

Development of liposomal drugs And Nano-Drugs: From academic research via incubators and startups to FDA and EMA approved products

Part I: Science and Technology

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Doxil: 80 – 100 nm SSL
remote loaded with
doxorubicin via ammonium
sulfate gradient. **18 years
to Doxil 1st FDA approved
nano-medicine (11.95)**

Today Agenda

- General difficulties in current drug development
- NMII MLV for osteoarthritis treatment
- Doxil the first FDA approved drug and its MOA (in short)
- Why 3 years after Doxil relevant patent expiration FDA approved only one generic product, Lipodox?
- Lessons learned for the development of novel nano-drugs
- LC100 new generation liposomal doxorubicin with less side effect and better efficacy than Doxil
- Scientists as entrepreneurs: a user's guide & personal experience

Drug development: from basic research to approved drug

The current chances

It was shown that for every 1,000 compounds that reach testing, only 5 make it to advanced clinical trials and less than 1 is ultimately approved by the U.S. Food and Drug Administration (FDA).

Namely chances for success are very low, the driving force for development in spite the failure is the large reward in case of success.

Ideas to increase of success chance will be discussed

How to improve the chances?

By orienting Research and R & D programs to focus on:

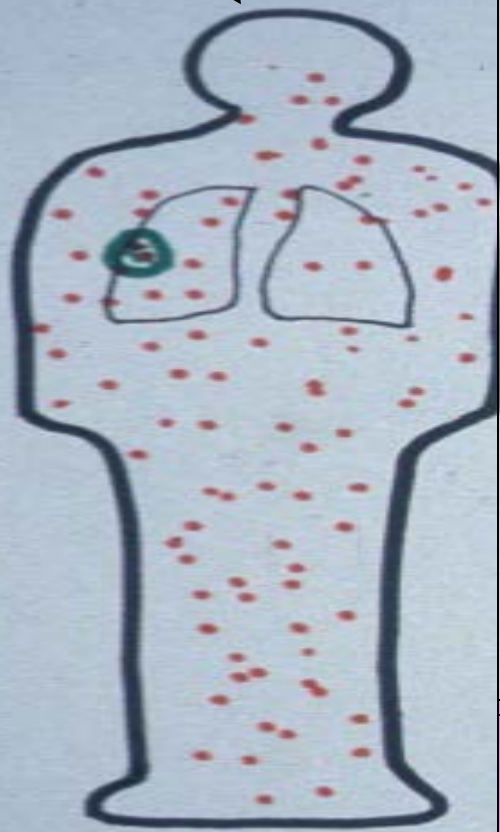
1. Leading emerging targets in specific therapeutic areas
2. Methods of breaking through the deficiencies in target-based drug discovery and drug performance.
3. Discovering better lead molecules and their optimal delivery for targeting unmet needs
4. Achieving proof of principal that can translate into human clinical trials
5. Good coordination between all involved in the development including good cross talk between basic and applied research!!! and between university researchers and TTO (technology transfer office) regarding I.P. and “marketing package” as well as the right strategic/financial partner(s).

Each of these points require having rational decision making processes, which will be demonstrated.

Barenholz Lab applied research and its status

- “Theoretical” and general aspects of DDS, science and technology
- QC methodologies
- *Cancer therapy, Doxil LTI (Sequus) to ALZA to J n J & LC100 Lipocure)*
- *Vaccinology (NasVax) → >*
- *Inflammatory and autoimmune diseases (Omri to Lipocure MS, RA, Lupus, **on the way to clinical trials**)*
- *Infectious diseases therapy*
- Lipid-based signal transduction
- *Local anesthetics (**Lipocure on the way to a clinical trial**)*
- *Osteoarthritis (cartilage lubrication and reduction of wear, Moebius Medical (**Finished clinical trial**))*
- Gene delivery (mostly basic)
- Environmental (LipoGreen)

What is all about? Why nano-drugs? Most current anticancer drugs are highly efficacious in cell culture however in vivo and especially in humans their performance is not good enough due to low efficacy and high toxicity, a result of inferior body distribution. Successful “magic bullet approach” (P. Ehrlich concept) is expected to change the body distribution thereby improving dramatically the drug performance.



3 types of parenteral targeting:

Promoter or enzyme specific targeting

**Cell surface specific (active)
By ligand**

**Disease specific
vasculature dependent
Targeting
EPR effect**

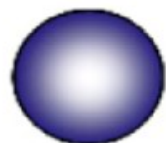


Another simpler approach is local administration



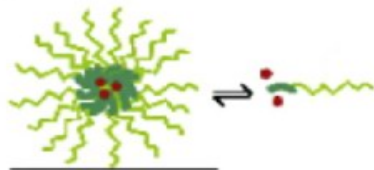
How to select the best nano-drug delivery systems (DDSs) from big list of available systems

Nanoparticles



50-200 nm

Polymeric micelles



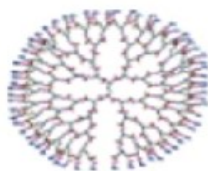
20 nm

Liposomes



40 nm - 400 nm

Dendrimers



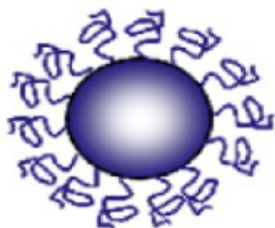
1-10 nm

Polymer-drug conjugates

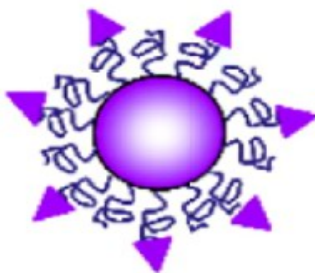


6-15 nm

PEGylated nanoparticles



Liganded PEGylated nanoparticles



Definition of a nano-drug

In agreement with definitions of Nanotechnology

In order to be referred to as nano-drugs the particles loaded with drugs have to be superior to larger particles carrying the drug as well as to the drug given “as is” in a none-particulate form

Liposomes are different from most other nano particles and carbon nanotubes

- Liposomes are:
- Biocompatible
- Biodegradable
- Not immunogenic
- Familiar to the scientific community
- Have known pharmacokinetics and biodistribution
- Have known metabolism

Therefore from Toxicology point of view they have advantages on most other nanoparticles and nanotubes for medical/pharmaceutical application and for consumer products including cosmetics and cleantech

Any drug development
is multidisciplinary in
nature

Liposome Based Drug
Delivery System

will be developed only if it is

*First important strategic
decision*

Advantageous to solve a clinical problem !!!

dependant upon

ENGINEERING OF DDS
FORMULATION

SUITABLE ANIMAL
MODEL

based upon

STABILITY
OPTIMIZATION

QUALITY
CONTROL
ASSAYS

OPTIMIZATION
Of CARRIER
TYPE and
COMPOSITION

BIDISTRIBUTION AND
PHARMACOKINETICS
of DRUG AND CARRIER
Personalized DDS

EFFICACY

Toxicology &
nano-
toxicology

include

CARRIER

DRUGS

determine

STABILIZATION
IN BODY
FLUID(S)

CHEMICAL
AND
PHYSICAL
STABILITY

DRUG\
AMPHIPHILE
RATIO

HYDRATION
PHASE AND
COMPRESIBILITY

ELECTRICAL
CHARGE

THERAPEUTIC
INDEX

Concept map describing development of DDS formulations

Today we have all the tools needed to apply a sensible approach for the design of Liposome based DDS:

The goal/means cause/effect relationship approach

Goal/means

Goals/means

Performance

Treatment of
metastatic
tumors

Properties

Structure

Processing

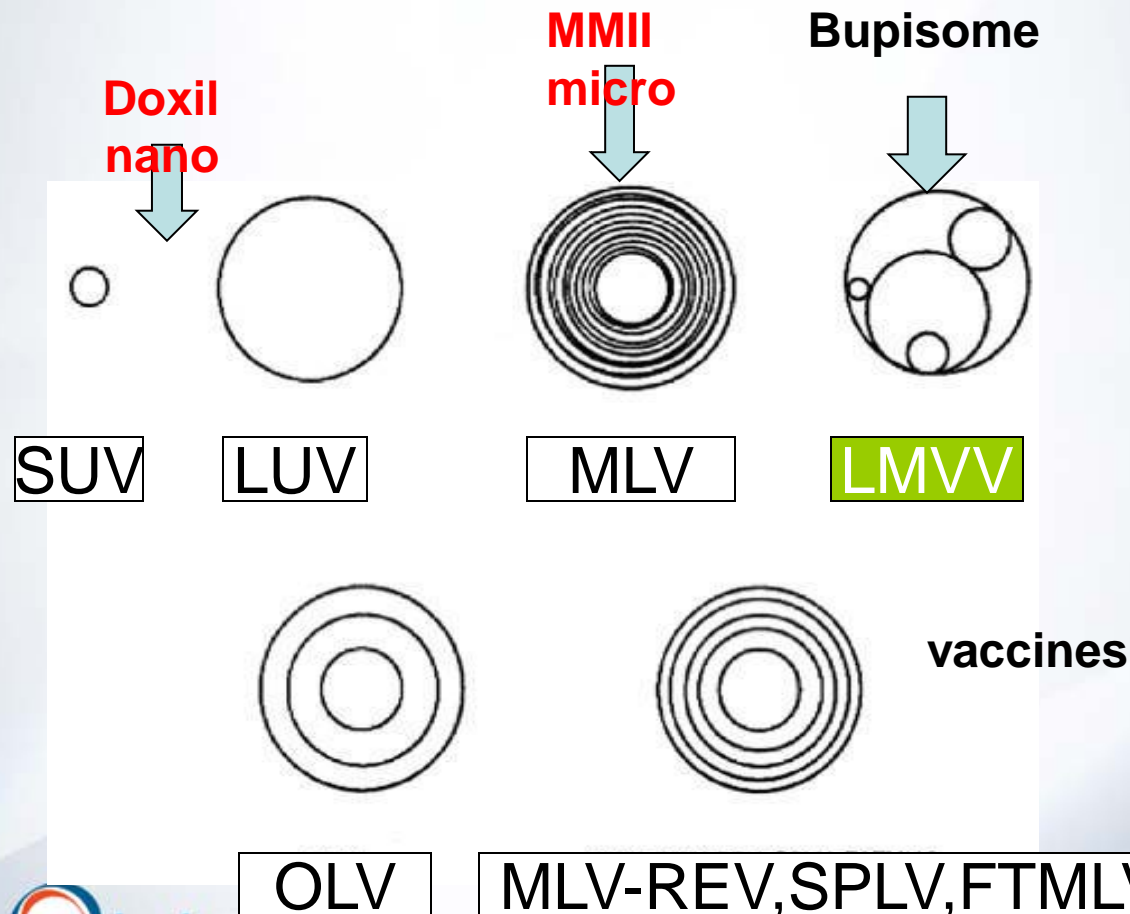
Cause/effect

Cause/effect

Liposomes

Are classified by their size and lamellarity.

Today we will deal with 2 liposomal drugs: the anti cancer nano-drug Doxil for systemic administration and the micro-medical device NMII for treatment of osteoarthritis

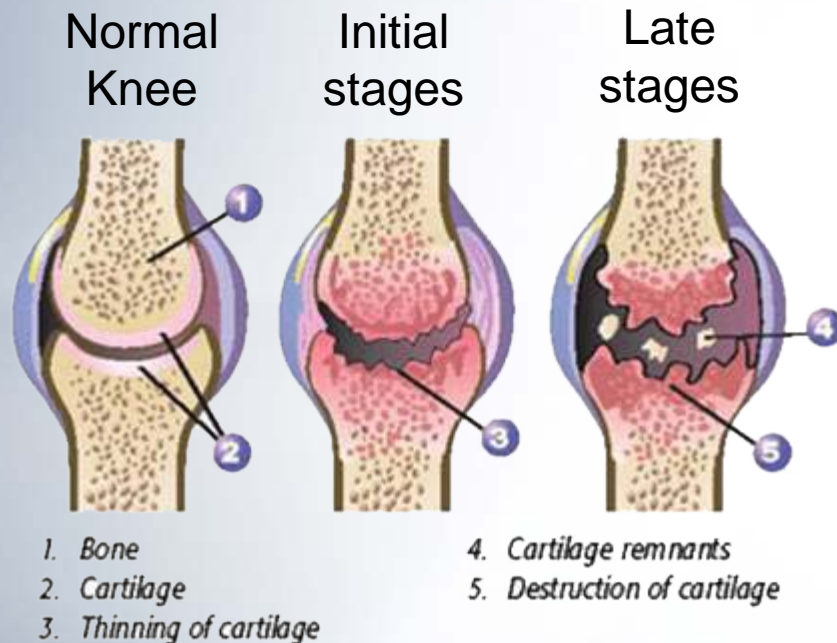


Osteoarthritis (OA) Treatment

NMII MLV Licensed to Moebius Medical LTD (incubator)

Osteoarthritis a major disease >1% of the population

The Issue: Cartilage destruction due to wear increases friction further accelerate wear and leads to pain & Inflammation (osteoarthritis = OA)



Treatments

Corticoids (oral) - ↓ inflammation

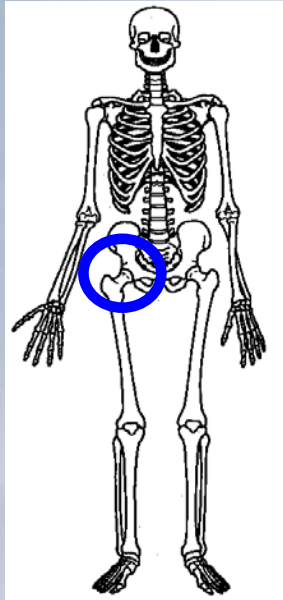
HA (I.A.) - ↑ viscosity

Liposomes – (I.A.) ↑ boundary

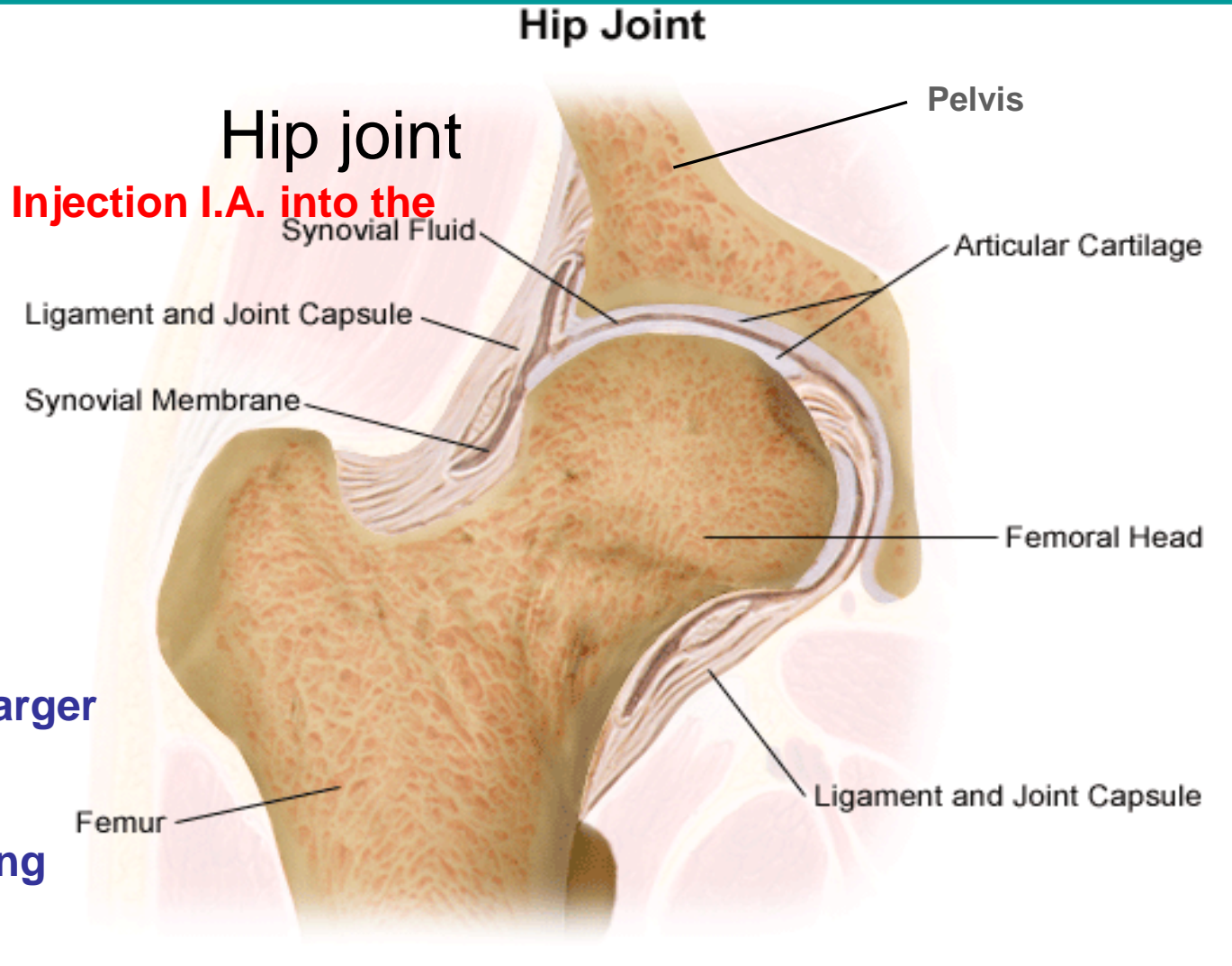
lubrication

We suggest that local liposome treatment may affect directly the disease cause by improving lubrication thereby and reducing cartilage wear !!!

Use of liposomes in joint tribology



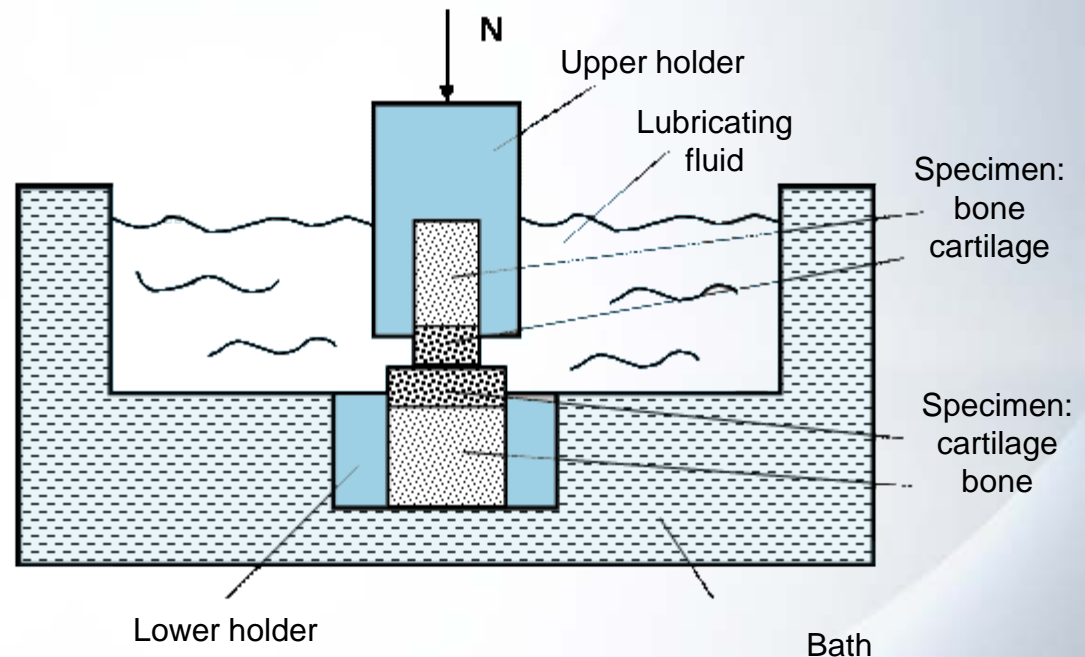
Size has to be larger than 250 nm as the synovial membrane having 250 nm pores!



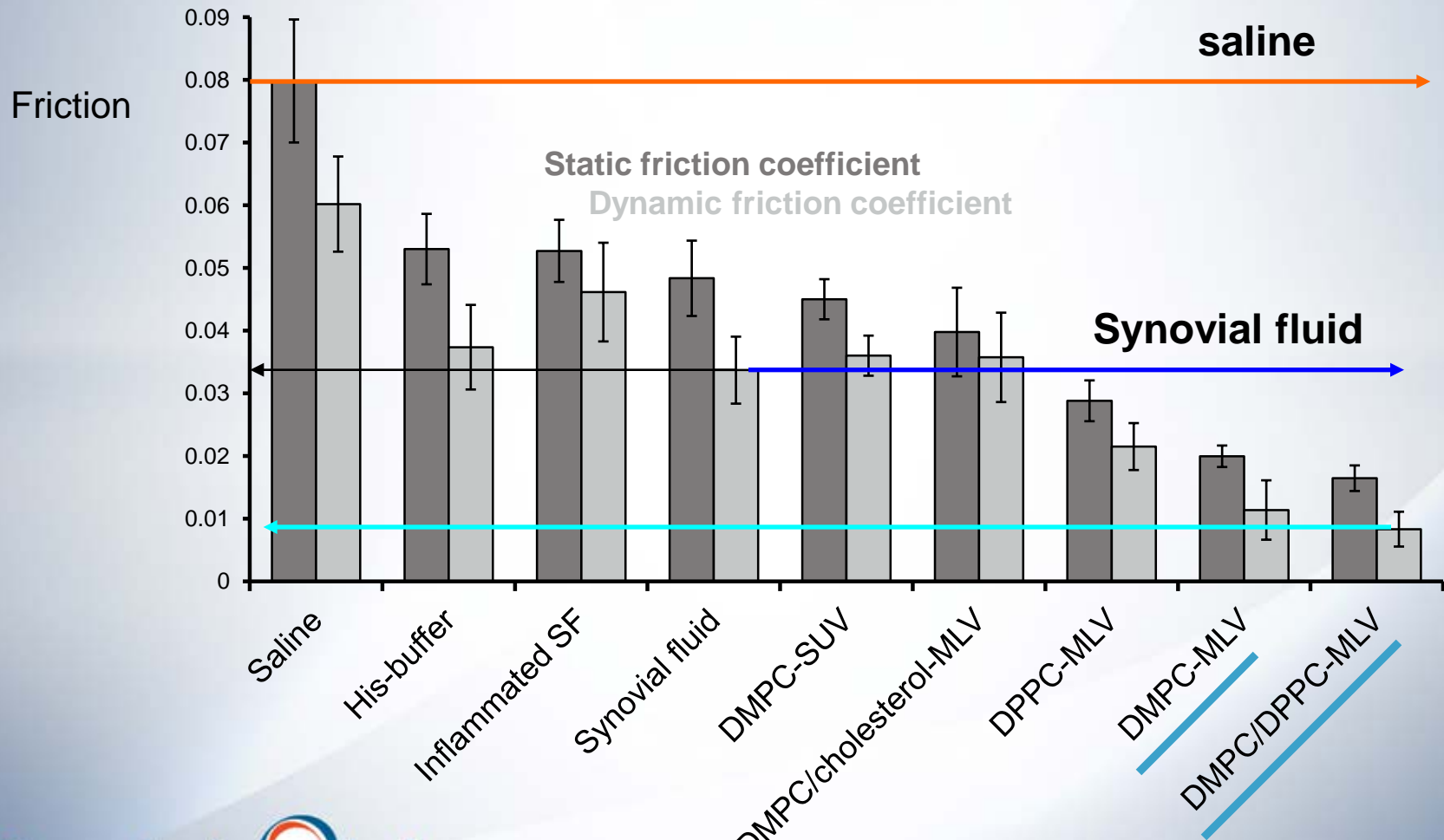
Screening for the optimal liposomes as lubricants and wear reducers

(Lipid composition and liposomes structure)

An ex-vivo cartilage-on-cartilage setup was developed in order to compare the lubrication and wear reducing capacity of different potential lubricants

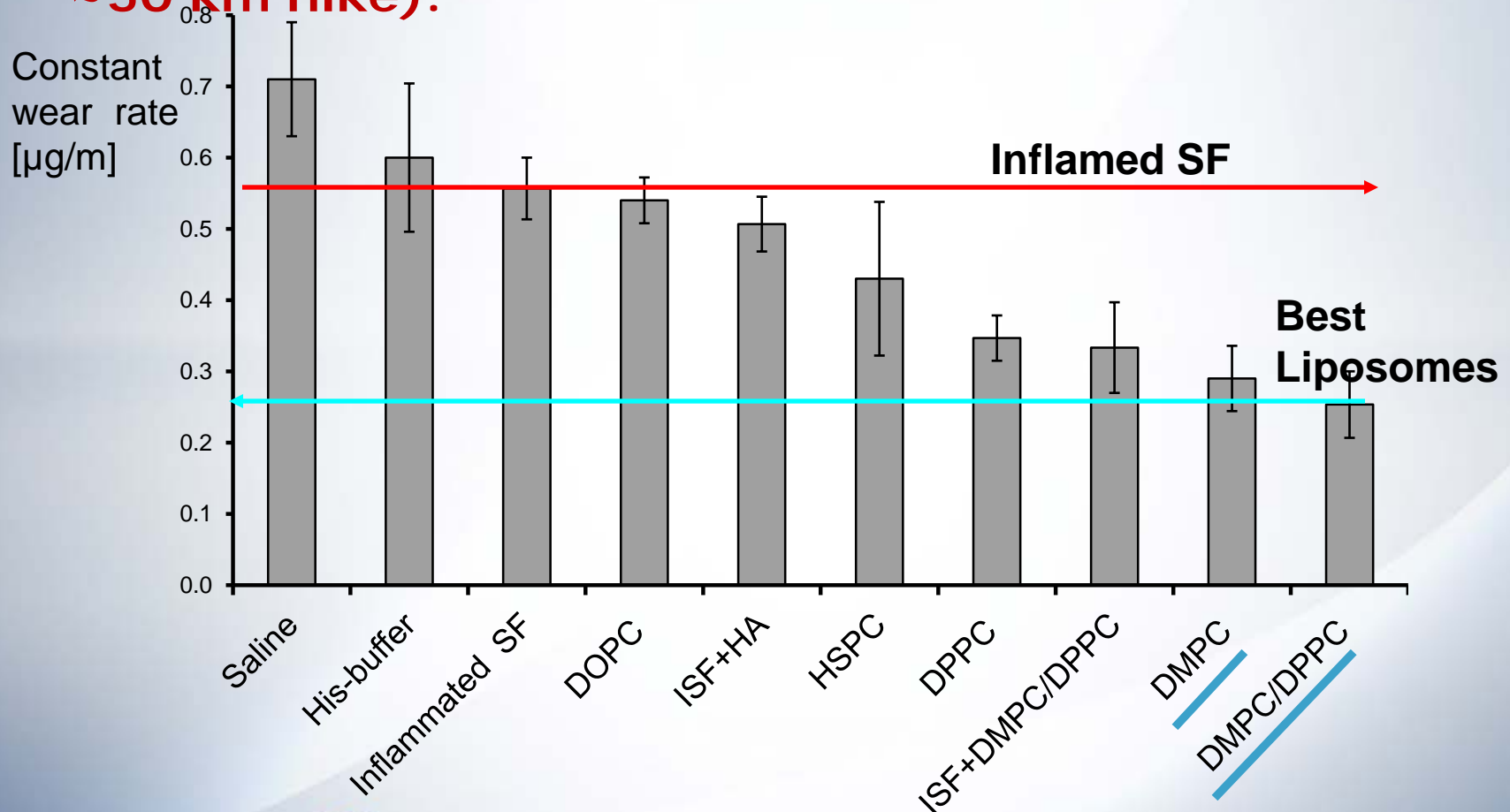


Finding the Optimal Liposomes – Lubrication: DMPC/DPPC- and DMPC-MLV are superior lubricants of human cartilage



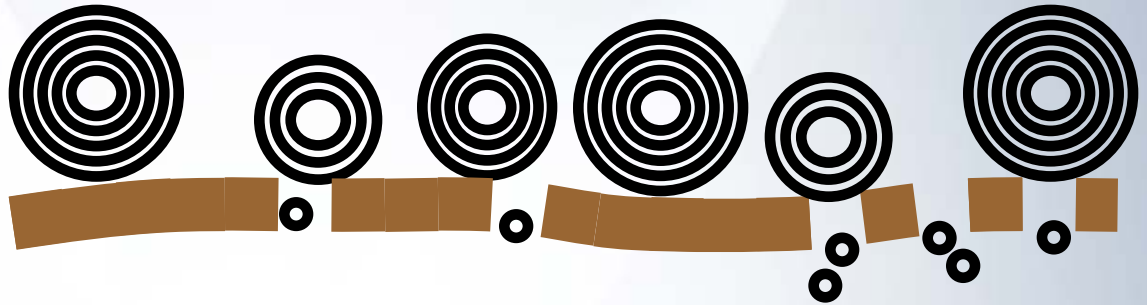
Finding the Optimal Liposomes – Wear Reduction

The liposomes which are the best lubricants
DMPC/DPPC and DMPC MLV are also the best cartilage
wear reducers (under condition which imitate long
~50 km hike).



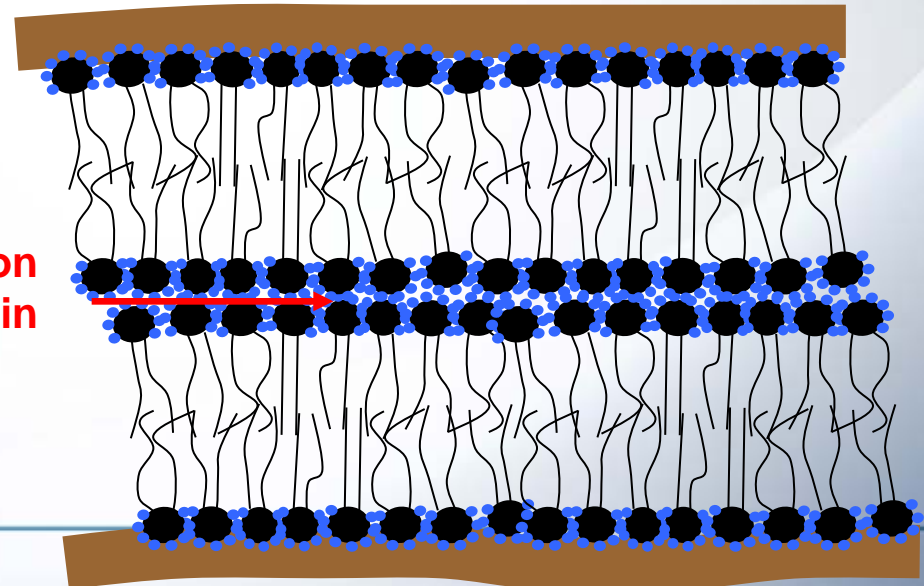
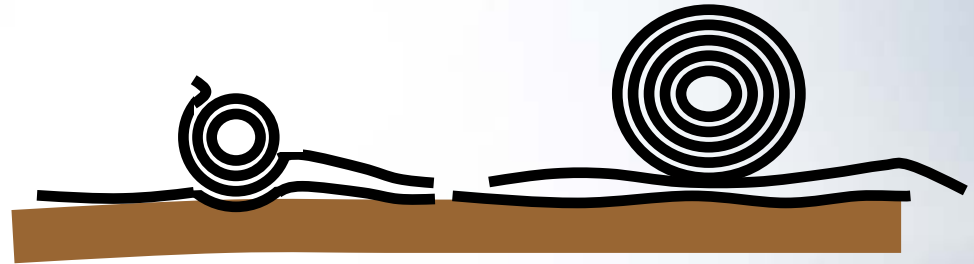
MoA: Hydrophilic Lubrication (fits J. Klein concept)

multi-lamellar vesicles (MLV) remain on and near cartilage surface, while small unilamellar vesicles (SUV) penetrate deeply into cartilage



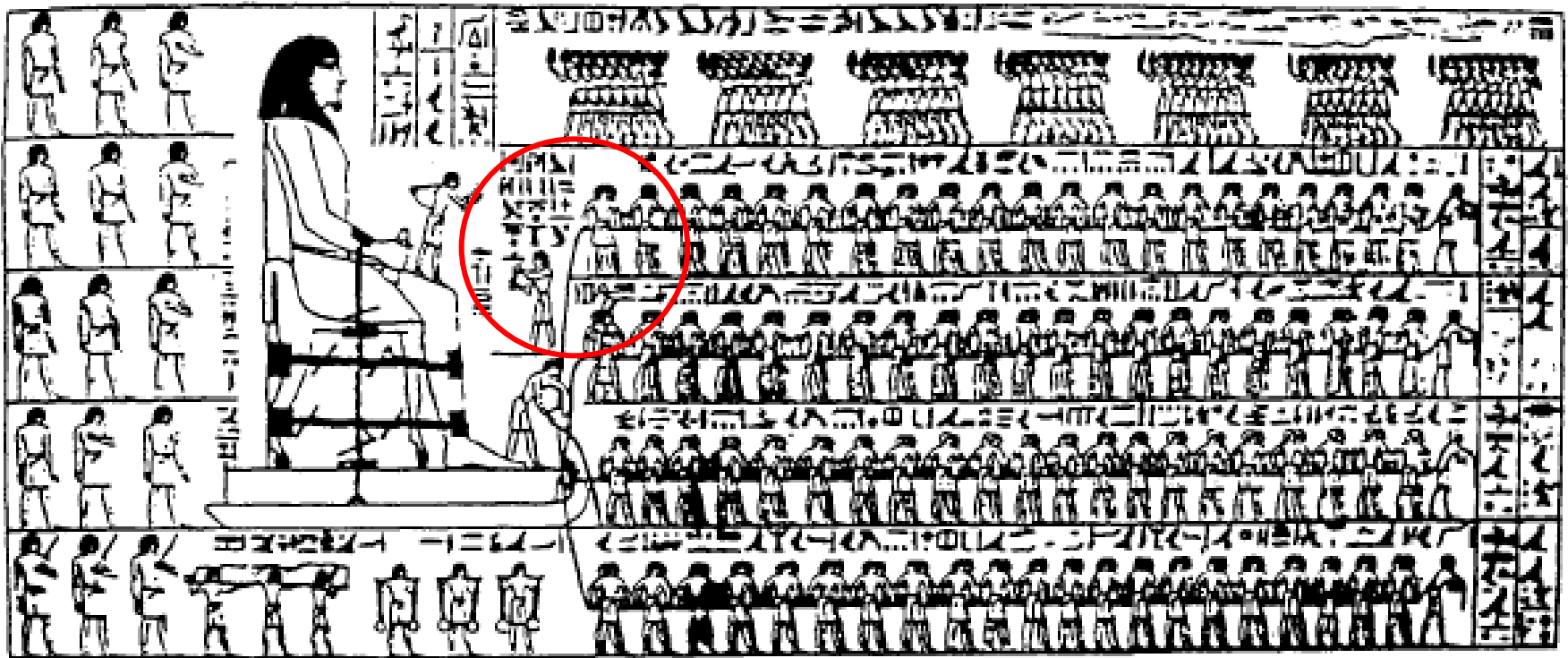
Hydrophilic Lubrication:

MLV, with phospholipid bilayers in the LD phase, readily delaminate by pressure deposition on opposing cartilage surfaces. Each of these 2 bilayers have highly hydrated 10-12 H₂O per PC head-groups (blue) which serve as **nano ball-bearings**, thereby facilitating very low friction joint motions



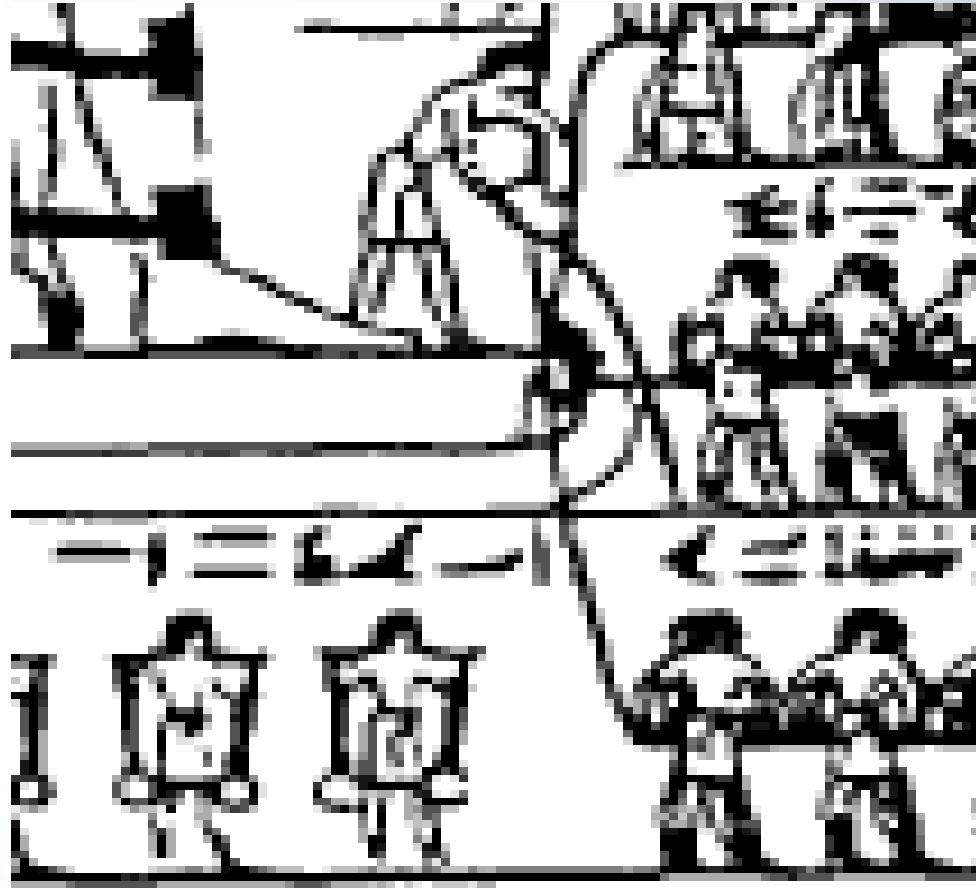
Based on biology hydrophilic lubrication may be better than the well known (to mechanics) hydrophobic lubrication. The Importance of wet (hydrated) surface is long known!
Old Egyptian description of how to overcome friction

(Prof' I. Etsion, Technion)



**Transporting an Egyptian colossus
from the tomb of Tehuti-Hetep 1880 B.C.**

Zooming on Lubricating by wetting



FIM as a Proof of Concept of OA Treatment

Phase I/IIa at Hadassah Medical Center in Jerusalem, Israel
Concluded in December 2012, by Prof Aryeh Kandel

- **40 participants** (av. age 64 years) suffering from symptomatic moderate osteoarthritis
- Randomized
- Compared / head to head standard of care (HA - Durolane) with **NMII** product (DMPC/DPPC MLV)
- Double-blind
- Single injection
- Follow-up 90 days

PRIMARY OBJECTIVE

Safety and tolerability

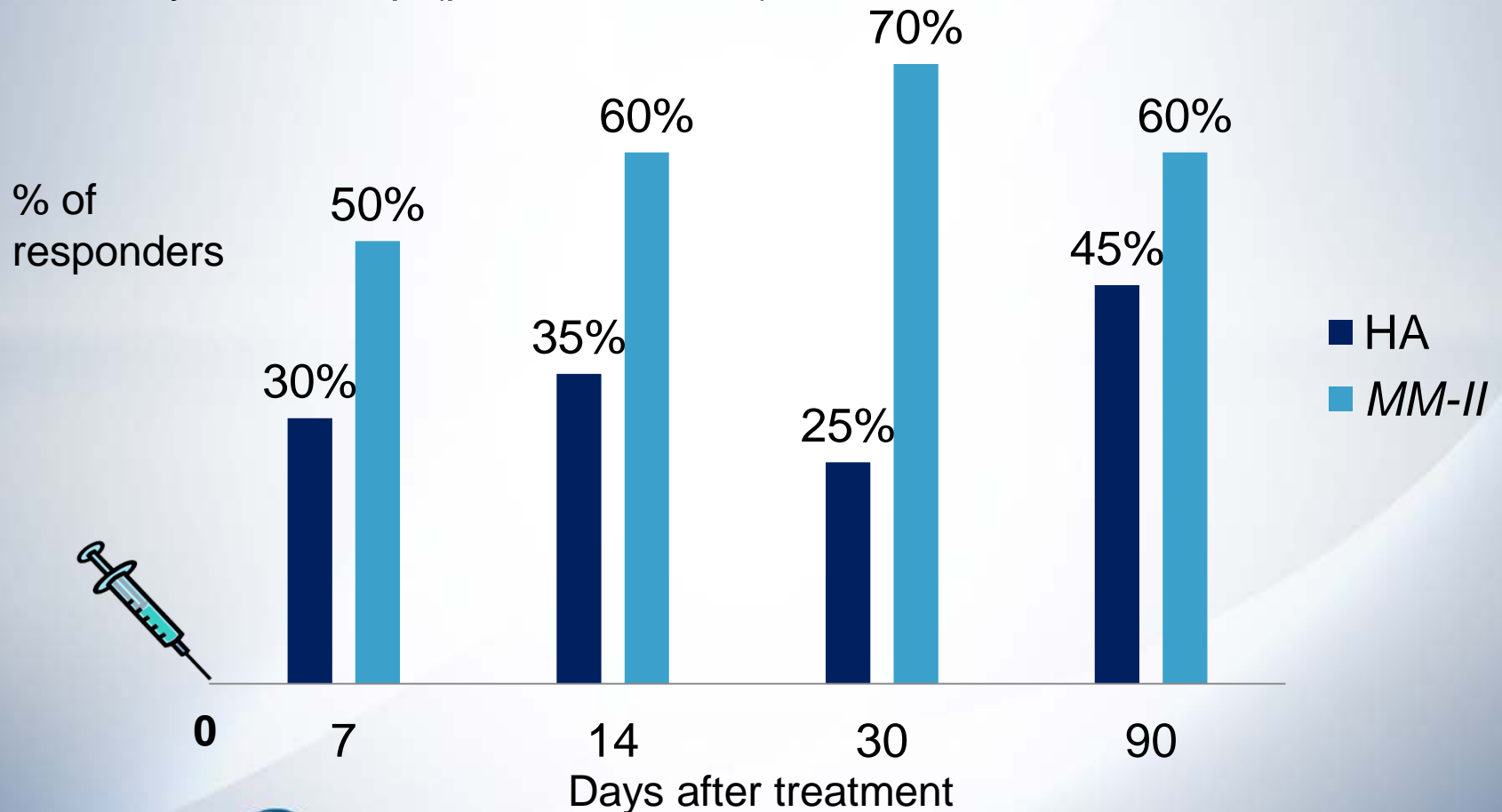
SECONDARY OBJECTIVE

Pain reduction and functionality improvements

The clinical trial was sponsored by Moebius Medical (Dr Yaniv Dolev , CEO) who licensed the technology from Yissum, HUJI (Barenholz) and Technion (Etsion)

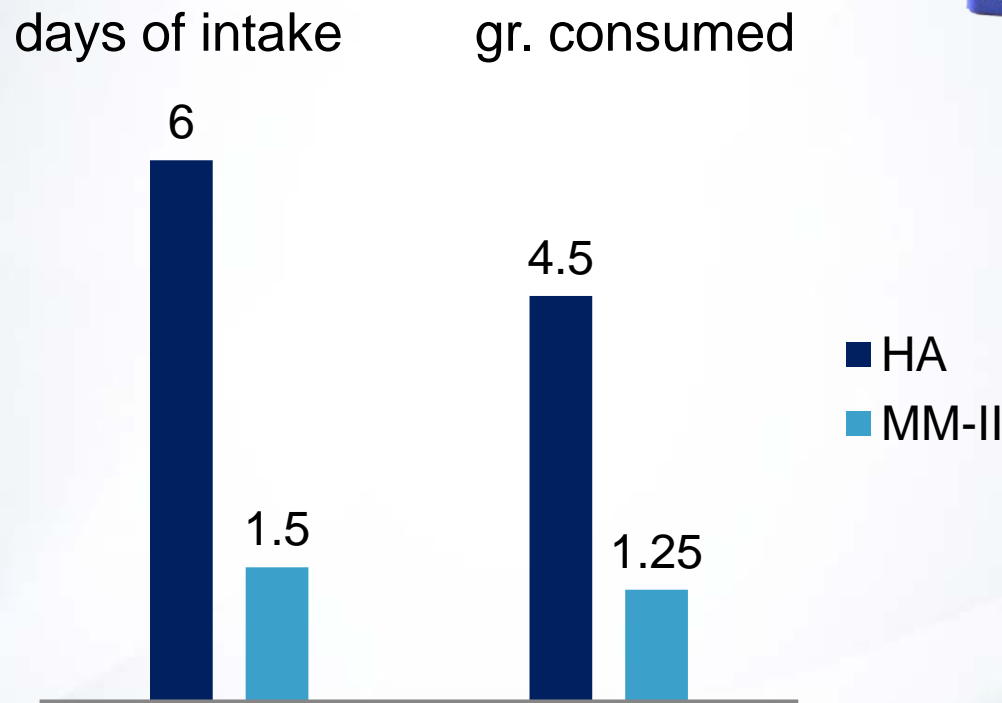
Proof of concept clinical trial highlights

OMERACT-OARSI criteria / index of responders to one treatment and 90 days follow up (pain & function)* NMII vs HA



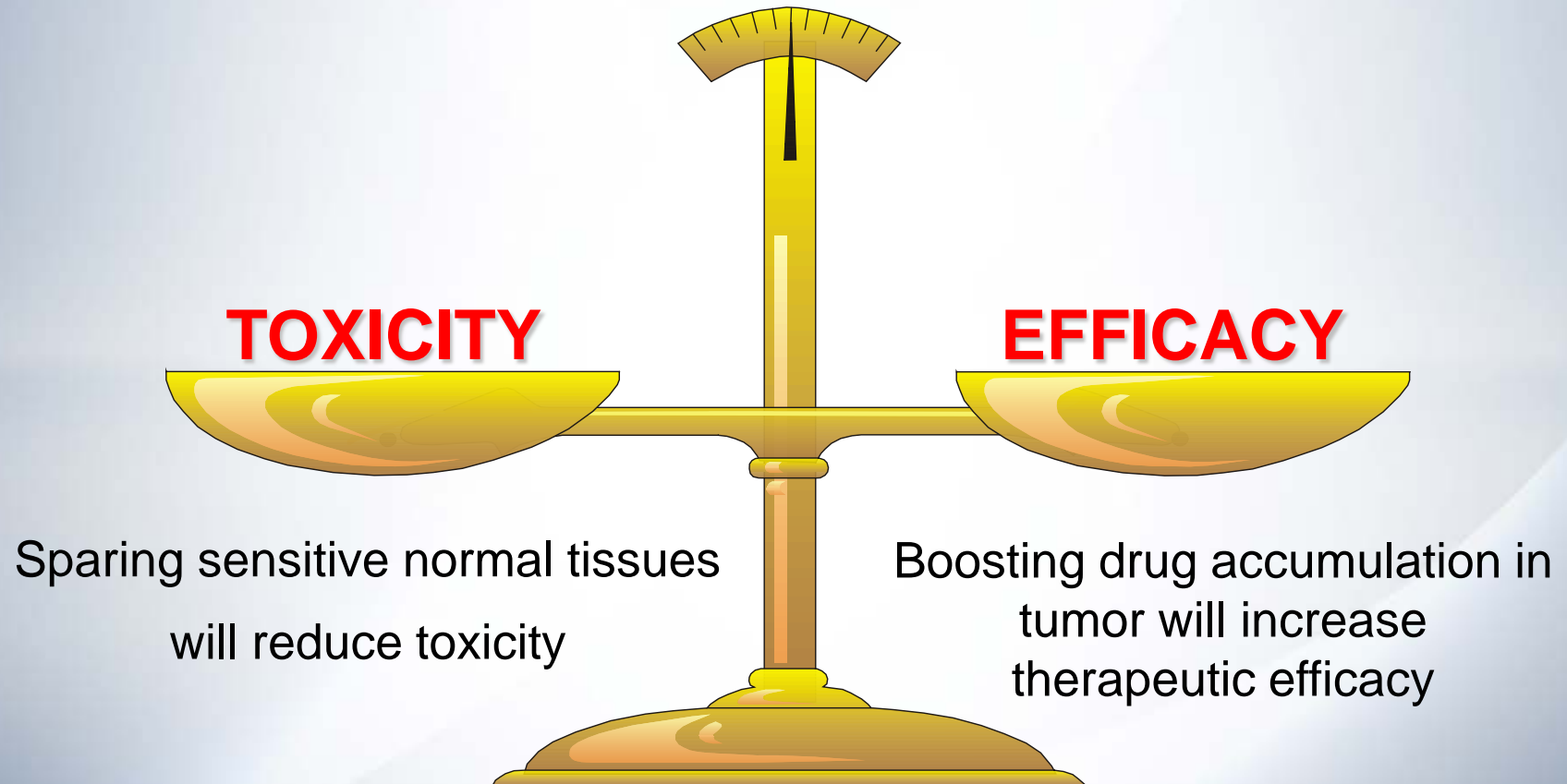
Proof of Concept Clinical Trial Highlights

Rescue Medication used between visits by the participants during the study



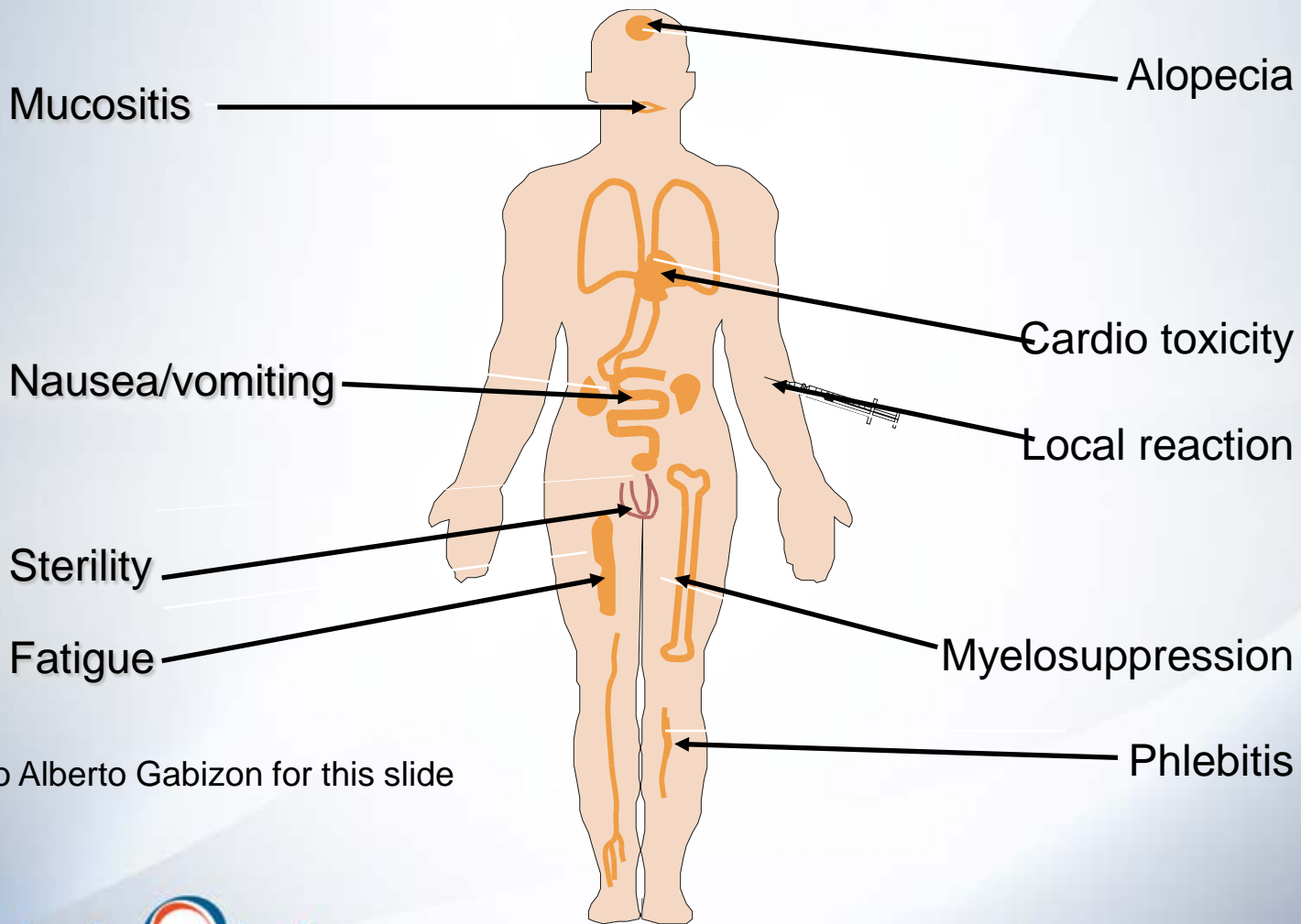
Doxil is an anticancer chemotherapeutic nano-drug aims to improve therapeutic index

INCREASING the THERAPEUTIC INDEX



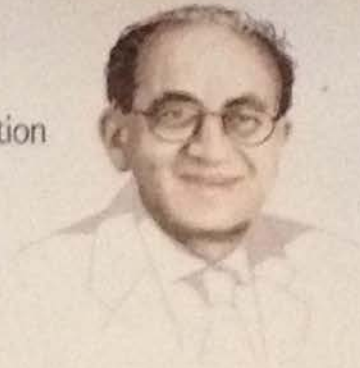
Thanks to Alberto Gabizon for this slide

Side effects of doxorubicin



Thanks to Alberto Gabizon for this slide

Rakesh K. Jain is Andrew Werk Cook Professor of Tumor Biology and director of the Edwin L. Steele Laboratory for Tumor Biology in the radiation oncology department of Massachusetts General Hospital and Harvard Medical School. He is a member of the National Academy of Sciences, the National Academy of Engineering and the Institute of Medicine, one of only 20 people ever to have been elected to all three bodies.



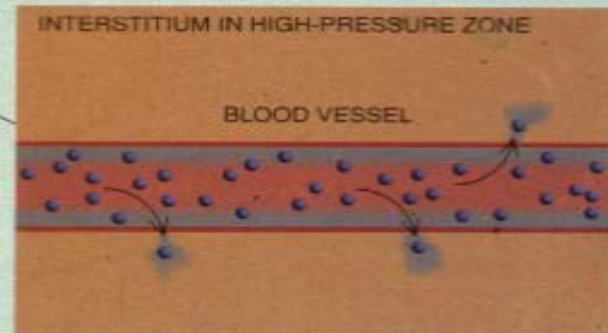
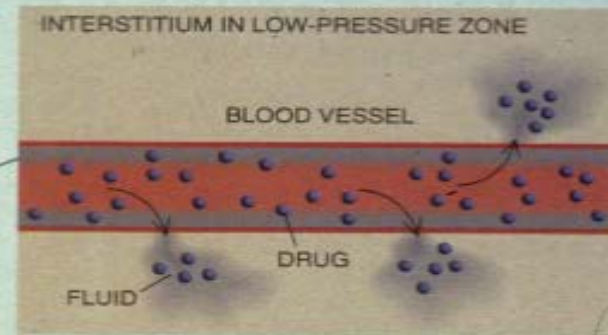
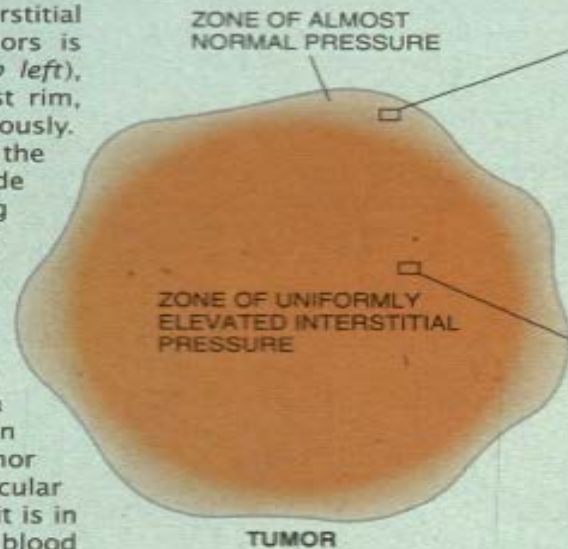
“To improve treatment, researchers need to not only gain a better understanding of cancer genetic underpinnings but also consider the physical forces in tumors”

R.K Jain in Scientific American, February 2014 p33-39

We are saying that : one also has to consider taking advantage on tumor tissue unique metabolism

Pressure Patterns in Human Tumors

Pressure in the interstitial tissue of solid tumors is uniformly elevated (*top left*), except in the outermost rim, where it drops precipitously. The elevated pressure in the inner zone can impede movement of large drug molecules into the matrix from the bloodstream—for a simple reason. Large molecules travel mainly by convection, flowing in fluid from a high- to a low-pressure region. In the outer zone of a tumor (*top detail*), the vascular pressure is higher than it is in the interstitium, and so blood fluid (*gray*) laden with drug molecules (*blue*) seeps (*arrows*) into the interstitium. In the inner zone (*bottom detail*), the interstitial pressure is about equal to that in the blood vessels; hence, convection virtually ceases. High interior pressure, which was predicted by a mathematical model, has now been found in humans (*table*) as well as in animals. The pressure readings listed are measured in millimeters of mercury.

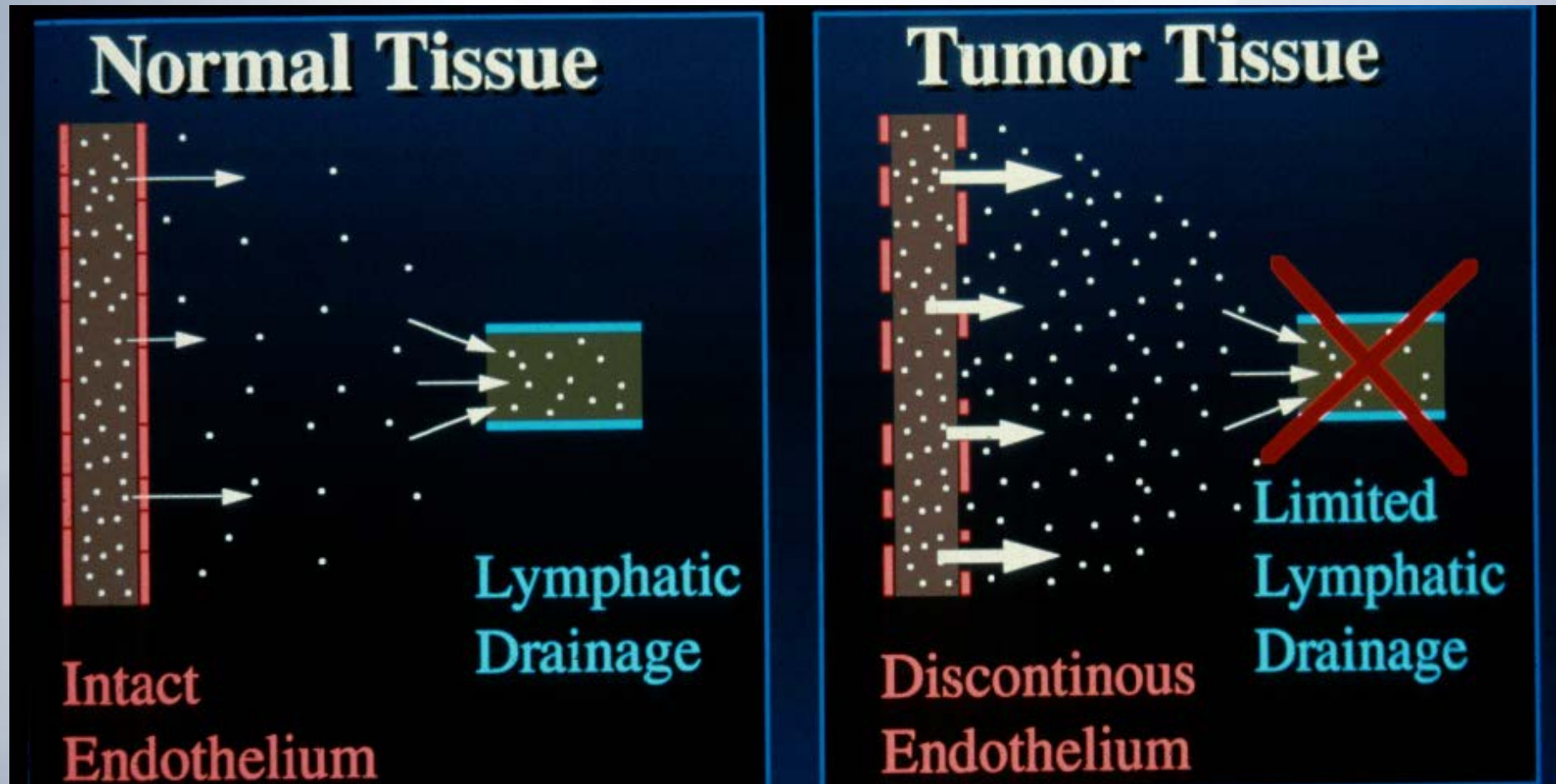


INTERSTITIAL PRESSURE IN HUMAN TUMORS

TYPE OF TISSUE	NUMBER OF PATIENTS	MEAN PRESSURE
NORMAL BREAST	8	0.0
NORMAL SKIN	5	0.4
RENAL CELL CARCINOMAS	1	38.0
CERVICAL CARCINOMAS	26	22.8
COLORECTAL LIVER METASTASES	8	21.0
HEAD AND NECK CARCINOMAS	27	19.0
BREAST CARCINOMAS	8	15.0
METASTATIC MELANOMAS	12	14.3
LUNG CARCINOMAS	26	10.0

One of the major obstacles to tumor efficacious chemotherapy is the physics of tumors. (Jain and Coworkers, Sci. Amer.)

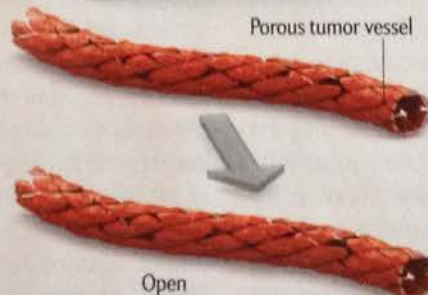
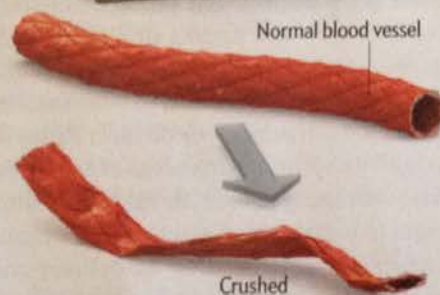
How can we take advantage of the “enhanced permeability and retention” (EPR) effect in tumors, the tumor “Achilles Heel” which explains nSSL selectivity into tumors



The working hypothesis is benefit of the tumor Achilles' Heel of the EPR effect which support use of nano <100 nm long circulating liposomes (nSSL) (adapted from: Maeda & Matsumura 1986)

Why Only Solids Squeeze Tumor Vessels

People are often surprised to learn that only the matrix and cells in tumors squeeze blood vessels shut; the fluid buildup does not play a role in that effect. The author likes to explain the logic by analogy to a plastic soft-drink bottle dropped into the ocean.



SCIENTIFIC AMERICAN ONLINE Watch a video demonstrating the compressive stress in tumors at ScientificAmerican.com/feb2014/matrix

Efficacious systemic cancer and inflammation chemotherapy by liposomes loaded drug requires enough such liposomes reaching the disease site with therapeutic drug levels, releasing the drug there. These can be achieved by optimizing the cross talk of lipid biophysics, nanotechnology, and biology.

[For doxorubicin a relatively large dose of 50 mg/m² dose is needed]

This requires:

Stable and high (but enabling release) drug encapsulation

Steric barrier to slow down interaction with blood components and RES

Extravasation into the tumor sites

Solutions:

Remote loading by ammonium sulfate encapsulation enables to deliver enough drug to the tumor **which can be released there at therapeutic levels**

2K PEG-DSPE grafted lipopolymer which increases circulation time and reduced RES uptake so enough liposomes can reach the tumor site

Size < 120 nm permit extravasation in the tumor (EPR effect) and increase of circulation time

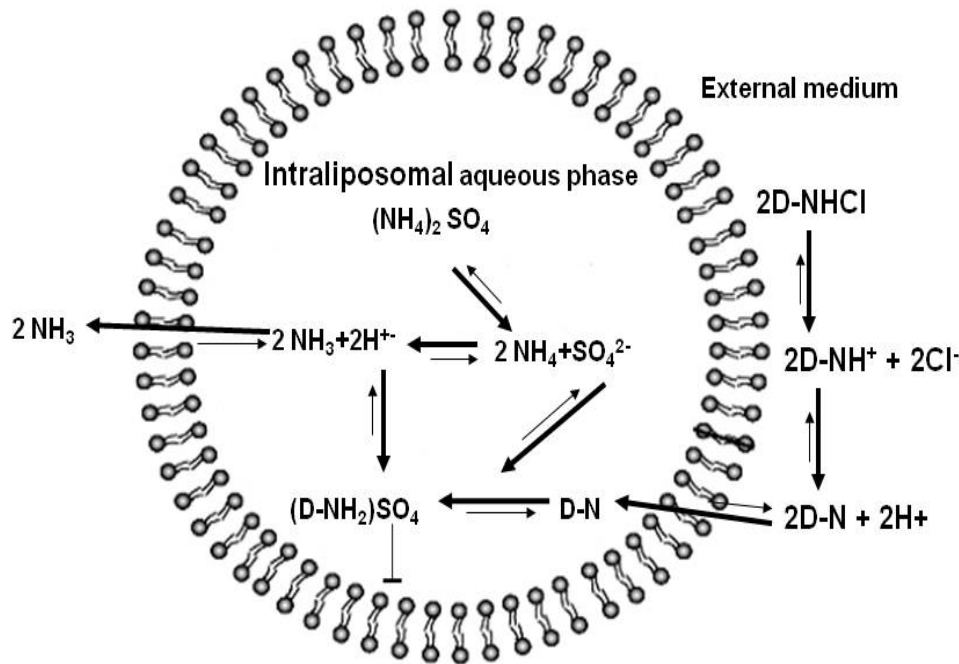
Design of a liposomal DDS requires the following decisions:
should be based on lipid biophysics, physical-chemistry and nanotechnology principles and these fields cross talk with disease biology

- Liposome type? Nano liposomes are preferred for systemic use and large liposomes (MLV or LMV) for local applications
- Desired zeta and surface electrical potential?
- Desired size distribution?
- desired in vivo release kinetic order (zero or other orders) and rate?
- Desired lipid composition? Steric barrier, Lipid phase? (LD, SO, LO)
- The preferred drug? (here mainly physicochemical considerations)
- Drug loading method? Passive? Remote (active)?
- Drug selection and/or design by Data Mining using many drug features. Creating an “training set, and evaluation by “test set”
Zucker et al JCR 2009, Cern et al JCR 2012, JCR 2013)
- Method of removal of non encapsulated drug?
- Liposomal storage conditions? Electrolyte versus no electrolyte? pH?
- Administration approach (oral, local, intravenous, topical, other)?

Remote loading by trans-membrane ammonium gradient is an essential component in Doxil clinical success

Trans membrane ammonium ion gradient is acting like a nano-pump and driving force for the uptake of doxorubicin into the intraliposome aqueous phase. There due to the excess of sulfate ion and protons it precipitates in a stable and reversible way as doxorubicin sulfate crystals allow for large drug accumulation without increasing of intra-liposome osmotic pressure

It also allow for drug release at the tumor site as will be discussed later



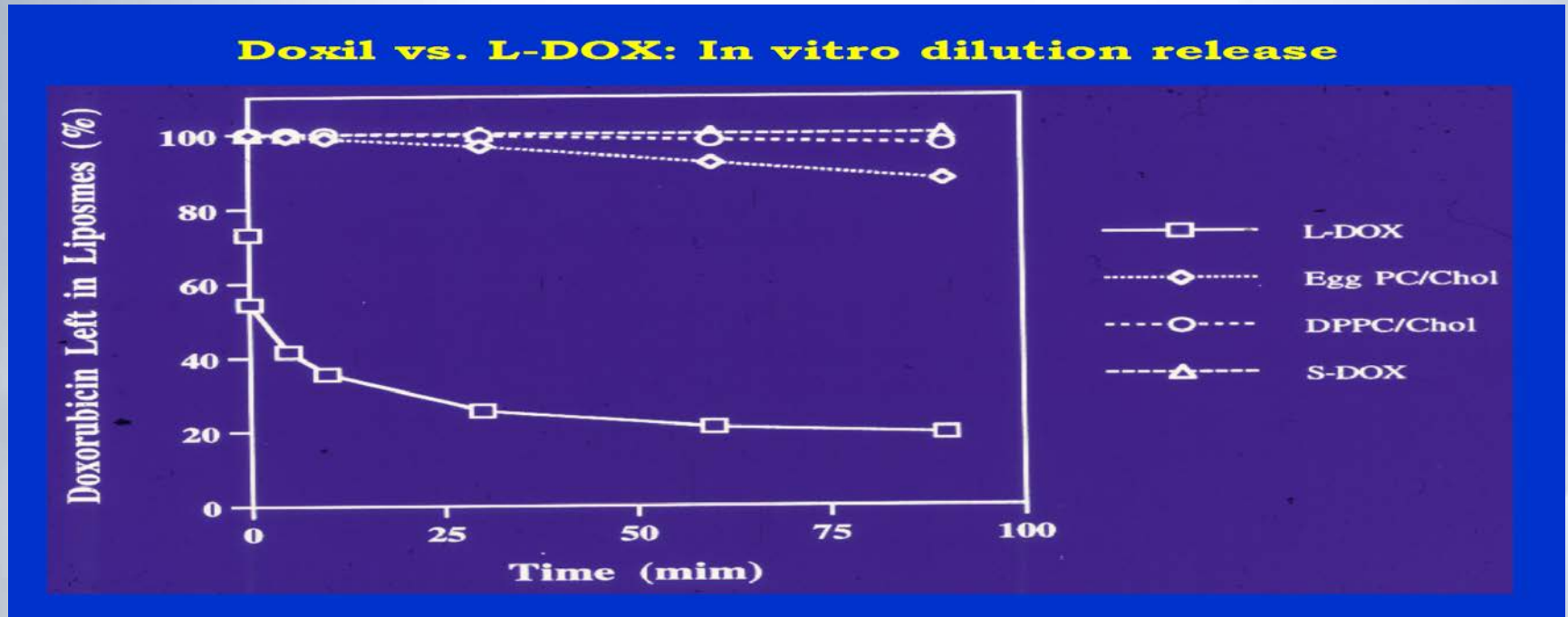
The sulfate counter ion lead to crystallization of DOX-sulfate which stabilizes drug loading

Un-ionized drug base (D-N) or acid (D-COOH) crosses the liposomal membrane and is trapped inside by its ionization and insoluble salt formation with the intraliposome counterion. **Active loading benefit from the nano volume of nano-liposomes.** Haran et al 1993, Zucker et al 2009, Cern et al 2012, Avnir et al 2008, 2011; Barenholz et al , US patents 5,192,549, 5,316,771 (1994)

Small angle X-ray diffraction demonstrates formation of DOX crystals-sulfate in the presence of sulfate anion and as result of ammonium sulfate gradient induced remote loading into SSL (to form Doxil) (Lasic et al 1992)

Sample	Reflections (Å)
Doxorubicin-HCl Crystalline	20.0, 10.1, 8.5, 7.6, 6.6 5.9, 5.3, 4.8, 4.5, 4.3
Empty liposomes	83, 55, 41.5
Loaded liposomes	27
3% Dox solution	none
3% Dox-(NH ₄) ₂ SO ₄	27
3% Dox-Na ₂ SO ₄	27

Stability of doxorubicin loading into liposomes: Comparing passive versus active (remote) trans- membrane ammonium sulfate gradient driven loading



Dilution (up to 10,000 fold) imitate the stability of drug loading upon infusion to human plasma. The first ml infused is diluted 3500 fold.

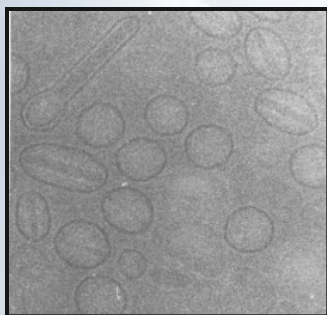
In such test we can discriminate between the effects of loading method (active vs passive and the contribution of liposome lipid composition

Relevance of Nanoism

Active Loading

Effect: Improving active loading of amphipathic weak bases and acids.

Mechanism: The smaller the liposome intraliposome aqueous phase the easier it is to form ammonium sulfate gradient for loading amphiphatic weak bases [Haran et al 1993 others, IP: Barenholz and Haran US patents 5,192,549 (1993), 5,316,771 (1994), others] applied for doxorubicin (DOX), tempamine (TMN), bupivacaine (BUP), others or calcium acetate gradient for loading amphiphatic weak acids [Clerc and Barenholz 1995 and US patent 5,939,096 (1999), others]. Both gradients are efficient to induce intraliposome drug loading and drug precipitation which correlates with high drug to lipid ratio and with loading stability. Applied for methyl- prednisolone hemisuccinate sodium salt (MPS = Solu-Medrol[®]).



Doxil[®] Experience

1. An example of successful passive targeting of liposomes to tumors

2. First patented liposomal drug

3. First nanomedicine reaching the clinic.
FDA approval 11.95

4. Annual Sales rate > \$700 millions 2010

-----INDICATIONS AND USAGE-----

DOXIL is an anthracycline topoisomerase inhibitor indicated for:

- **Ovarian cancer (1.1)**

After failure of platinum-based chemotherapy.

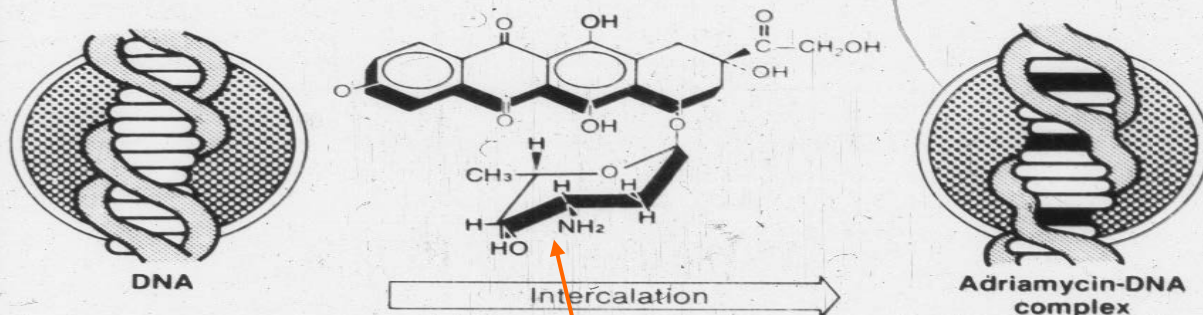
- **AIDS-related Kaposi's Sarcoma (1.2)**

After failure of prior combination chemotherapy or intolerance to such therapy. Results are based on objective response rate; no results are available from controlled trials that demonstrate clinical benefit.

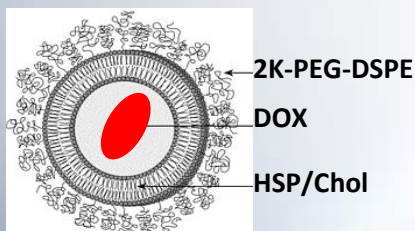
- **Multiple Myeloma (1.3)**

In combination with bortezomib in patients who have not previously received bortezomib and have received at least one prior therapy.

1 ml of the Doxil dispersion has 2.3×10^{14} liposomes and each liposome contain ~10000 molecules of doxorubicin.

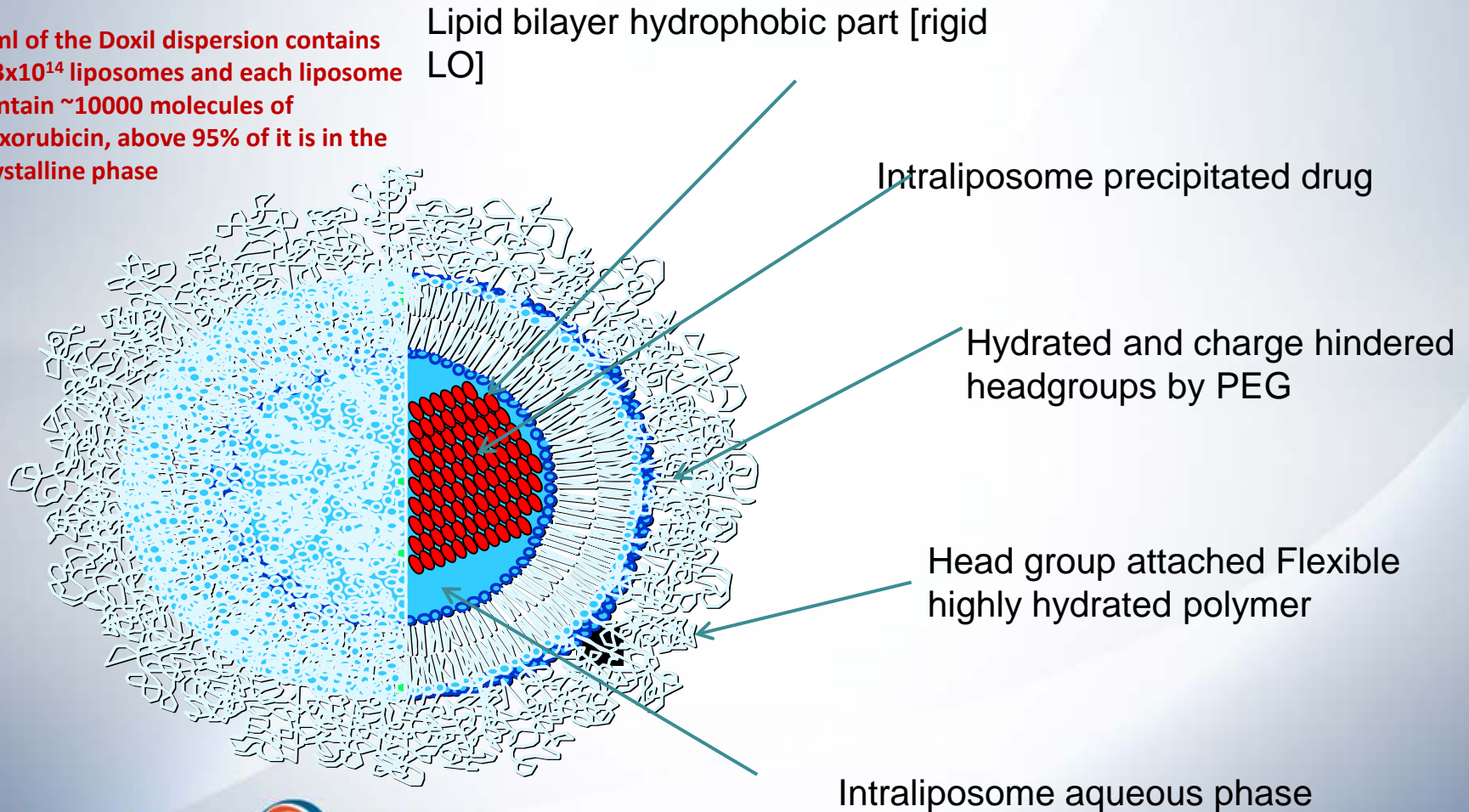


DOXORUBICIN DUE TO THE PRIMARY AMINE OF ITS MANOSE AMINE IS AN AMPHIPHATIC WEAK BASE AND THEREFORE CAN BE REMOTE LOADED INTO LIPOSOMES HAVING AN AMMONIUM ION GRADIENT

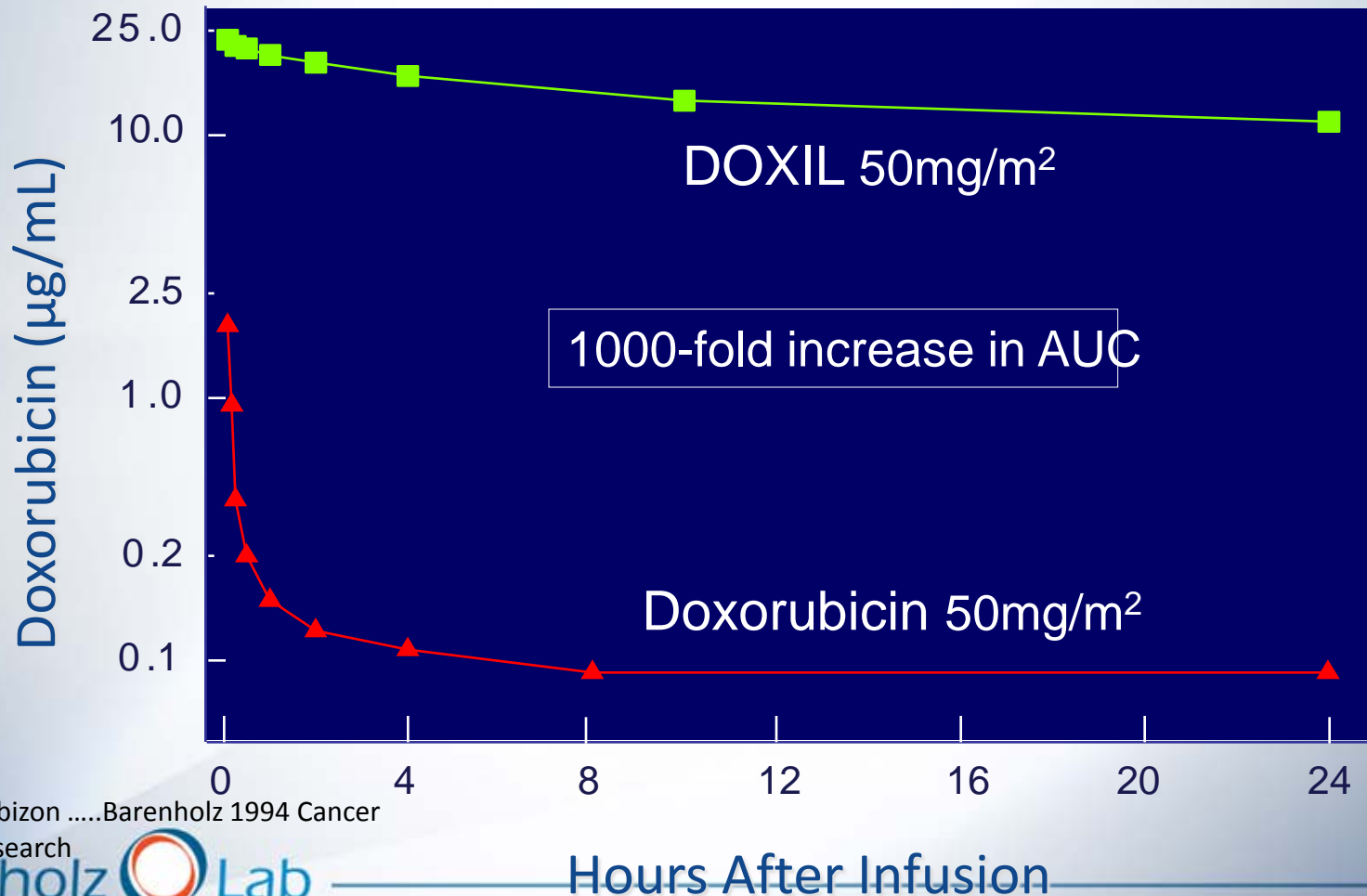


Doxil[®]: Structure-Performance Relationships (Every detail matters)

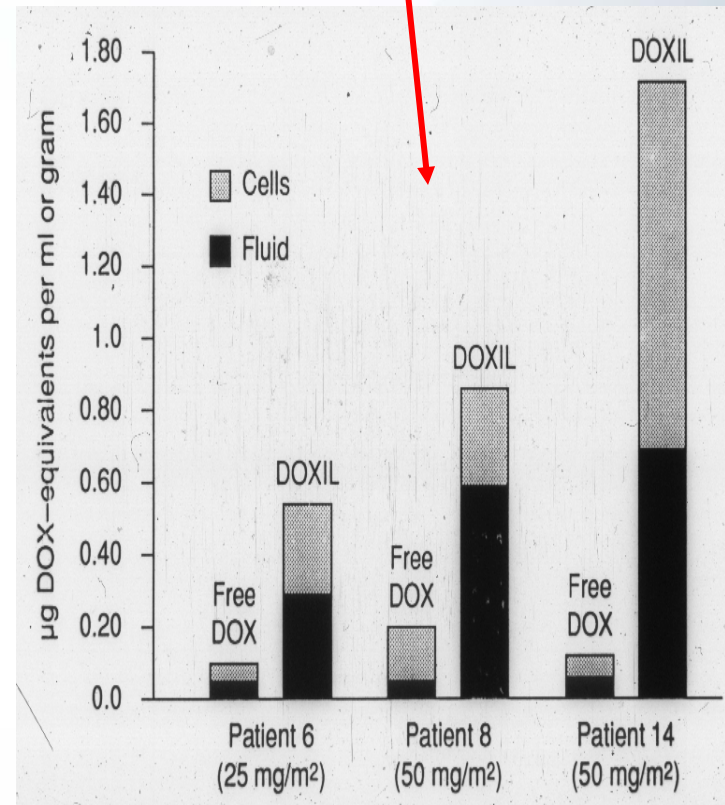
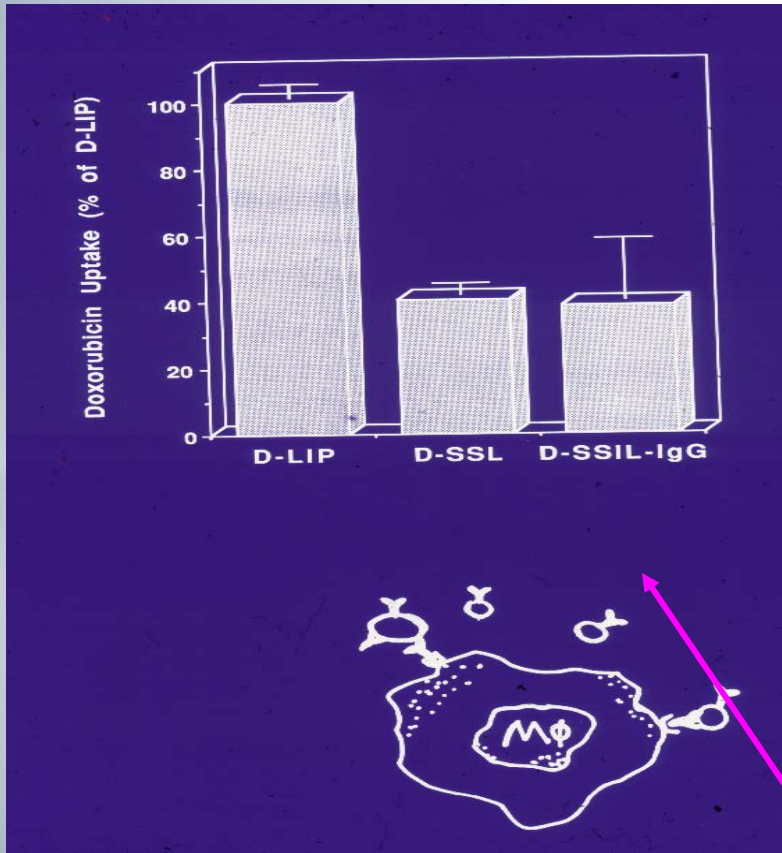
1 ml of the Doxil dispersion contains
 2.3×10^{14} liposomes and each liposome
contain ~10000 molecules of
doxorubicin, above 95% of it is in the
crystalline phase



Doxil[®] Prolonged PK and being nano allow for tumor accumulation



The first proof for human tumor passive targeting and accumulation of Doxil's doxorubicin due EPR effect in cancer patients. Demonstrating superior bio-distribution of Doxil versus free doxorubicin (Based on Gabizon & Barenholz, Cancer Research 1994)



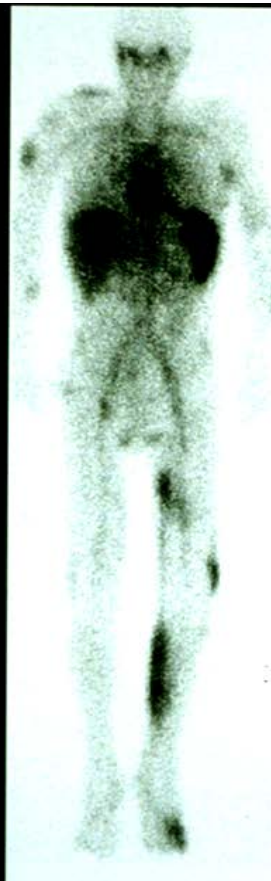
Doxil being nSSL show much lower uptake by macrophages leading to its prolonged and superior doxorubicin pharmacokinetics in all animal species tested including humans

(Emanuel et al Pharma Res 1976)

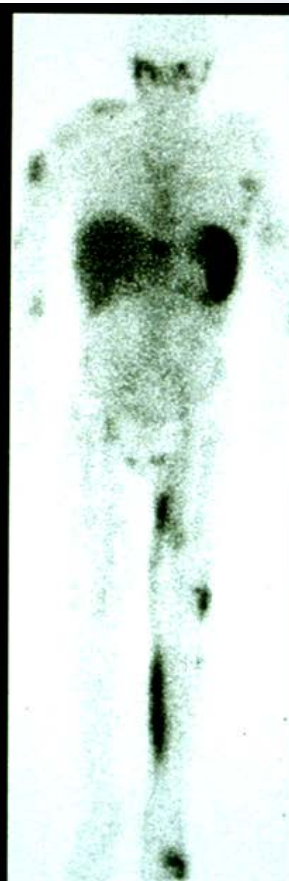
Serial Gamma Scintigrams of KS Patient after Pegylated Liposomes Containing ^{111}In -DTPA (remote loaded)



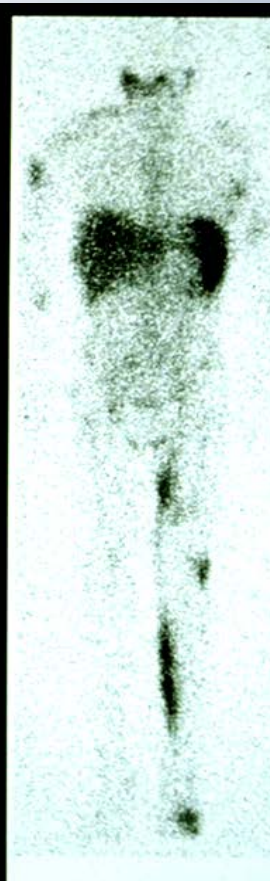
4 hrs.



24 hrs.



48 hrs.



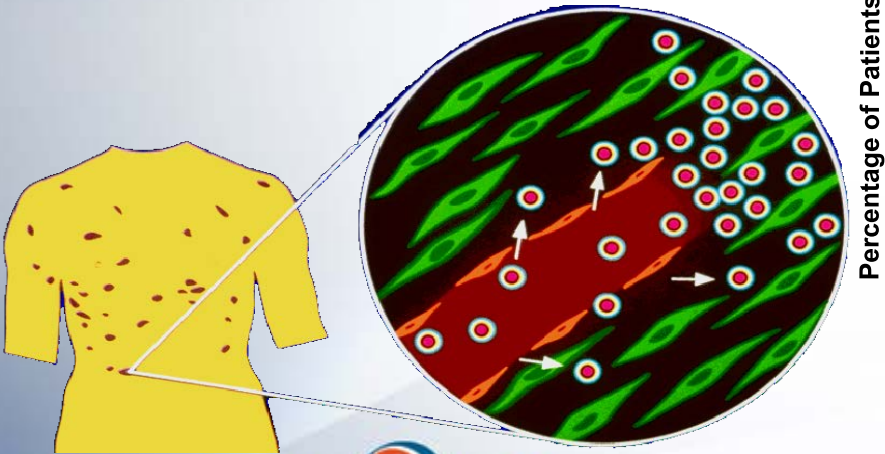
96 hrs.

Personalized DDS

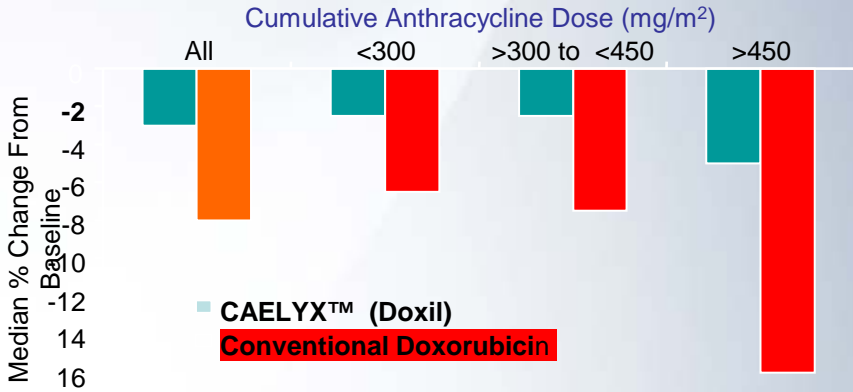
Doxil side effects are lower than of doxorubicin

	Doxorubicin	Doxil
Vesicant effect	+++	+/-
Infusion reaction	-	+*
Nausea/Vomiting	++	+/-
Myelosuppression	+++	+ (no gr. 4)
Stomatitis/Mucositis	++	+++
Hand-Foot (PPE)	-	+++
Cardiotoxicity	+++	+
Alopecia	+++	+
Max. Tolerated Dose	60 mg/m ²	50 mg/m ²
Dose Intensity	20 mg/m ² /wk	12.5 mg/m ² /wk
Max. Cum. Dose	450 mg/m ²	Undefined >650 mg/m ²

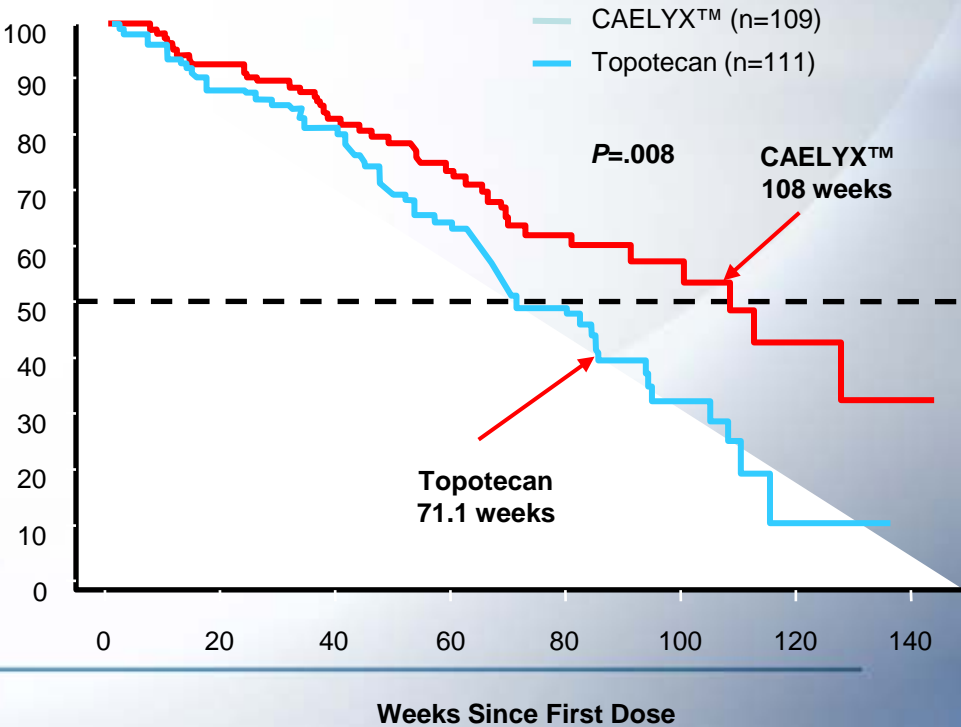
Doxil Mechanism of action: EPR effect and what next?



LVEF show that cardio-toxicity of cumulative drug dose is lower for Doxil than for doxorubicin



In ovarian cancer Doxil is superior over topotecan



What is the MoA of doxorubicin release of Doxil at tumors ? (2)

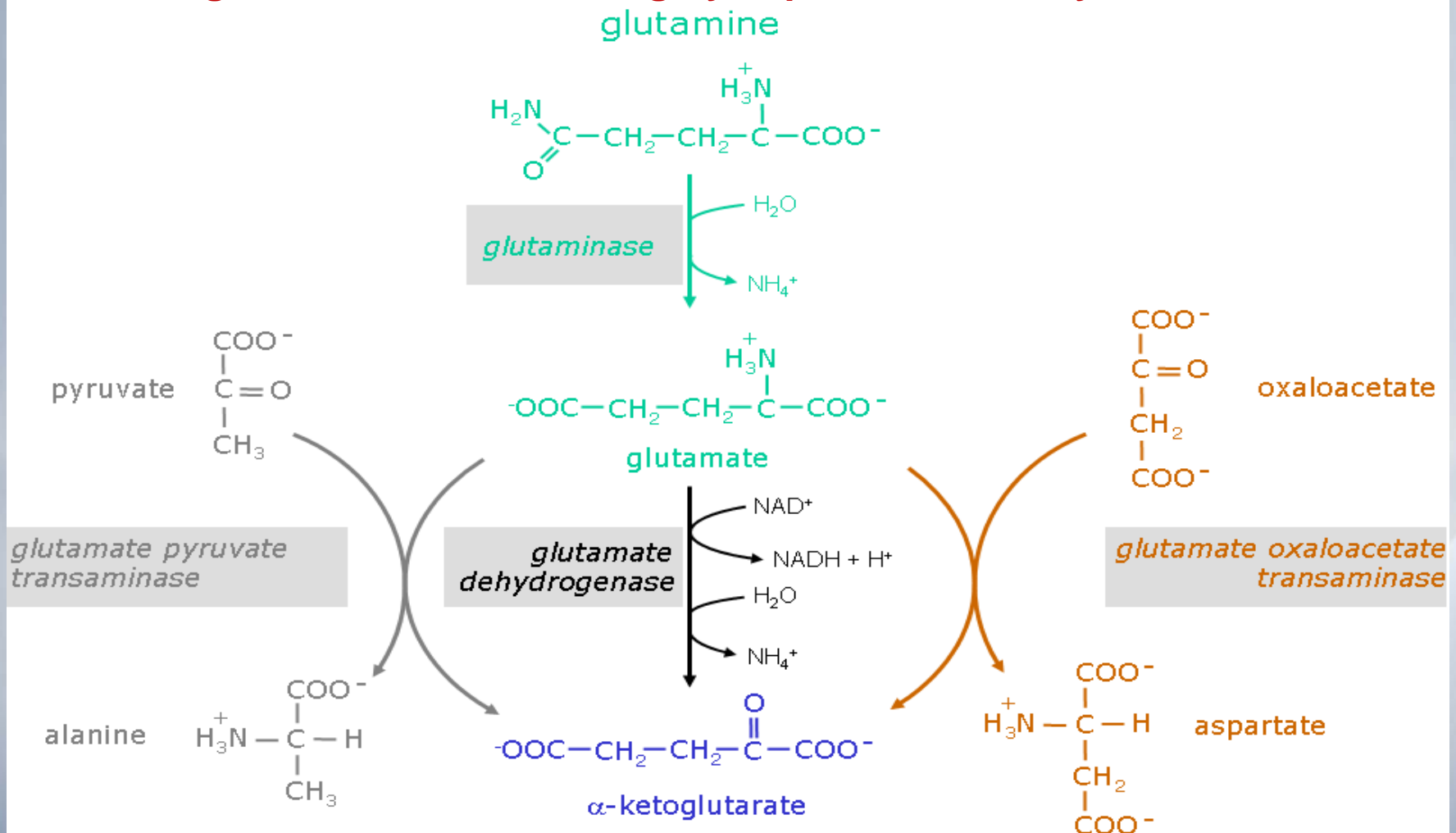
There are few options to explain tumor interstitial doxorubicin release of Doxil

- Breakdown of liposomes by phospholipases such as secretory PLASE A2 ?

This is not applied to Doxil liposomes:

1. Due to their high mole% of cholesterol (Mouritsen, K. Jorgensen, Pharm. Res. 15 (1998) 1507–1519; Andresen et al Prog. Lipid Res. 2005).
 2. Also confirmed by the lack of therapeutic effects and cis platin release of Stealth cisplatin (ref. in previous slide)
- Therefore it must be assumed that some factors typical (or unique) to tumor interstitial induce doxorubicin release of Doxil at the tumor sites.
 - **A very attractive option is our hypothesis that ammonium/ammonia produced due to the tumor unique glutaminolysis (Moreadith and Lehninger JBC, 259, 6215-6221, 1984; Eng et al Science Signaling 3, 1-9, 2010) induces the doxorubicin release of Doxil either by collapse of the ammonium gradient or other mechanisms. This presentation is focused on the evaluation of the mechanism by which ammonium/ammonia induce doxorubicin release of Doxil**

Glutaminolysis in tumor cells is a response by which tumor cells overcome inhibition of Krebs cycle aconitase by ROS and creating α ketoglutarate to move the highly important Krebs cycle forward.



Ammonia Derived from Glutaminolysis Is a Diffusible Regulator of Autophagy

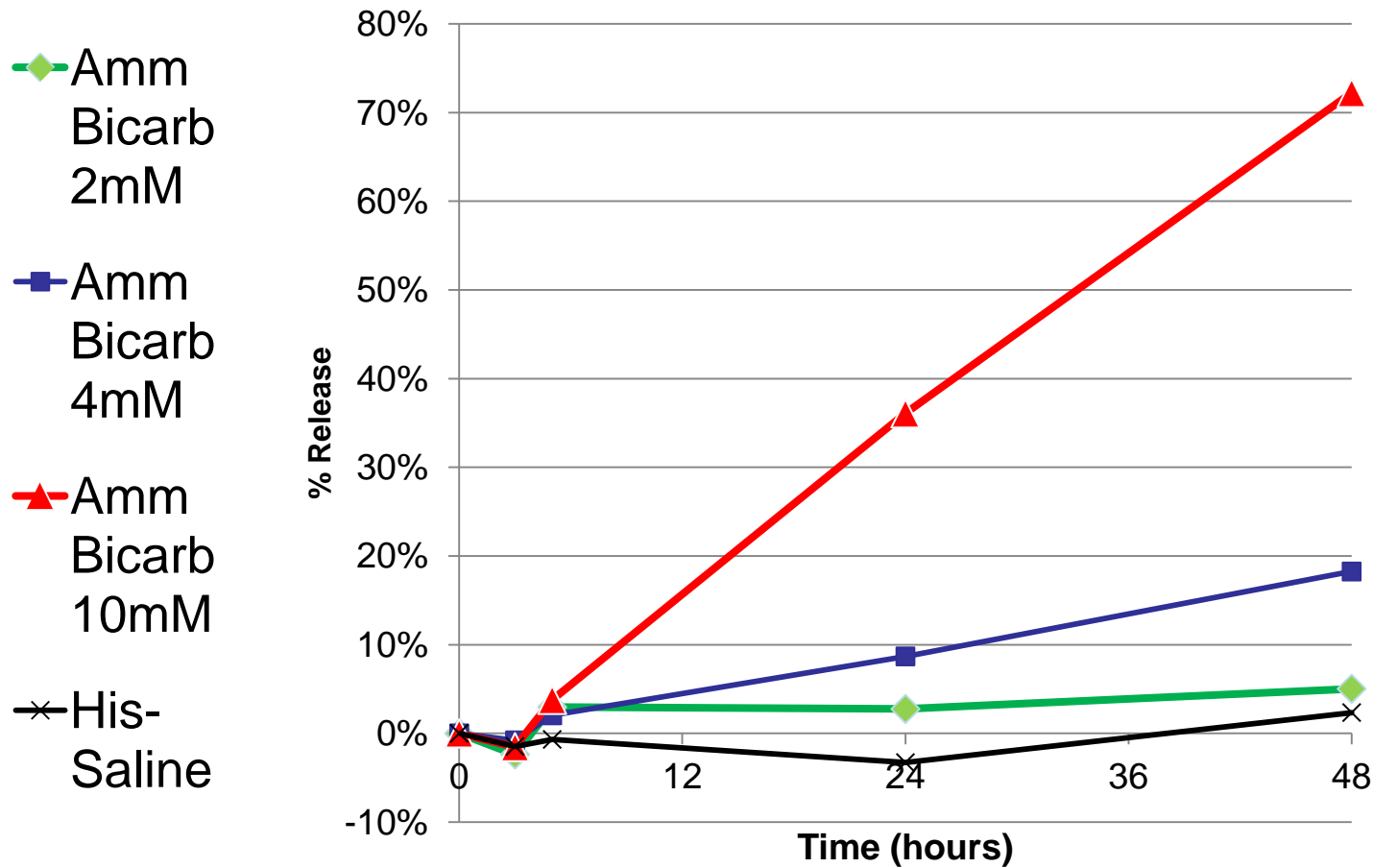
Christina H. Eng,¹ Ker Yu,¹ Judy Lucas,¹ Eileen White,^{2,3} Robert T. Abraham^{1*}

(Published 27 April 2010; Volume 3 Issue 119 ra31)

Autophagy is a tightly regulated catabolic process that plays key roles in normal cellular homeostasis and survival during periods of extracellular nutrient limitation and stress. The environmental signals that regulate autophagic activity are only partially understood. Here, we report a direct link between glutamine (Gln) metabolism and autophagic activity in both transformed and nontransformed human cells. Cells cultured for more than 2 days in Gln-containing medium showed increases in autophagy that were not attributable to nutrient depletion or to inhibition of mammalian target of rapamycin. Conditioned medium from these cells contained a volatile factor that triggered autophagy in secondary cell cultures. We identified this factor as ammonia derived from the deamination of Gln by glutaminolysis. Gln-dependent ammonia production supported basal autophagy and protected cells from tumor necrosis factor- α (TNF- α)-induced cell death. Thus, Gln metabolism not only fuels cell growth, but also generates an autocrine- and paracrine-acting regulator of autophagic flux in proliferating cells.

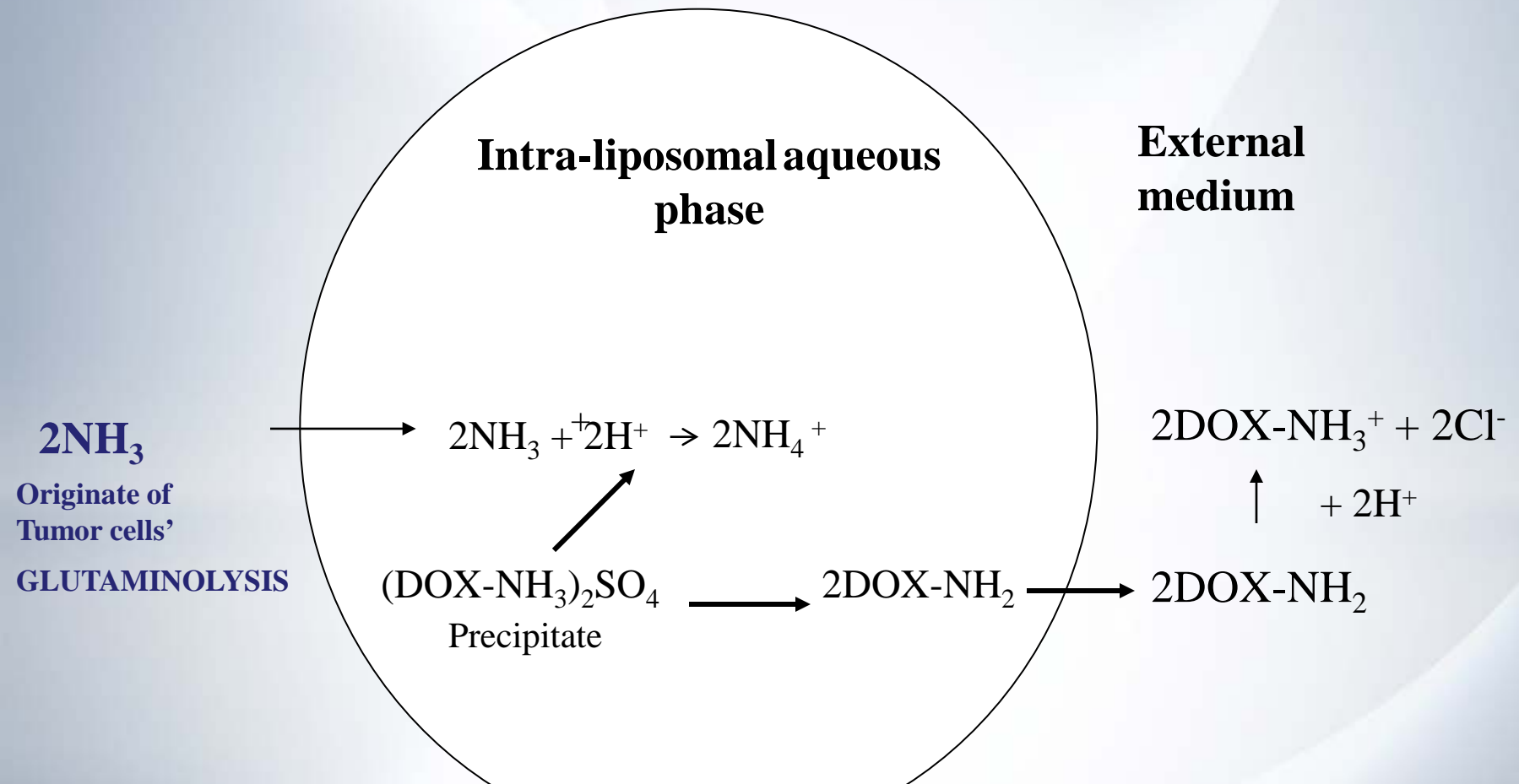
www.SCIENCESIGNALING.org 27 April 2010 Vol 3 Issue 119 ra31

At pH 6.8 in the presence of bicarbonate a substantial release occurs already at physiological ammonium concentrations of tumor microenvironment



Physiological concentration of ammonium at tumor site

MoA of ammonia induced doxorubicin release from PLD.



Accordingly ammonia produced continuously by the tumor cells as a result of glutaminolysis is released to the tumor microenvironment from where it is taken up by the PLD into the intra-liposome aqueous phase where it get protonated by a “proton transfer reaction” from the protonated doxorubicin. The resulted un-protonated uncharged doxorubicin is than released from the liposomes enable it to be taken up by the tumor cells.

Ammonia form in tumor is reaching a tumor tissue concentration of 5mM (Eng et al 2010). The ammonia results of the of glutaminolysis which is a unique pathway specific to tumor cells.

Wikipedia describes glutaminolysis as follows:

Glutamine is the most abundant amino acid in the plasma and an additional energy source in tumor cells especially when glycolytic energy production is low due to a high amount of the [dimeric form of M2-PK](#). Glutamine and its degradation products glutamate and aspartate are precursors for [nucleic acid](#) and [serine](#) synthesis.

Glutaminolysis is insensitive to high concentrations of reactive oxygen species (ROS).

Due to the truncation of the citric acid cycle the amount of [acetyl-CoA](#) infiltrated in the citric acid cycle is low and acetyl-CoA is available for de novo synthesis of [fatty acids](#) and [cholesterol](#). The fatty acids can be used for phospholipid synthesis or can be released.^[31]

Doxil[®]- Short History

Advantages:

- Doxorubicin in liposomes (Doxil[®]) has much lower cardio-toxicity and most other side effects than conventional doxorubicin.
- Overall Doxil improves patient compliance and quality of life.

Indications:

- Approved by the FDA (1995) and world wide (as Caelyx) for:
 - AIDS-related Kaposi's sarcoma (KS), Nov, 1995
 - Relapsed ovarian cancer, 1999; after platinum-based treatment, 2005
 - Metastatic breast cancer with cardiac risk, Europe, 2003
 - Multiple myeloma in combination with VELCADE[®] (bortezomib), 2007

History and I.P.

- Doxil is based on 2 patent families (1988/1989) those of Liposome Technology Inc. (LTI) on the pegylated liposomes, and those of Yisum (Barenholz and Haran) on drug loading Licensed to LTI which was acquired by ALZA which was acquired by J & J. Both patent families were expired before April 2014.
- Doxil is (was) produced by Ben Venue Laboratories in the United States for Janssen Products LP, a subsidiary of Johnson & Johnson for global distribution. 2011 onward Doxil Shortage, August 2013 Doxil production stopped.
- February 2013 FDA approved the Lipodox of Sun Pharma 1st generic Doxil

Doxil[®]- Reasons for Success

1. High and stable remote loading that enable release in the tumor
2. Steric stabilization by the pegylated lipopolymer
3. Nano ~100nm size which enable benefit from EPR effect)
4. High T_m based L_O phase of the lipid bilayer which enables to achieve zero order slow release
5. **We do not know the MoA of doxorubicin release from Doxil at the tumor site?**
6. **We also do not know what is the optimal release rate?**

Doxil Teams' Acknowledgment

Department of Oncology
Hadassah — Hebrew University
Hospital

Alberto Gabizon

Laboratory

Dorit Goren
Aviva Horowitz
Michal Shemla
Dinah Tzemach
Zohar Yehoshua

Neuro-Oncology: Tali Slegal and team

Clinics

Raphael Catane
Roland Chisin (*Nuclear Medicine*)
Rut Isacson
Bella Kaufman
Eugene Libson (*Radiology*)
Tamar Peretz
Aaron Sulkes
Beatrice Uziely

Nursing Team of Oncology Ward and Day Care
Pharmacy Services — Cytotoxic Unit

Other Locations

Franco Muggia
and Colleagues, USC, Los Angeles
(*Clinical Research*)

Demetrios Papahadjopoulos
and Colleagues, UCSF, San Francisco
(*Basic Research*)

Department of Biochemistry
Hebrew University — Hadassah
Medical School

Chezy Barenholz

Major Participants

Shimon Amselem
Lily Bar
Rivka Cohen
Gillad Haran

Others

Meir Blaler
Eli Kedar
Elijah Bolotin
Stephane Clerc
Arie Dagan
Shula Druckman
Noam Emanuel
Oren Tirosh

SEQUUS Pharmaceuticals
(Liposome Technology Inc. — LTI)

Bob Abra
Tony Huang
Danilo Lasic
Frank Martin

and their teams

Why two years after expiration of Doxil® patents and with drug sales exceeding \$ 700 millions and at least 12 companies that try to make it there is still only one FDA approved Doxil equivalent generic pegylated liposomal doxorubicin (Lipodox of Sun Pharma was very recently approved in US (Not yet in Europe)



Understanding the difficulties will help a lot in the development of new nano-drugs

It seems generic Doxil is a big challenge

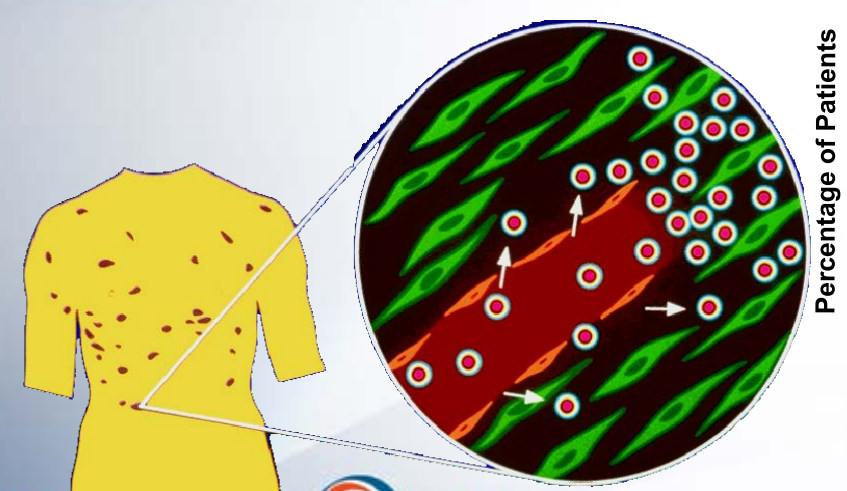
The lack of FDA approved generic Doxil is also the opportunity to develop an Improved formulation which will overcome Doxil main drawbacks but will retain Doxil advantages.

For this we have firstly to identify Doxil main drawbacks

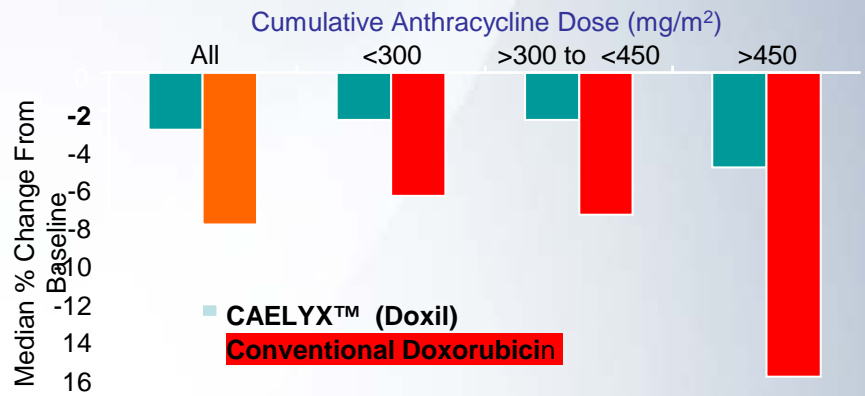
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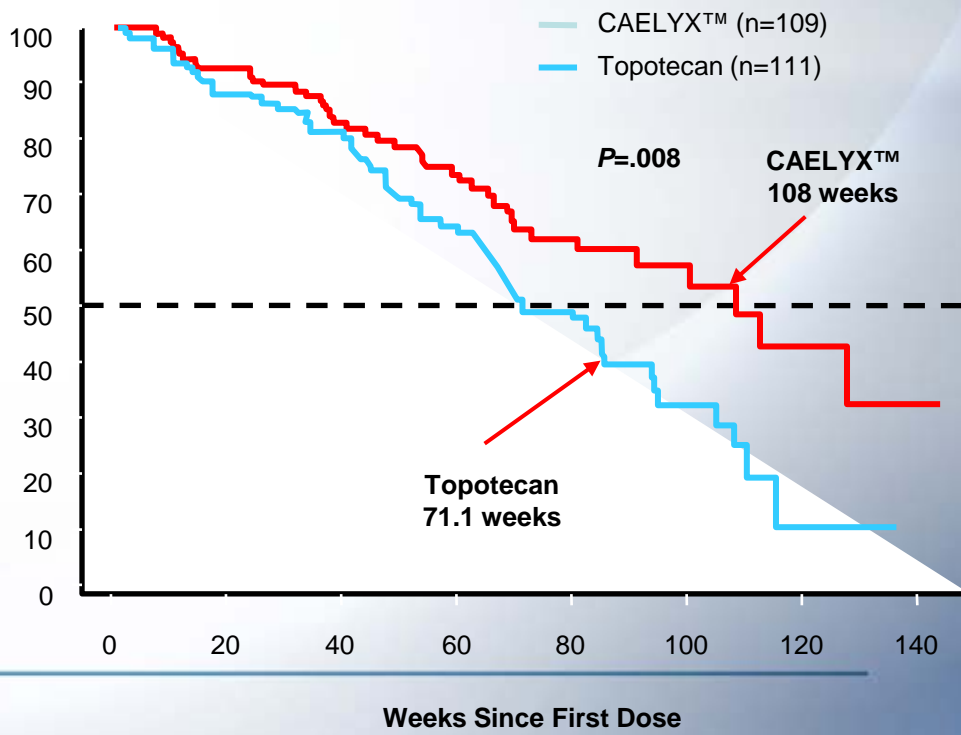
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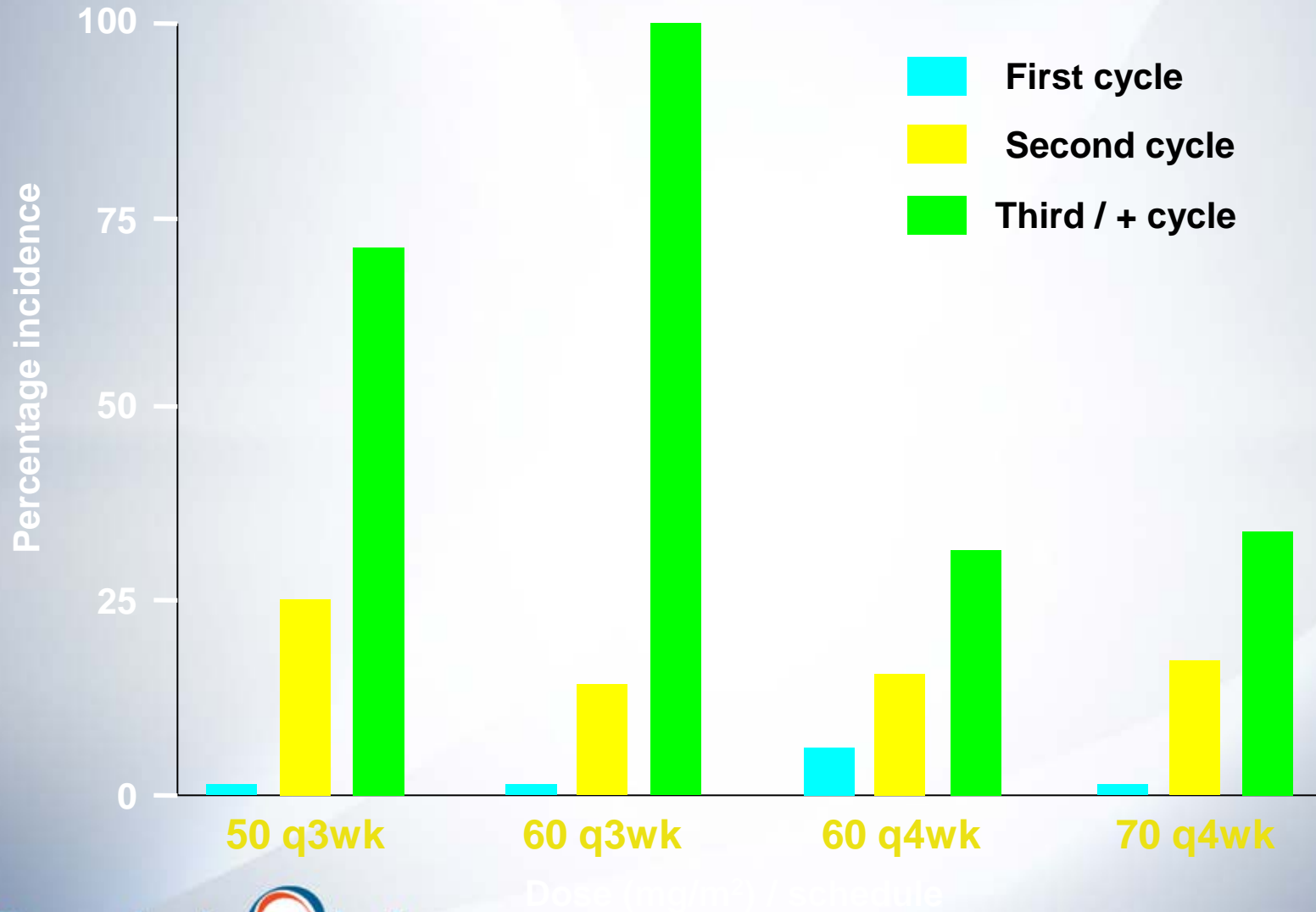
Hand-Foot Syndrome (Palmar-Plantar Erythema)

A major issue of patient compliance



Hand-Foot syndrome (PPE) in PLD-treated patients

Increased incidence with repeated cycles and short intervals



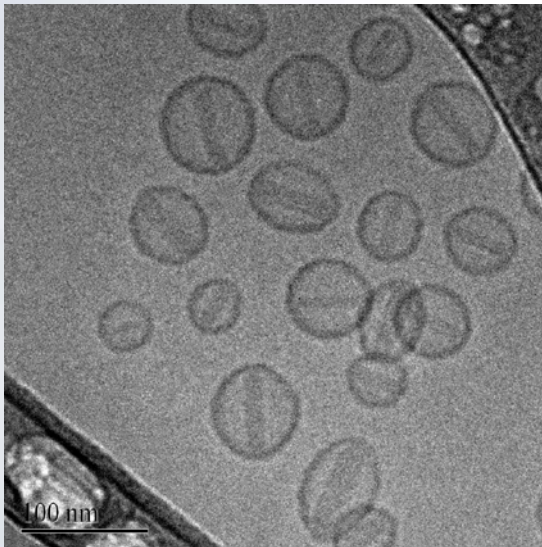
What is expected of an improved liposomal doxorubicin?

- It should have all the clinical benefits of Doxorubicin and Doxil™ concomitantly with much better safety profile regarding unwanted side effects
- Over all it should improve cancer patient compliance and quality of life without compromising therapeutic efficacy

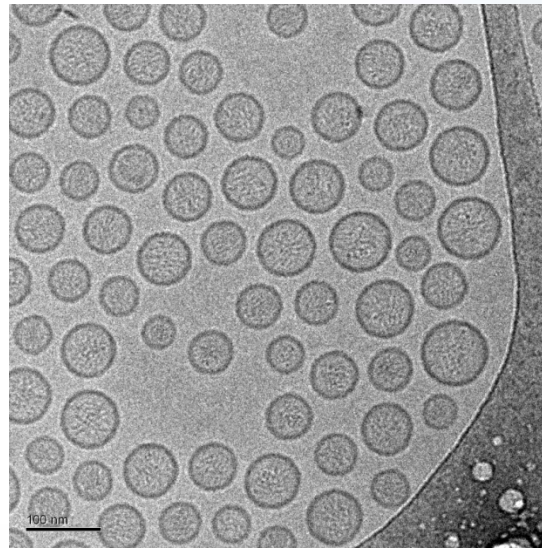
Our LC-100 a novel (patent protected) liposomal doxorubicin nano-drug with is expected to meet the above 2 conditions

LC100 features

A Long circulating sterically stabilized Doxorubicin liposomes (like Doxil liposomes) but with an improved safety profile



Doxil™ (Dox -Sulfate)
Scale bar: 100nm

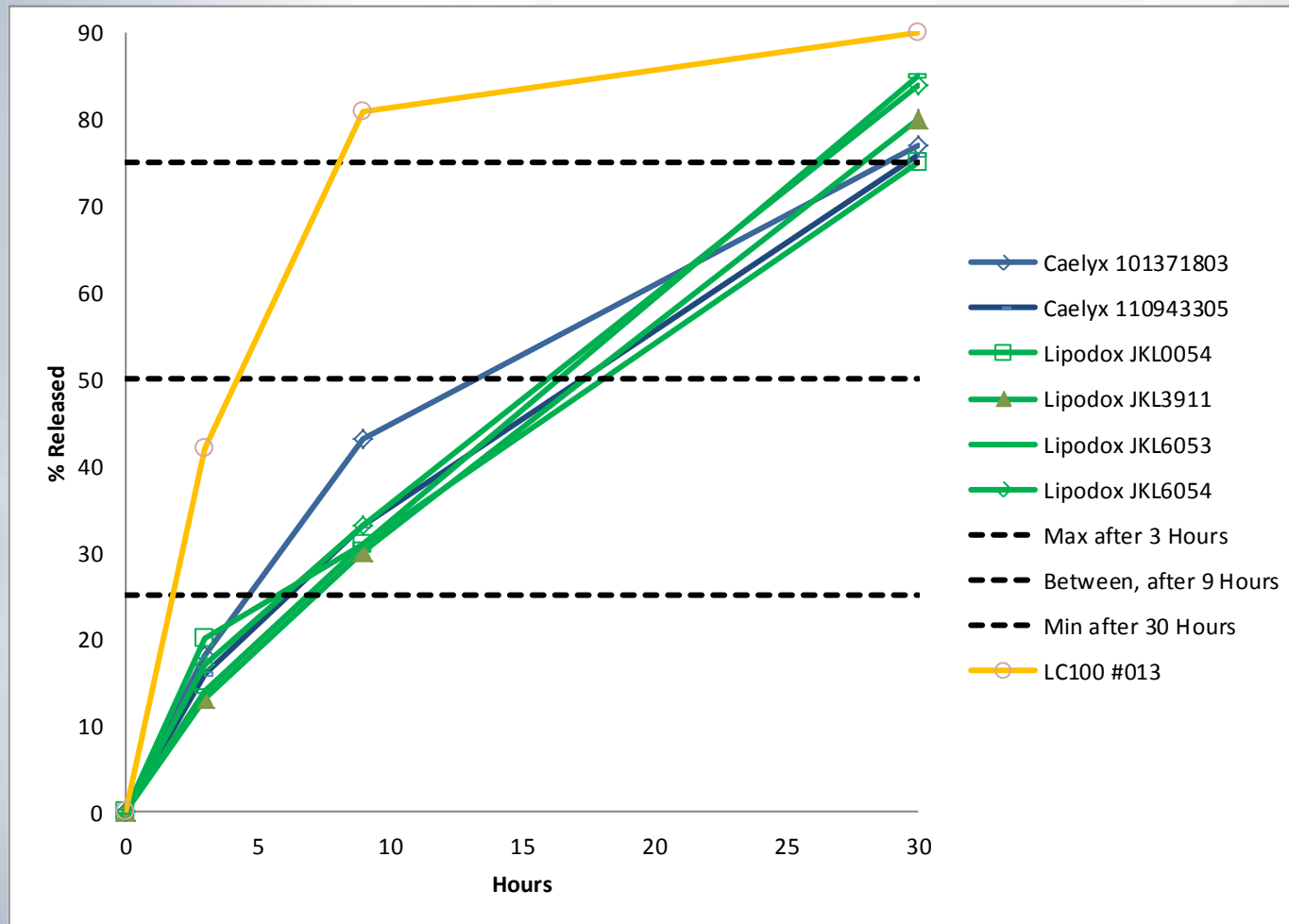


LC-100 (Dox NEW)
Scale bar: 100nm

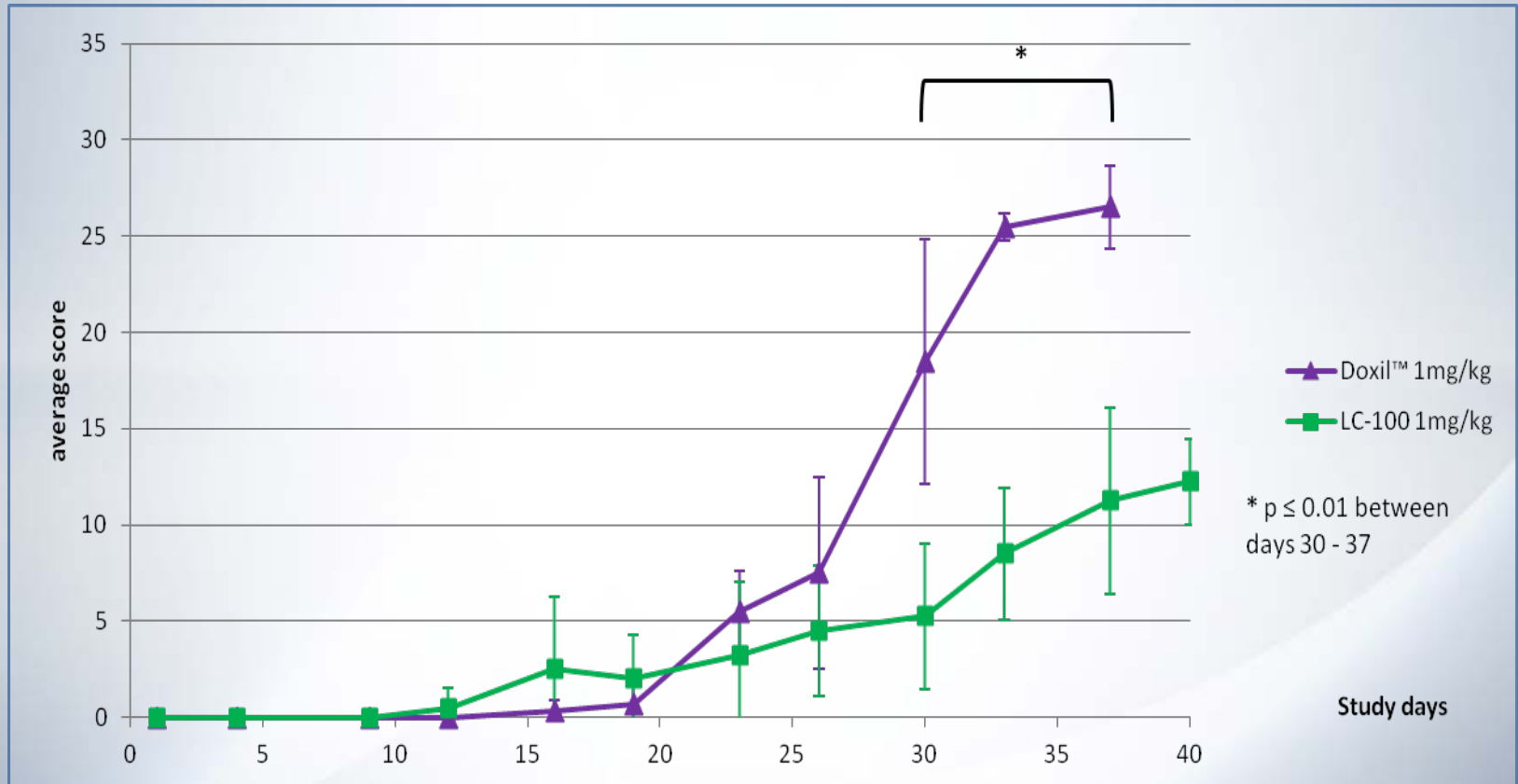
LC 100 are spherical liposomes and their intraliposomal doxorubicin does not show a crystal formation as one can seen in Doxil

Dissolution (PLD release kinetics) evaluation of PLD

Comparing Doxil and Lipodox™ to LC100 at tumor microenvironment conditions (at 37°C)

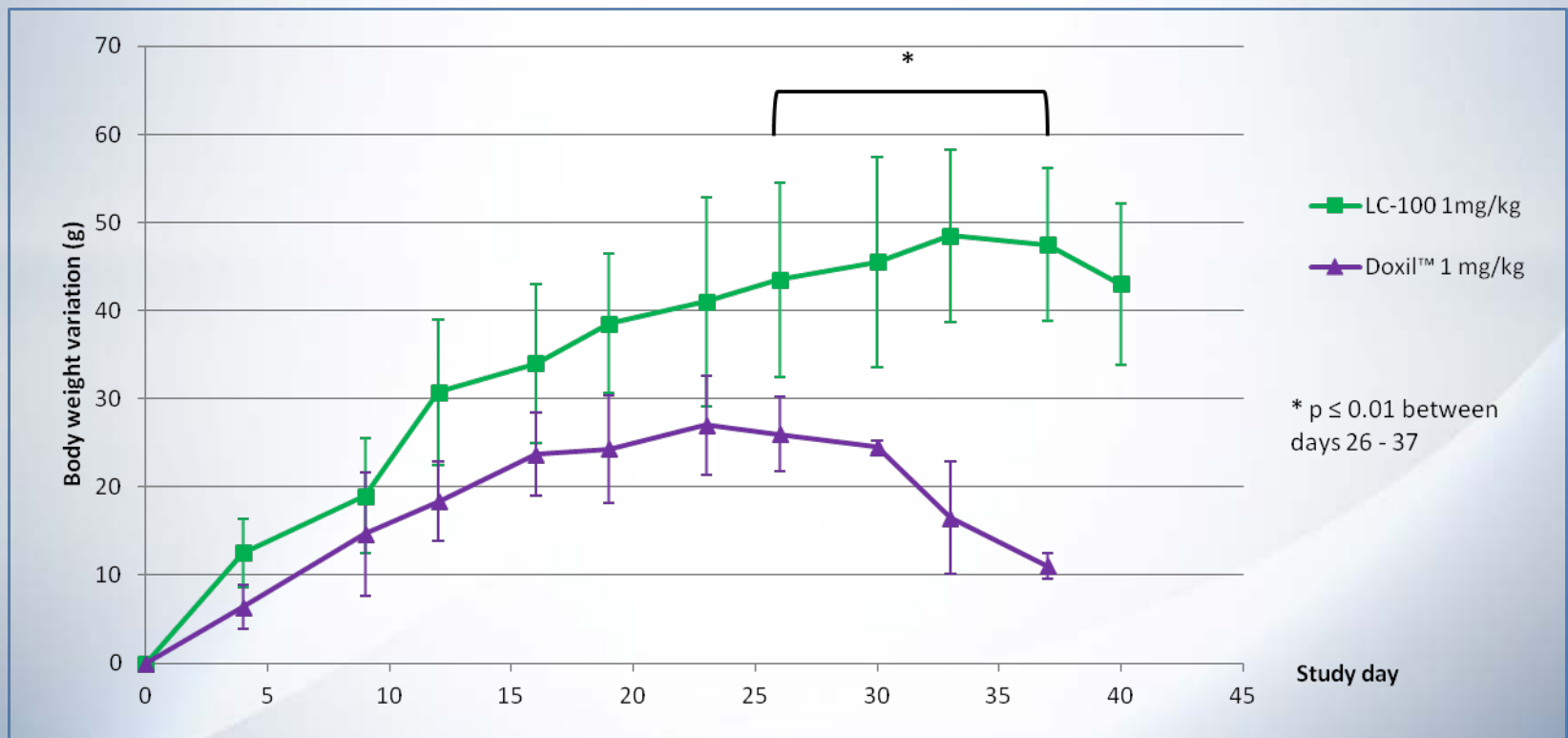


LC-100 versus Doxil™ - reduced rat Hand and Foot (PPE) toxicity (as accumulative score)



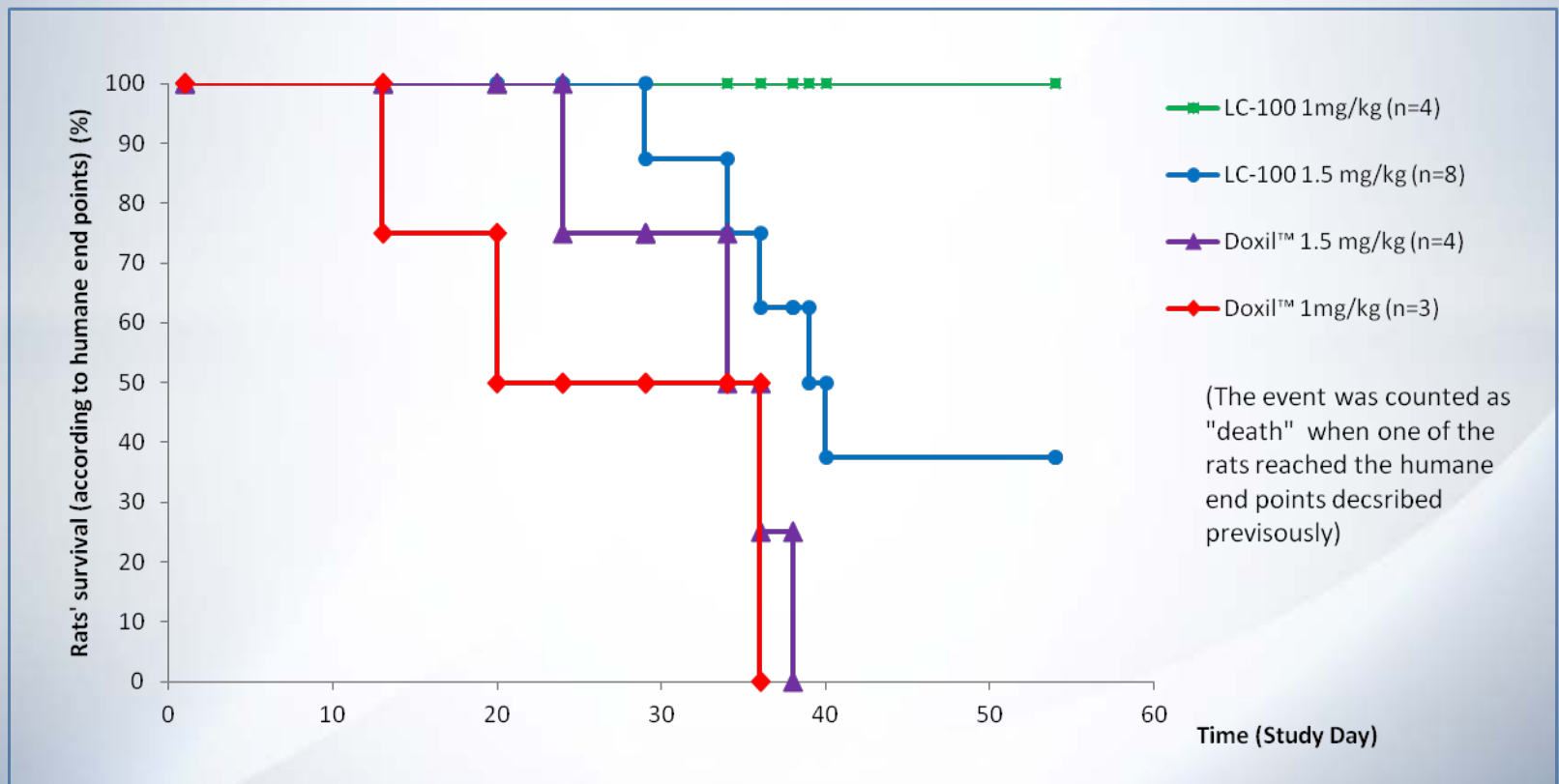
LC-100 versus Doxil™ - Effect on Body Weight gain (general toxicity)

(our novel pegylated liposomal doxorubicin nano-drug) showed **better growth** based on body weight gain in comparison to Doxil™ treatment



Survival of Rats “humane end points”

LC-100 (our novel pegylated liposomal doxorubicin nano-drug) treatment showed much better **“QUALITY OF LIFE”** as compared to Doxil™ treatment



“Humane end points”: signs of severe pain (usually associated with a scoring above 20), excessive porphyrin secretion from the eyes and/or nose, excessive aggressiveness, severe signs of infections, etc...

Conclusions

- LC-100 at repetitive injection of 1mg/kg twice weekly over 12 weeks has an improved safety profile over Doxil™
 1. lower “Hand & Foot Syndrome” score
 2. Lower General Toxicity (body weight)
 3. Both should translate into better “quality of life”

LC-100 Product Development Status

- Current CMC status:
 - Established product specifications and production process
 - Stability for over one year
 - Closure selection and specifications
 - Developed in-vitro release method
- Status pre-clinical (Comparison with Doxil):
 - PPE rats model demonstrating superiority over Doxil
 - General Toxicity demonstrating superiority (Body Weight)
 - Supportive mice PK mice studies with similar tumor drug accumulation
 - Similar (or even slightly better) therapeutic efficacy in the two tested mice models
 - On the way to phase I/IIa clinical trials in ovarian and breast tumors

Special Thanks

to my LC 100 team

Dr Doron Friedman

Tal Berman

Jackie Toledo

Yaelle Felsen

Michael Raslin

Wolf Rajchenbach

Alexander Lyskin

Lisa Silverman

And Janos Szebeni my partner in the development of Doxebo used to reduce Doxil induced Complement activation

Development of liposomal drugs And Nano-Drugs: From academic research via incubators and startups to FDA and EMA approved products

Part II: entrepreneurs and entrepreneurships

**Professor Yechezkel (Chezy) Barenholz,
Laboratory of Membrane and
Liposome Research,
The Hebrew University –
Hadassah Medical School,
Jerusalem, Israel**

**Barcelona NanoMed
March 4-5 2014**



Doxil: 80 – 100 nm SSL
remote loaded with
doxorubicin via ammonium
sulfate gradient. **18 years
to Doxil 1st FDA approved
nano-medicine (11.95)**

Today Agenda

- General difficulties in current drug development
- NMII MLV for osteoarthritis treatment
- Doxil the first FDA approved drug and its MOA (in short)
- Why 3 years after Doxil relevant patent expiration FDA approved only one generic product, Lipodox?
- Lessons learned for the development of novel nano-drugs
- LC100 new generation liposomal doxorubicin with less side effect and better efficacy than Doxil
- **Scientists as entrepreneurs : a user's guide**
35 years of personal experience

The Harvard MBA/Dentist Joke

Tech Transfer from Barenholz Lab HUJI

- *Cancer therapeutic, Doxil licensed to LTI (Sequus), a medium size company (1985) to ALZA to J & J (a very large Pharma)*
- *LC100 a new generation improved “Doxil” licensed to Lipocure a medium size start-up with capacity to produce clinical materials)*
- *Vaccinology Licensed to Nas[→]Vax as inc[→]ubator later a start up*
- *Inflammatory and autoimmune diseases LC200 licensed to Omri transferred to Lipocure (MS, RA, Lupus, on the way to clinical trials)*
- *Local anesthetics ultra long local anesthetics licensed to Lipocure on the way to a clinical trial)*
- *Osteoarthritis (cartilage lubrication and reduction of wear licensed as a medical device to Moebius Medical an incubator Finished successfully proof of concept clinical trial , on the way to the pivotal study*
- *Large scale production of cytotoxic and non cytotoxic liposome based drugs was licensed to Ayana LTD*
- *Cleantech: Use of “cheap” liposomes to clean the environment was licensed to LipoGreen an incubator*

Drug development: from basic research to approved drug

The current chances

It was shown that for every 1,000 compounds that reach testing, only 5 make it to advanced clinical trials and less than 1 is ultimately approved by the U.S. Food and Drug Administration (FDA).

Namely chances for success are very low, the driving force for development in spite the failure is the large reward in case of success.

The Motivation to the development of good nano-drugs is obvious from looking at Doxil[®] Sales on

- Annual sales rate exceeded \$700 M in H1 2011, before Ben Venue production shutdown by FDA
- Cost of Doxil[®] course of treatment
 - 50 mg/m² every 4 weeks
 - \$4000/50 mg vial (\$80/mg)
 - \$8,000/month (\$ 96.000 per year)

Based on adding new (now evaluated) indications sales
may reach \$ 1.0 Billion

Doxil® market is expected to increase due to many new indications in clinical trials

Drugs	Indication	Phase
DOXIL + temsirolimus (mTOR inhibitor)	recurrent sarcoma	I/II
DOXIL + LY573636 (mitochondrial apoptosis)	advanced solid tumors	I
DOXIL	before mastectomy in invasive breast cancer	I
DOXIL + vorinostat & bortezomib	relapsed or refractory multiple myeloma	I
DOXIL + dexamethasone & lenalidomide (thalidomide derivative)	newly diagnosed multiple myeloma	II
DOXIL + ixabepilone (microtubule stabilizer)	advanced ovarian, fallopian tubem or metastatic breast	I/II
DOXIL + bortezomib, cyclophosphamide, dexamethasone	multiple myeloma	I/II
DOXIL + bortezomib, dexamathasone, lenalidomide	relapsed/refractory multiple myeloma	II
+ ~40 additional clinical trials		

Efforts to Make Generic Doxil®

Name	Company	Comments
Myocet®	Enzon Pharmaceuticals/Cephalon	Non-pegylated, approved in Europe for breast cancer
TLC Lipo-Dox®	Taiwan Liposome Company/TTY Biopharm	Non-pegylated, launched in Taiwan in 2001 for AIDS-Kaposi sarcoma, breast cancer and ovarian cancer
Doxisome	Taiwan Liposome Company	In planning of bioequivalence studies as Doxil®/CAELYX® substitute
Lyodox	Amronco Life Sciences Ltd.	Sold in Latin America. poor quality
Doxoget	Getwell pharmaceuticals	Sold in Indi. Poor quality
Lipodox	Sun Pharma	Sold in India, failed EMA and FDA recently FDA approved
Generic Doxil®/CAELYX® Early stage	Teva Pharmaceutical Industries, Ltd.	Israeli-based generic company; in development
Generic Doxil®/CAELYX® early stage	Gedeon Richter PLC	Hungary-based generic company; in development

However as can be learned from the FDA draft guidelines of 2010 on generic Doxil this is not easy

- :
 - Have the same drug product composition
 - Manufactured by an active liposome loading process with an ammonium sulfate gradient and,
 - have equivalent liposome characteristics including liposome composition, state of encapsulated drug, internal environment of liposome, liposome size distribution, number of lamellar, grafted PEG at the liposome surface, electrical surface potential or charge, and in vitro leakage rates.
- Requires *In-Vitro and* clinical Studies

<http://www.fda.gov/downloads/Drugs/.../Guidances/UCM199635.pdf>

Why three years after expiration of Doxil® patents and with drug sales exceeding \$ 700 millions and at least 12 companies that try to make it there is still only one FDA approved Doxil equivalent generic pegylated liposomal doxorubicin (Lipodox of Sun Pharma was very recently approved in US (Not yet in Europe)



Understanding the difficulties will help a lot in the development of new nano-drugs

Technical Hurdles

- Liposomes with the same physicochemical characteristics can have different therapeutic outcomes and toxicities, because measurements of bioequivalence measure only the average properties
- Small changes in manufacturing processes, such as lipids, excipients, equipment, exact method of preparation, or facilities can result in significant changes to therapeutic outcome or toxicities
- The PK/PD of therapeutic nanoparticles is complex

FDA guidelines draft, 2010

<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM199635.pdf>

Require a complex CMC and simple clinical trials

Identity in CMC which is based on detailed physico-chemical parameters

Equivalent composition:

Chemical: drug-to-lipid ratios, amounts of free and encapsulated drug, percent drug encapsulation, lipid bilayer phase transitions, excipients

Liposome characterization

liposome size distribution and morphology as demonstrated on multiple batches and samples of test and reference products (number of lamellae, lipid bilayer phase transition and X ray diffraction pattern, entrapped volume)

Internal liposome environment

drug loading using an ammonium sulfate gradient, internal pH, magnitude of the pH gradient across the membrane, equivalence in the doxorubicin sulfate level, presence and structure by SAXS, WAXS and cryo-TEM of DOX-sulfate precipitate inside the liposomes

Equivalent surface properties

electrical surface and zeta potential, PEG layer thickness, equivalent concentrations and size of grafted PEG at the surface, equivalent PEG-lipid chemistry to prevent premature cleavage of the PEG from the liposome surface

Equivalent drug release rates in a variety of conditions that result in equivalent drug delivery to target (tumor) cells

different physiologically relevant solutions, e.g., human plasma, a range of pH values, a range of temperatures, under low frequency ultrasound

Clinical Requirements

- A single-dose, two-way crossover pharmacokinetic study, in ovarian cancer patients whose disease has recurred or progressed after platinum-based chemotherapy
- AUC and C_{\max}
- V_{dss} and Cl
- Not easy as it require a BE in both encapsulated and free doxorubicin may require many patients which are not easy to get

Failures of Generic Doxil[®] (QbD)

- Smaller liposomes (75 nm, 300 mM ammonium sulfate)

identical lipid composition

identical PK parameters in normal mice

rapid drug release for the 75 nm liposomes

Doxil liposomes had higher AUC and C_{\max} in S-180 sarcoma-bearing mice

75 nm liposomes were more active, therapeutically

75 nm liposomes had greater toxicity (decreases in body weight)

Cui J et al. J Control Release. 2007 Apr 2;118(2):204-15.

- 6 different PEGylated liposomal doxorubicin formulations were compared to Doxil[®] in a murine breast cancer model, and in tumor-free monkeys

counter ion in the loading process was changed from sulfate to dextran sulfate (DSAS)

same doxorubicin plasma PK as Doxil

greater decreases in tumor volume

3.2-fold increased aspartate transaminase levels (hepato-toxicity)

5.0-fold increased cardiac troponin I levels (cardiac toxicity)

increased bone marrow hypocellularity (bone marrow toxicity)

increased kidney toxicity

Basic vs Applied Research: Major points to remember

There is a statement made by the famous French scientist L. Pasteur that “there is no Basic Science and Applied Science but only Good and Bad Science” which is still 100% correct today.

However differences between the two do exist with the good Basic Science being a pre-requisite for a good Applied Research. These differences are important for the optimization of the both R & D and the drug development processes.

The main differences between the basic and applied research are that applied research require to deal with:

Long term stability and large scale production issues

I.P. and “regulation” (a lot of paper work according to requirements of agencies such as FDA)

In applied research which aims to develop product “the best may the enemy of the good” and in order to save money and time the good is enough to get to an approved product.

In applied research ego should not play a major role

Requirements of Industry from Scientists and drug developer at the 21 century are much larger than at the end of the 20th century.

I.P. and know-how related decisions

The I.P. and Know-How are our actual 
assets

**Only I.P. but not know-how can be protected,
however:**

- 1. Patents are very costly. Many times saving I.P. expenses ends up in great losses and even total loss.**
- 2. Patents has to be made so what is licensed can be controlled and will not be too broad limiting other options of the inventor.**
- 3. Many times it will be advantageous (in spite cost) to split patent according to specific applications so each of the applications will not be too broad and can be licensed to a separate company.**
- 4. When is the best time to file a patent application?**

Patent will be in force 21 years after date of filing of the provisional application

Other important points to remember

Every Scientist believe his project is unique and he forget that Industry has many options.

Scientist has to understand industry and investors needs and language

Scientists, University, and TTO require knowing well the competition, and understanding the needs and limitations.

External help to the Scientist is a must especially on I.P. and regulatory issues.

approaching the right people in Industry can make make the “click”. (**Connections, connections, connections**)

Important roles of thumb:

The I.P. clock has a limit of 21 years (from patent filing), therefore shortening development time or postponing as long as possible mean a lot of money

Development of nano-drugs is multidisciplinary Therefore productive sharing is a must.

100% of Zero is equal exactly to Zero!!!

Major Strategic Decisions to be Taken

How to select a strategic/financial partner ?

What are the options?

1. Big Pharma (royalty agreement and maybe upfront payment plus research and consulting). The main advantages: good and long term financial and professional support. Disadvantages: rigidity and competition with many other company projects, also the inventor may have only a small or no say in the development program.
2. A middle size Pharma or existing start-up. Advantages and disadvantages are the opposite to the situation exists for Big Pharma. The scientist project may become the main focus of the company
3. Building a start-up around the TTO I.P. (royalties and/or equity, consulting, research)? See NasVax example. Always short on money wasting IP time
4. Starting with an incubator (Mobeius OA project), hardly enough for feasibility studies unless larger that required investment was made (Polypid)
5. Trying to take it forward to include production, fits only very specific projects

The complex triangle involved in a productive applied science

Three sides of a triangle: Industry, (and/or financial enterprise); University plus Technology Transfer company (TTO); and the Scientist (researcher) constitute the three sides combine to perform a project and commercialize it.

Although all three parties share a common interest: the project success, they also have many opposing interests.

Relationships between the 3 parties are therefore complex and common interests varied, like: University (TTO) – Scientist; Industry – Scientist; University (TTO) - Industry etc.

Each of the 3 sides should perform what he knows best, but help other sides on all need (good interaction and collaboration)

In this short presentation I will discuss the role of each side and demonstrate it on my own >20 years of experience

Roles of Each of the 3 Parties

Role of Industry (strategic/financial partner) :

To supply the financial support of the R & D program; being responsible (or sharing responsibility with Scientist) on all what involved in clinical trials or equivalent steps (such as beta sites),

Finish R & D of the drug prototype and the final form of the drug

To finance Scientist research and I.P.

Roles of Each of the 3 Parties

Role of Technology Transfer Company (TTO):

To represent Scientist (and University);

To deal on behalf of the scientist and University with all legal and administrative issues of the project.

To make sure all I.P. (patentability and “freedom to operate” issues were dealt with properly

To make sure that Scientist get all his needs to perform his duties in the project.

To make sure all I.P. issues were dealt with properly

To maximize interests of Scientist and University in a fair way

Roles of Each of the 3 Parties

Role of scientist/researcher

To perform the research, proof of concept, and feasibility studies. These may require minimal R & D to produce a prototype according to the needs

To help with supplying the basic information required to respond to I.P. issues

To keep good records of research methods and results

To supply Industry with all the needs for advancing the program smoothly

To interact with and be open to advice from Industry

The 3 parties in my case are:

The Technology Transfer Company: Yissum Ltd

R & D company of the Hebrew University Jerusalem Israel

The Scientist: Professor Yechezkel Barenholz Ph.D.

**Head Laboratory of Membrane and Liposome Research at
the Hebrew University – Hadassah Medical School,
Jerusalem, Israel**

The Industry: for Doxil - LTI (Sequus), ALZA, J & J

**For other projects: Various, including large Pharma,
Biotech companies, Start-ups, and Incubators.**

TTO - Scientist Relationship

What I got from TTO during Doxil development?

1. Help (financial and “package design”) with feasibility studies (**NO**)
2. Help on IP issues (patentability and freedom to operate) to find suitable patent attorney (**YES**)
3. Help in finding funding (**NO**)
4. Help in contractual arrangements with respect to IP issues, research support and benefits (**YES, partial**)
- 5. Guidance on how to keep records (NO)**
6. Help in presentations (**NO**)
7. Criticism!!! (**NO**)

Today Yissum is better on most items but the scientist has to be on guard all the time

A cartoon character with green hair, wearing a pink jacket, white shirt, and blue pants, waving with one hand.

THANK YOU!

From Jerusalem

