Liposomes

Daan Crommelin, Dutch Top Institute Pharma
Gert Storm, Utrecht University

EMA 1st international workshop on nanomedicines
2-3 September, London

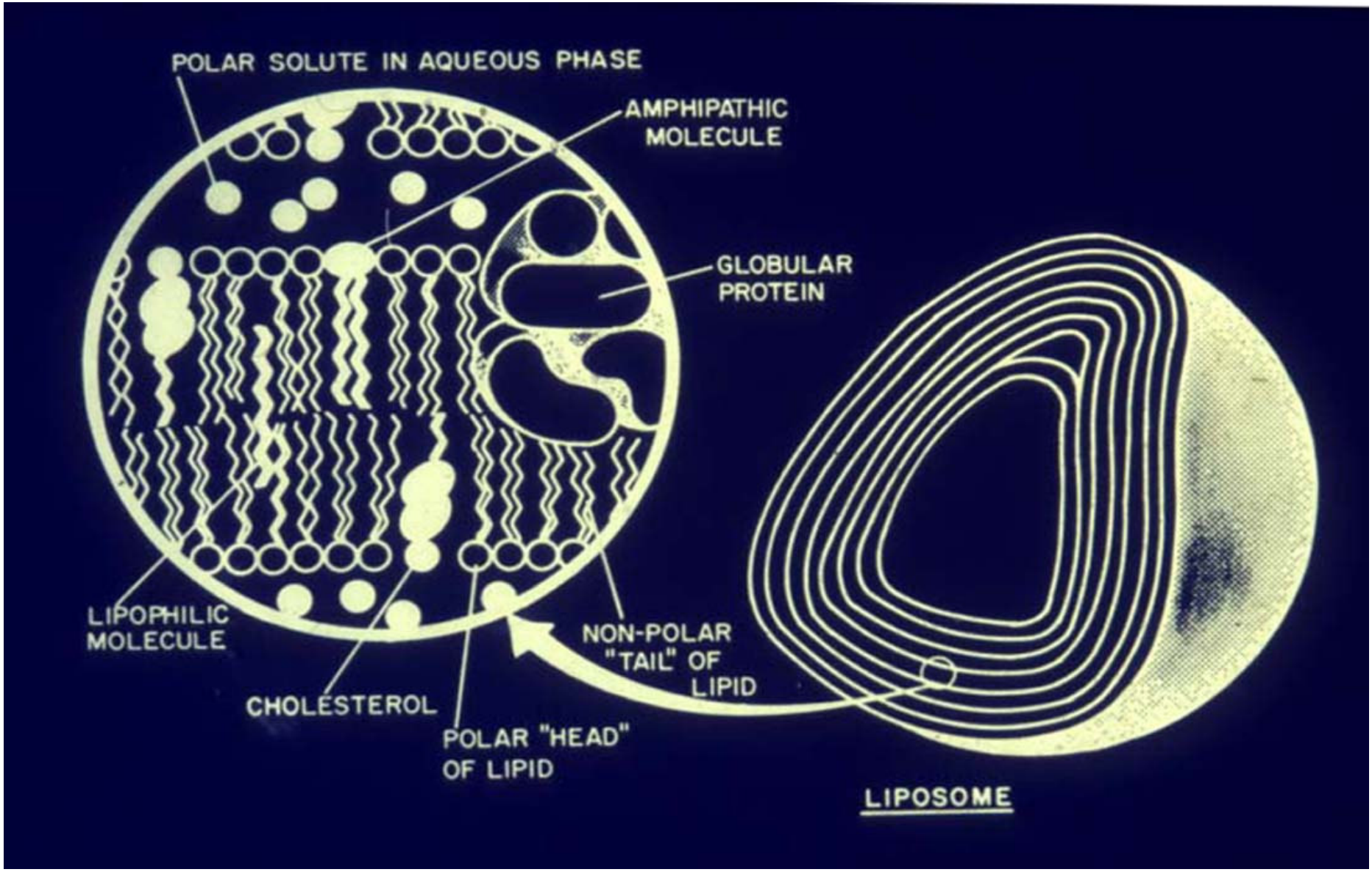
jointly shaping the future of medicine

.

Declaration of interest Crommelin: See my CV in folder

THE liposome does not exist.....

TI PHARMA

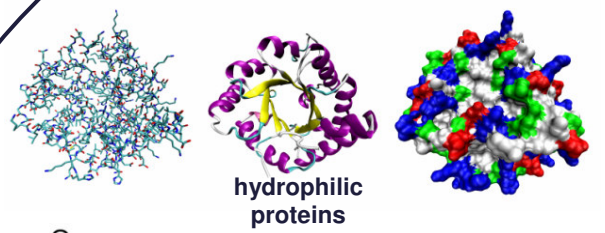


From Gregoriadis, 1979

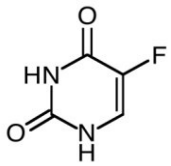
Liposomes: which actives?

TI PHARMA

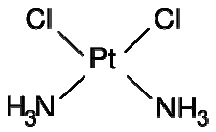
Hydrophilic molecules



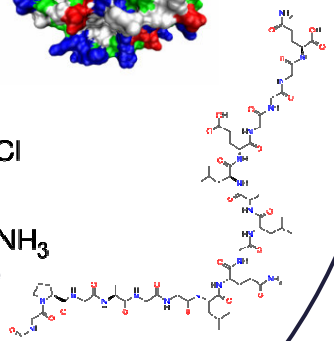
hydrophilic proteins



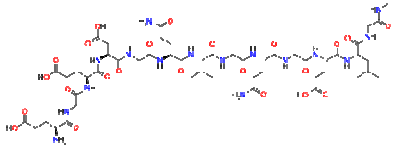
5'-fluorouracil



cisplatine



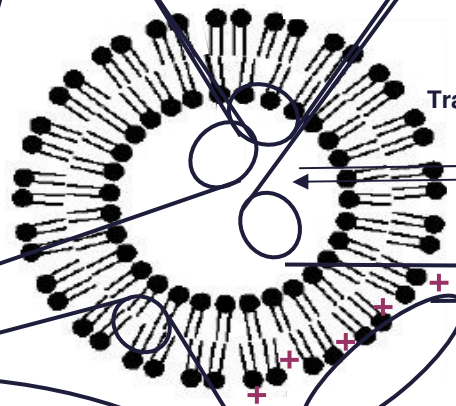
hydrophilic peptides



Amphiphilic molecules

Depending on the partition coefficient

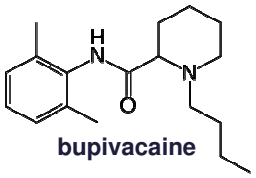
Covers a wide range of molecules



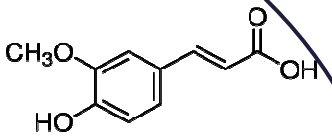
Transmembrane gradient

(Δ pH, chemical salts)

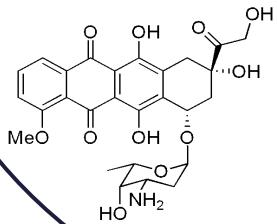
Weak bases and weak acids



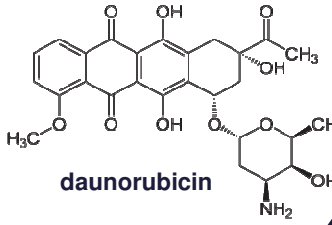
bupivacaine



ferulic acid

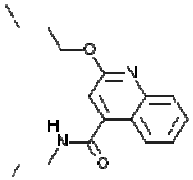


doxorubicin

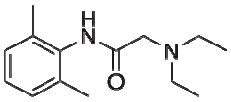


daunorubicin

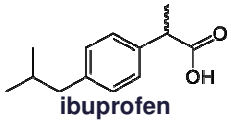
Hydrophobic molecules



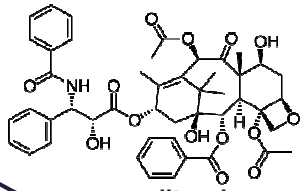
dibucaine



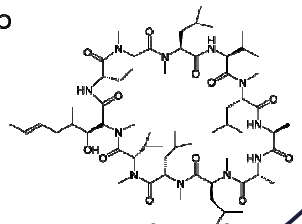
lidocaine



ibuprofen

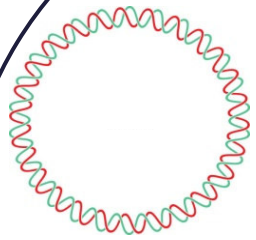


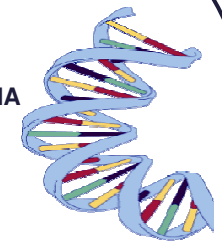
paclitaxel



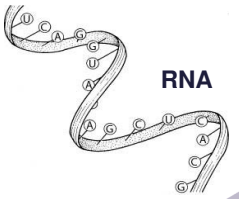
cyclosporin

Nucleic acid based-drugs





DNA



RNA

Courtesy of OctoPlus

Approved 'Liposome'-based Drug Products**

Product	Year Approved	API	Sparingly Soluble	Revenue
Visudyne®	2000	Verteporfrin	Yes	90 M $*
DOXIL/Caelyx®	1995	Doxorubicin	No	550 M $*
AmBisome®	1990	Amphotericin B	Yes	400 M $*
ABELCET®	1995	Amphotericin B	Yes	
Definity®	2001	Octafluoropropane	Yes	
Myocet®	2001	Doxorubicin	No	
DepoCyte®	2002	Cytarabine	No	
DepoDur®	2004	Morphine	No	
Daunoxome®	1996	Daunorubicin	No	
Octocog alfa®	2009	Factor VIII	No	

* Thomson Pharma www.northernlipids.com ** not exhaustive

What is in the pipeline?

liposomes | Search

PVP-I **liposomes**, Mundipharma	(PVP-ILH liposomes, Mundipharma)
PVP-ILH **liposomes**, Mundipharma	
ciprofloxacin **liposomes** (STEAL...	
heat-sensitive **liposomes** (doxo...	(doxorubicin (heat-sensitive li...)
non-phospholipid **liposomes**, No...	(BCTP)
E1A/DC-cholesterol **liposomes**, ...	(tgDCC-E1A)
111In-VNB-pegylated **liposomes** ...	(NanoVNB)
CKD-602 (injectable **liposomes**,...	(AP-30)
vincristine sulfate **liposomes** ...	(vincristine sulfate (liposomal...)
vincristine sulfate **liposomes** ...	(vincristine sulfate (liposomal...)
anti-her2 doxorubicin **liposome**...	(doxorubicin-her2 antibody frag...)
belotecan (injectable **liposome**...	(AP-30)
111Indium-VNB-pegylated **liposo**...	(NanoVNB)
docetaxel (heat-sensitive **lipo**...	
carboplatin (heat-sensitive **li**...	
doxorubicin (heat-sensitive **li**...	

mal | Search

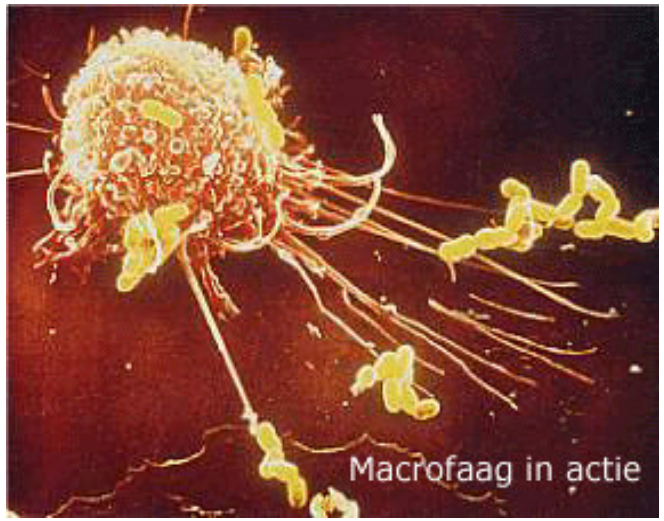
somal Alzheimer's disease ...	(ACI-24)
somal Bcl-2 (lymphoma/soli...	(BP-100-1.02)
somal CTL/CpG vaccine (can...	(HPV E7 cancer vaccine (liposom...)
somal EphA2 siRNA	
somal GW-1843-U89 (cancer)...	(OVI-237)
somal Grb-2 modulator (can...	(BP-100-1.01)
somal IL-2, Biomira	(interleukin-2 (liposomal), Onc...)
somal KSA vaccine, IDM Pha...	
somal NDDP	(AR-726)
somal PGE-1, Endovasc	(liposomal prostaglandin E-1, E...)
somal Raf-1 siRNA (antican...	(Raf-1 LE-siRNA)
somal SOD (Peyronie's dise...	(Lipoxysan)
somal SOD (wound healing),...	(Lipoxysan)
somal T4 endonuclease V, A...	(T4N5 liposome lotion, AGI Derm...)
somal adjuvant system	(TLC A-60)
somal adjuvant, Statens Se...	(CAF-01)

Thomson-Pharma, 2010

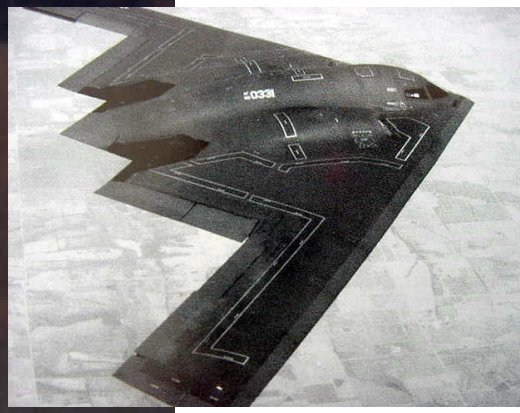
Factors controling the fate of liposomes *in vivo* after intravenous administration:

- size of the liposomes (0.03 - 20 µm)
- type (morphology) (unilamellar, multilamellar, multivesicular)
- charge of the bilayer (negative, neutral, positive)
- rigidity of the bilayer (gel/fluid state)
- route of administration

Liver and spleen (macrophages) are major 'consumers' of liposomes.....



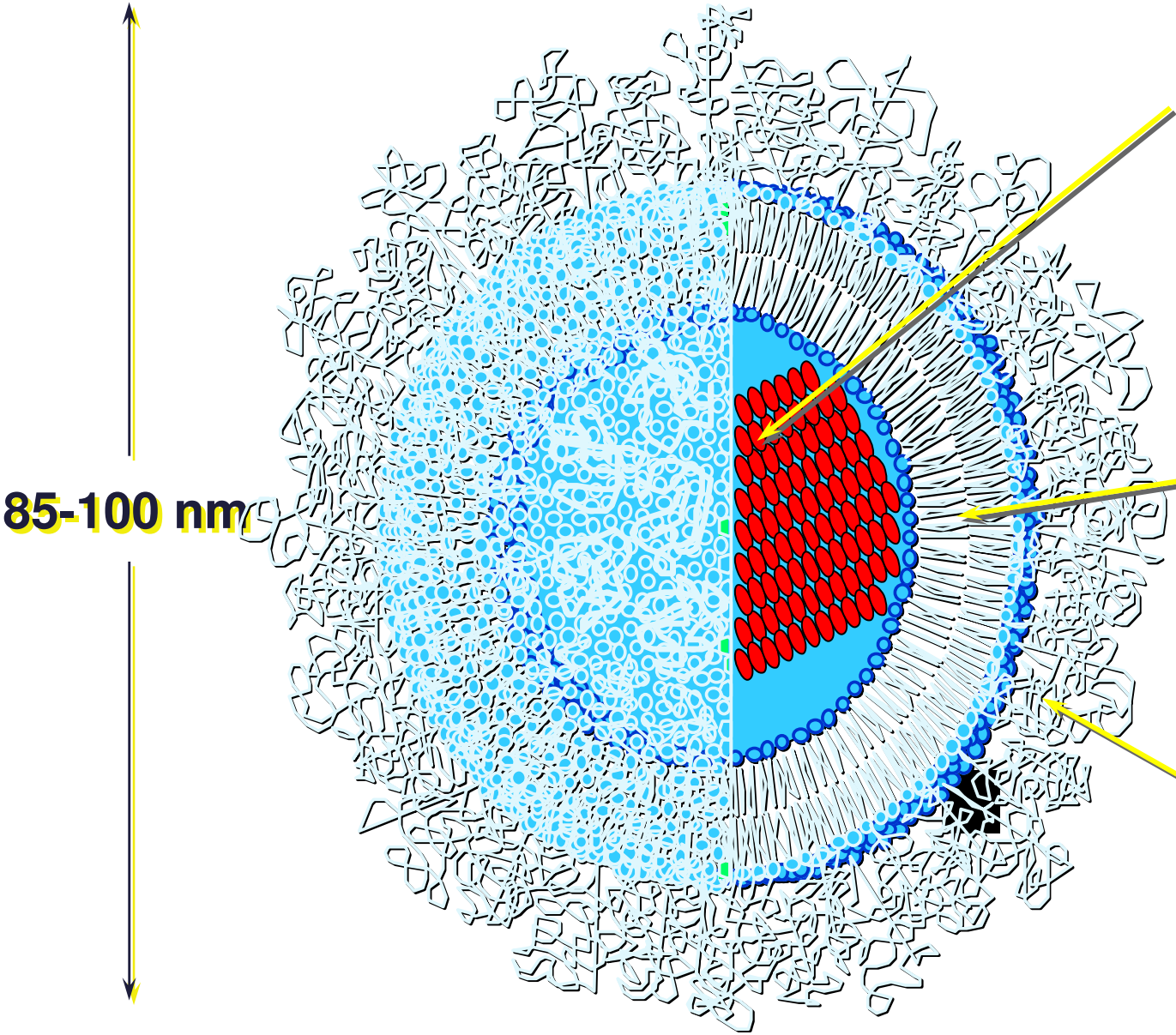
Reduction of 'non-target site uptake'



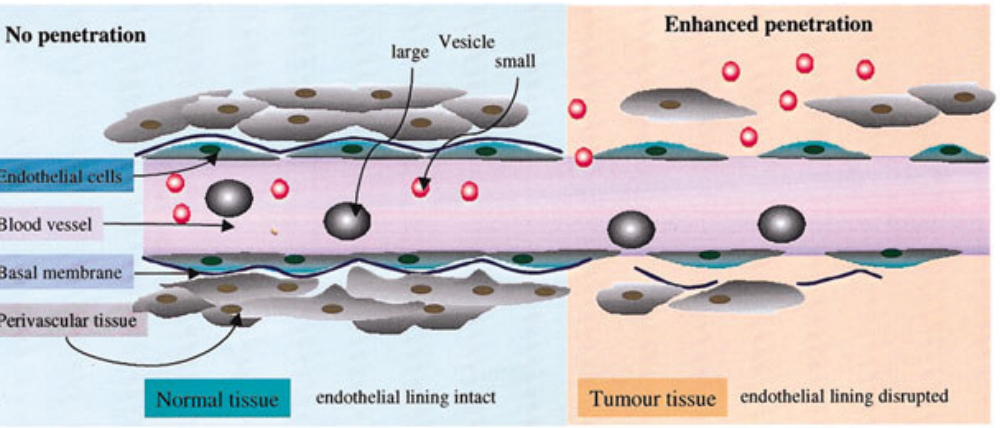
PEGylation:

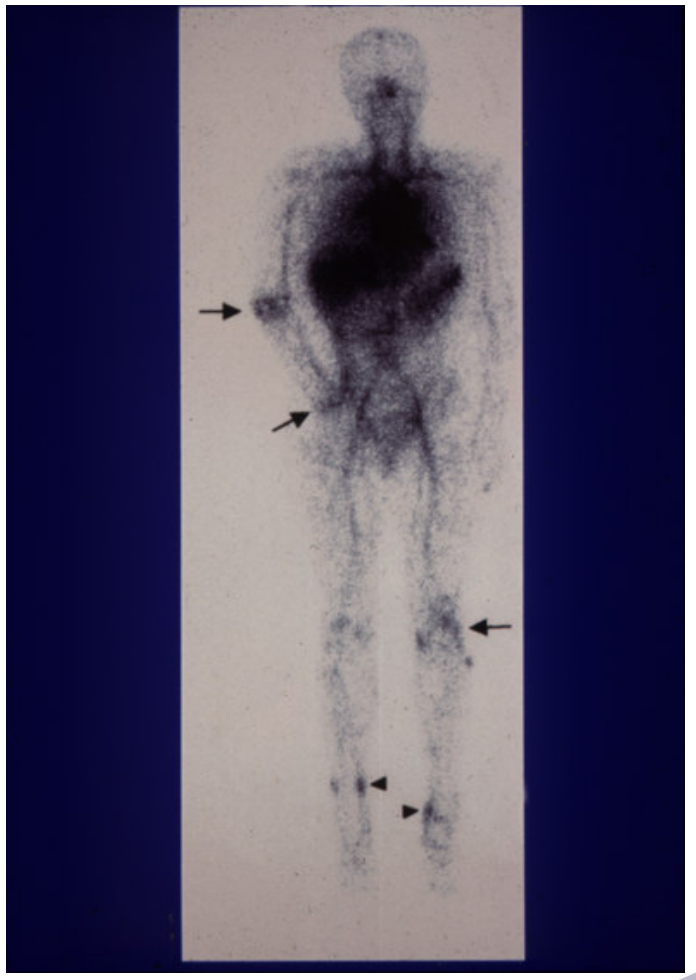
- **Masking uptake–receptor sites**
- **Reducing clearance by glomerular filtration**
- **Reducing immunogenicity (?)**

Structure of Doxil®



Enhanced Permeation and Retention Effect (EPR)

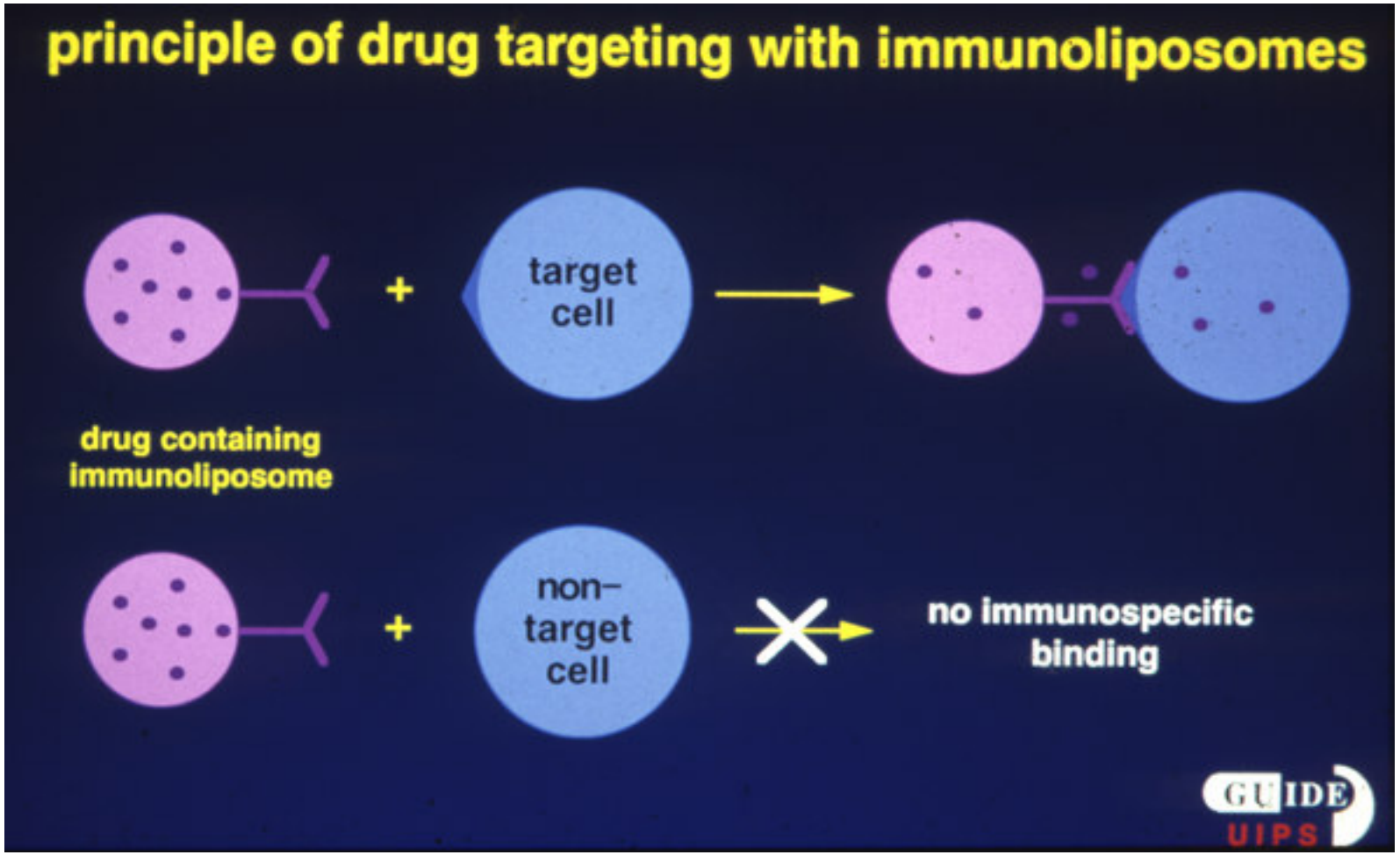




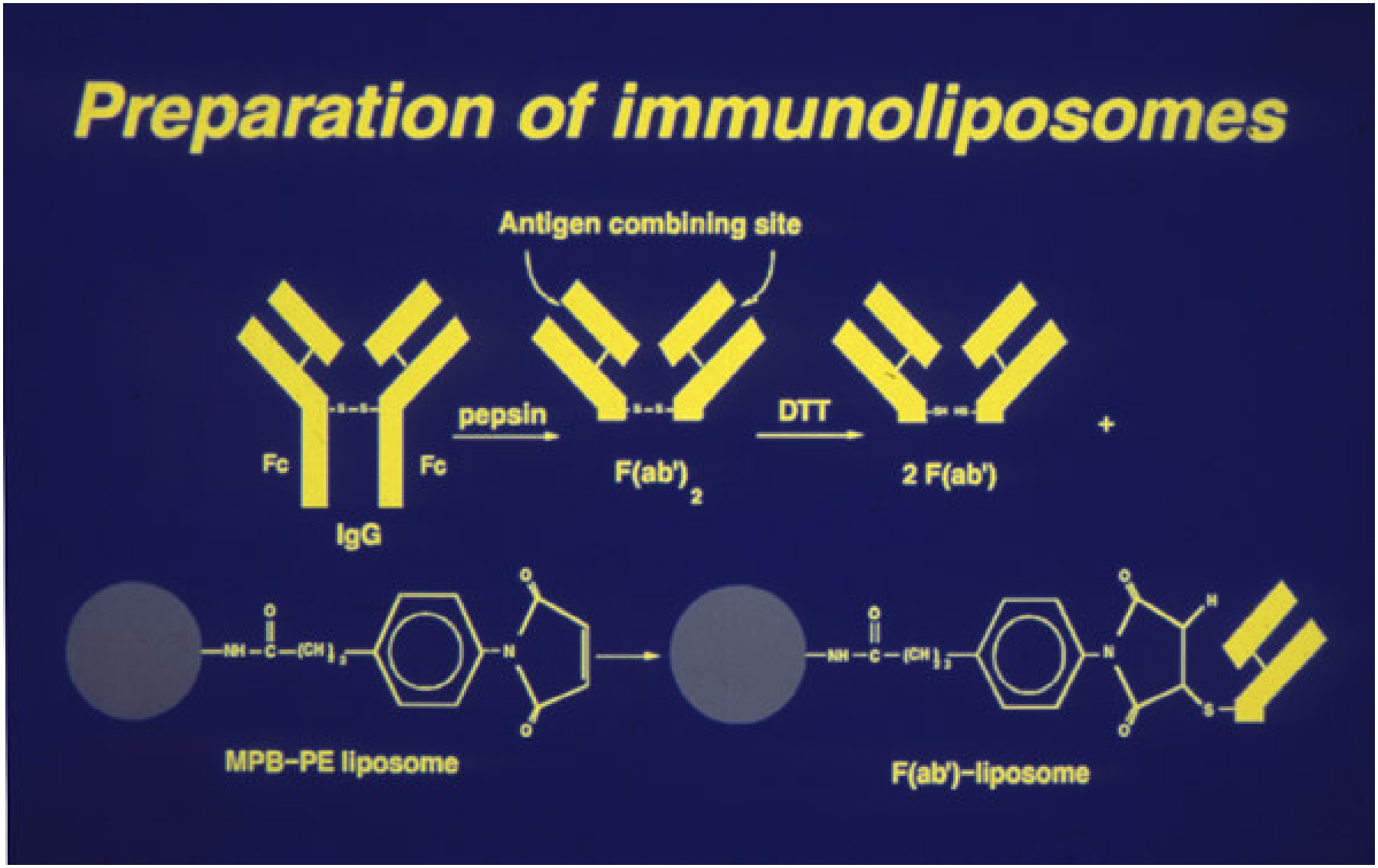
Tc-liposomes,
PEG type
100 nm
46 year female patient (Dams/Storm et al.)

- synovial lining elbow, wrist, knee, ankles

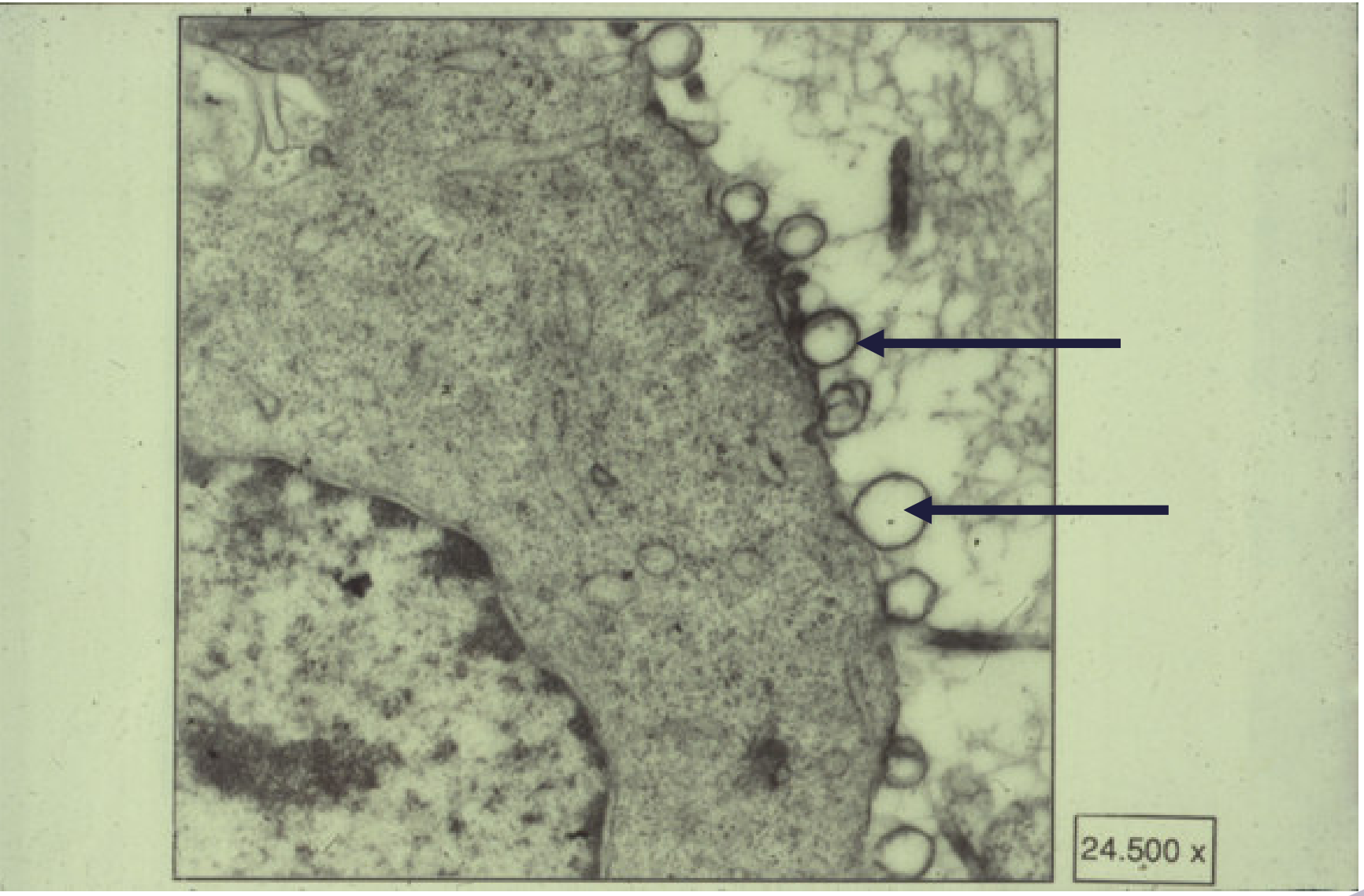
Targeted delivery of drugs..... Can't we do better?



Preparation of immunoliposomes



Immunoliposomes on human ovarian tumor cell (Nassander et al)



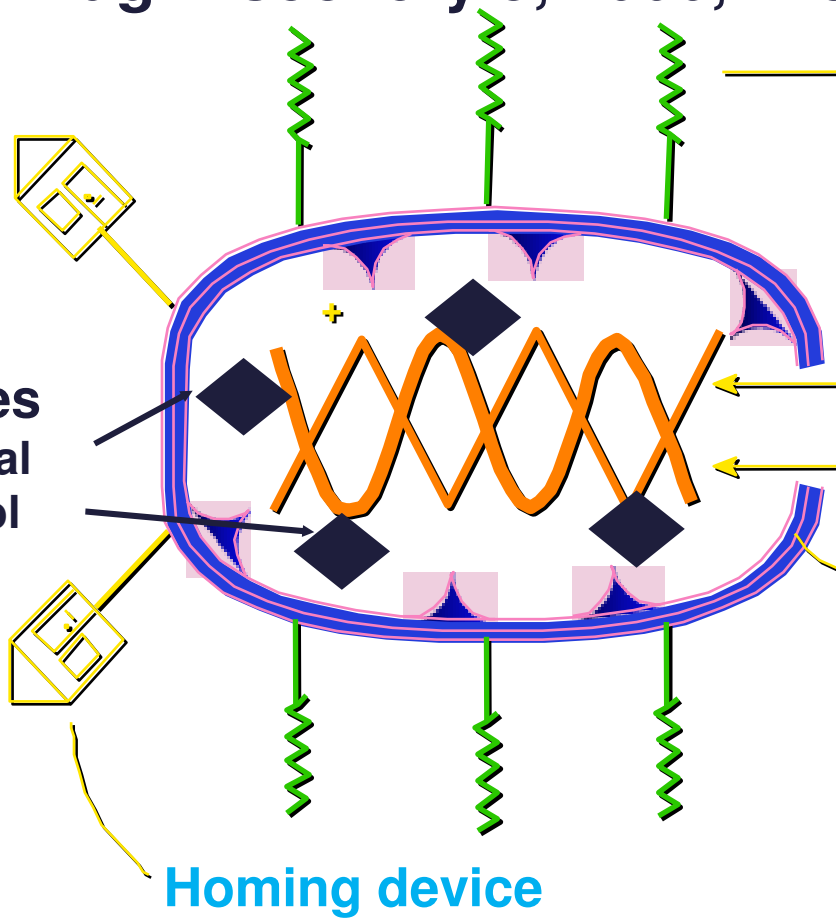
INNOVATION

Artificial viruses: a nanotechnological approach to gene delivery

Enrico Mastrobattista, Marieke A. E. M. van der Aa, Wim E. Hennink and Daan J. A. Crommelin

Nanotechnology: for Perfect Delivery

Nature Reviews Drug Discovery 5, 2006, 115



ell trafficking devices
uclear localization signal
icrotubular hook-up mol

The ‘artificial virus’ approach: U-t(r)ech(t)-nology

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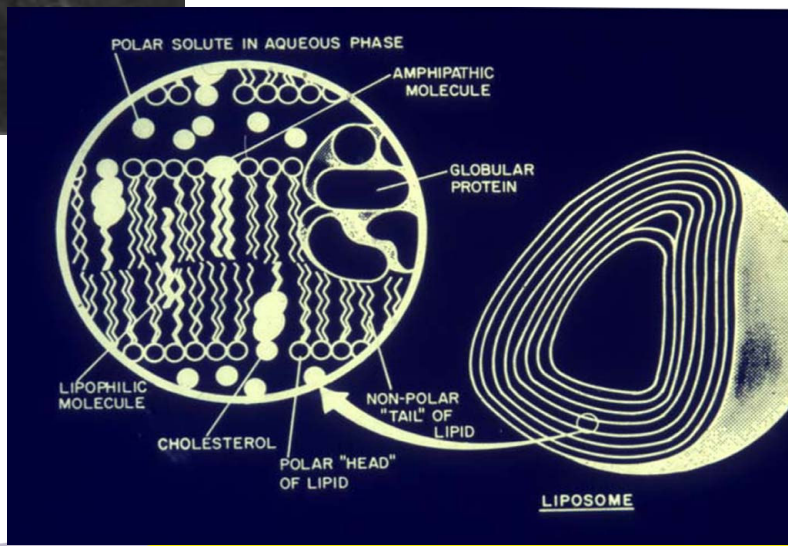
KISS approach?



Liposomal Drug Products: Detailed Characterization A Must!

composition and physicochemical characteristics

↓

in vivo fate

↓

efficacy and safety

To assure the quality of liposomal formulations a number of evaluation tests are available:

Assay/ Characterization	*Methodology/Analytical Target*
pH	pH meter
Osmolarity	Osmometer
Phospholipid concentration	Lipid phosphorus content/HPLC
Phospholipid composition	TLC, HPLC
Cholesterol concentration	Cholesterol oxidase assay, HPLC
Drug concentration	.....
Chemical stability	
pH	pH meter
Phospholipid peroxidation	conjugated dienes, lipid peroxides
	FA composition (GLC)
Phospholipid hydrolysis	HPLC, TLC, FA concentration
Cholesterol autooxidation	HPLC, TLC
Antioxidant degradation	HPLC, TLC

To assure the quality of liposomal formulations a number of evaluation tests are available:

- *Physical stability*

Vesicle size distribution	
submicron range	DLS
micron range	Coulter Counter, light microscopy laser diffraction, GEC
Electrical surface potential, surface	pH/zeta-potential measurements, pH sensitive probes
Numbers of bilayers	SAXS, NMR
Percentage of free drug	GEC, IEC, protamine precipitation
Dilution-dependent drug release	retention loss on dilution
Relevant body fluid induced leakage	GEC, IEC, protamine precipitation

Biological characterization

Sterility	aerobic and anaerobic cultures
Pyrogenicity	rabbit or LAL test
Animal toxicity	monitor survival, histology, pathology

(Based on Barenholz and Crommelin, 1994)

SAXS = small angle X-ray scattering, DLS = dynamic light scattering, GEC = gel exclusion chromatography, IEC = ion exchange chromatography, LAL = Limulus Amoebecyte Lysate, NMR = nuclear magnetic resonance, SAXS = small angle X-ray scattering, TLC = thin layer chromatography.

Guidance for Industry

Liposome Drug Products

Submission of Chemistry, Manufacturing, and Controls, Human Pharmacokinetics and Bioavailability and Labeling Information

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

Comments and suggestions regarding this draft document should be submitted within 90 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit comments to Dockets Management Branch (HFA-305), Food and Drug Administration, 12420 Parklawn Dr., rm. 1-23, Rockville, MD 20857. All comments should be identified with the docket number listed in the notice of availability that publishes in the *Federal Register*. For questions regarding this draft document contact XXX.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
XXX, 2001

Mei-Ling Chen

Contains Nonbinding Recommendations

Draft Guidance on Doxorubicin Hydrochloride

This draft guidance, once finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the Office of Generic Drugs.

Active ingredient: Doxorubicin Hydrochloride

Form/Route: Liposome injection/Intravenous

Recommended studies: 2 Studies

When the test and reference pegylated liposome products

- have the same drug product composition and
- are 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

February, 2010

ttp://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM199635.pdf

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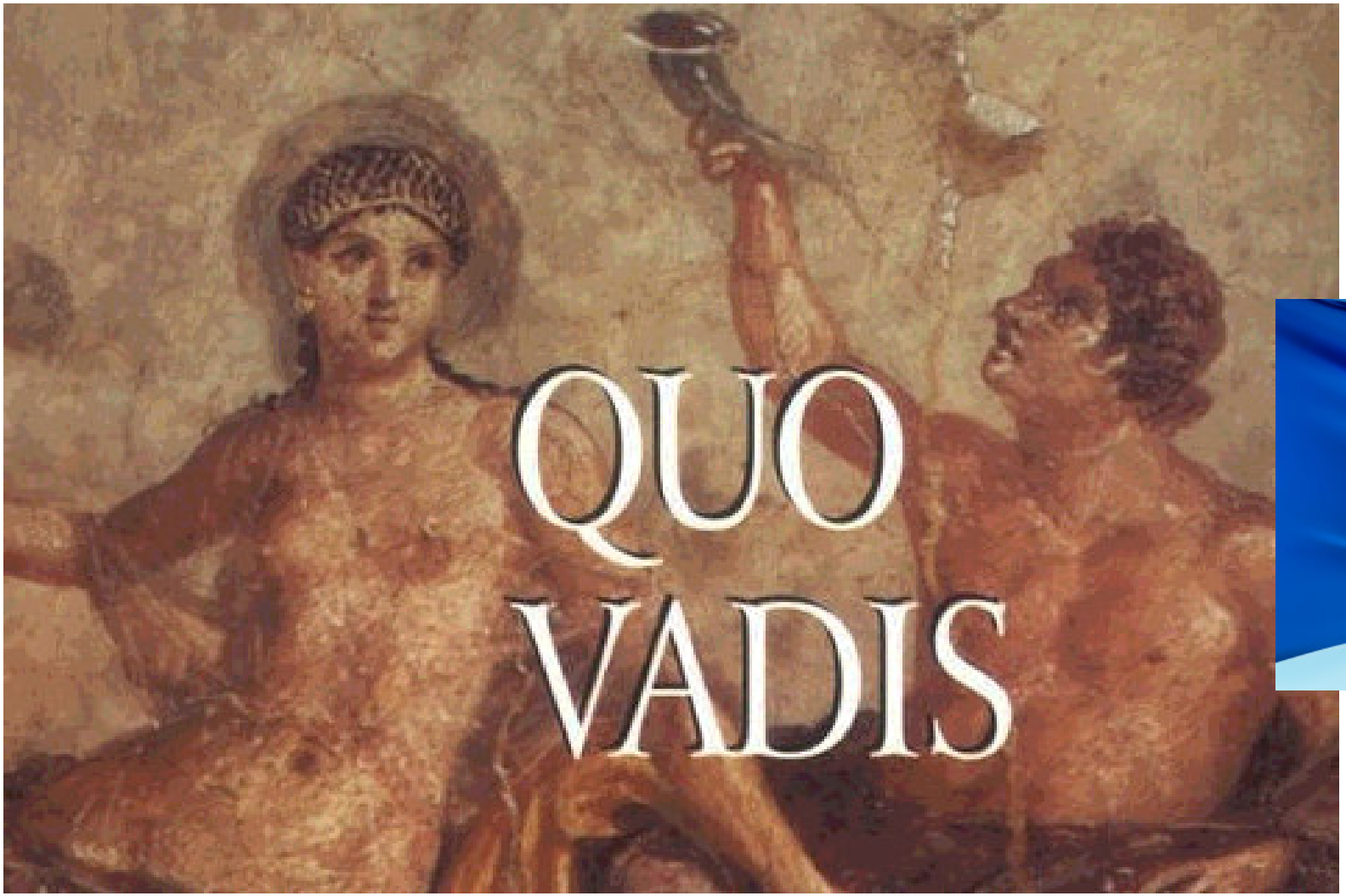

Henryk Sienkiewicz