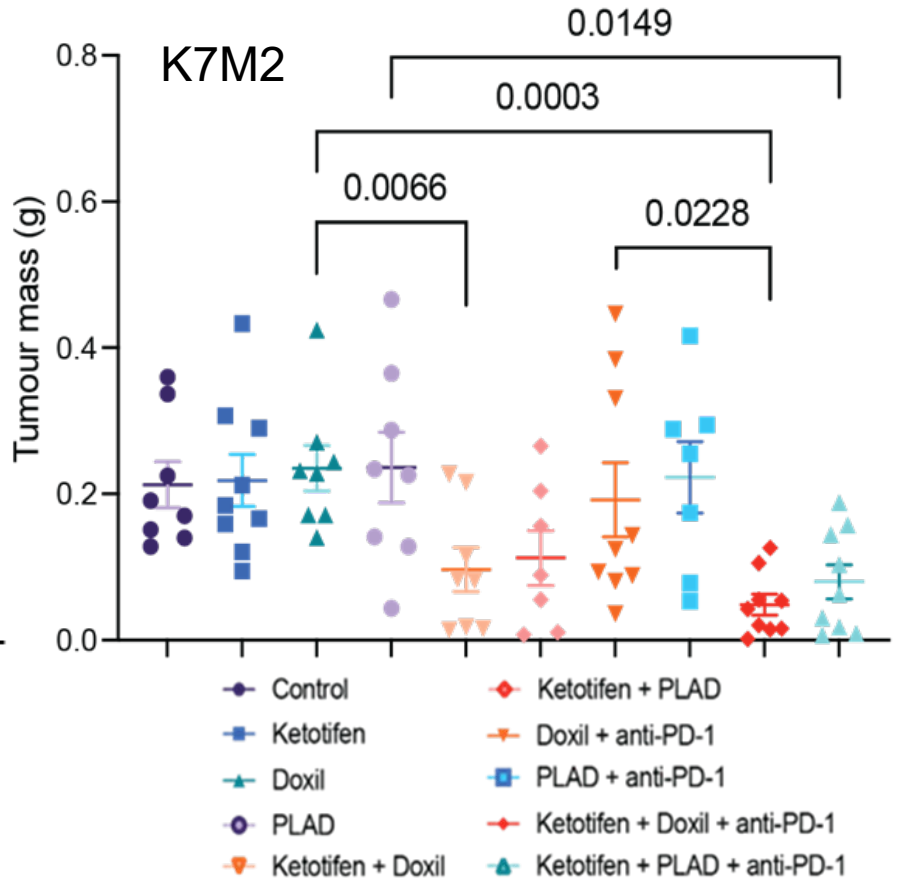
Antonia Charalambous



Ketotifen TME reprogramming increases drug delivery and improves nano-immunotherapy in MCA205 sarcoma and K7M2wt osteosarcoma







Ketotifen+PLAD/Doxil+aPD1 are the most effective arms in these studies

Molec Cancer Ther., in press

Future Prospects -Take-Home Message

- Liposomes and LNP offer an attractive delivery tool, yet underexploited, for Lipid-based Drugs and Prodrugs.

- A rational approach to multimodality therapy can help realize the full pharmacological potential of nanomedicines and transform a low value-added product to a highly successful therapeutic tool with qualitative edge over single agent therapy.

- The interaction of nanomedicines with the immune system has significant advantages over that of standard chemo-immunotherapy

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THANK YOU

PRAYING FOR PEACE