Rediscovering Potential of Liposome in Advanced Drug Discovery in Oncology

AAPS 2024
October 22, 2024

Naoki Yamada ,PhD
Director of Strategy and Operations
FUJFILM Pharmaceutical U.S.A., Inc.

FUJIFILM
Value from Innovation

Session Description and Objectives

Description

Small molecules are a promising modality for anticancer drugs such as Targeting Protein Degrader (TPD). The complicated design of advanced small molecules has inherited significant difficulties in biodistribution. In this session, the presenter will show how liposomes improve the biodistribution of small molecule drugs and discuss the potential of liposomes to create new value in the growing field of molecular targeted therapy, immunotherapy and TPD by presenting in-house data.

Learning objectives

- ✓ To understand the emerging potential of liposome formulation in advanced cancer therapies, such as molecular targeted therapy, immunotherapy and TPD field.
- ✓ To learn the liposome API encapsulation mechanism and AI prediction method for encapsulation success rate.
- ✓ To learn about the CMC challenges in liposomal drug development.

Biography and Contact Information

Naoki Yamada is a Director of Strategy and Operation at FUJIFILM Pharmaceuticals U.S.A., Inc. Through 19 years at Fujifilm working as a formulation and process development scientist.

After working on silver halide crystal photographic film for camera and movies in 2000s, Naoki has been at the forefront of the company's expansion into the pharmaceutical industry, demonstrating his deep knowledge in drug formulation, as well as the company's fine chemical technology cultivated through photographic film manufacturing.

His achievements include the following: development of proprietary manufacturing system for nanoparticle-based drugs; leading formulation development for liposome/LNP-based pipelines, including two in clinical stage; and scale-up studies supporting a newly established GMP facility for nanoparticle-based drugs.

Naoki Yamada, Ph.D.
Director of Strategy and Operations
FUJIFILM Pharmaceuticals U.S.A., Inc
Email: naoki.yamada@fujifilm.com

Forward Looking Statements and Regulatory Matters

This presentation contains certain statements which constitute "forward-looking statements". These forward-looking statements may be identified by words such as 'believes', 'expects', 'anticipates', 'projects', 'intends', 'should', 'seeks', 'estimates', 'future' or similar expressions or by discussion of, among other things, development and manufacturing strategies and goals, plans or intentions. The forward-looking statements involve risks and uncertainties that could cause actual business, financial, and further technology, manufacturing, preclinical, clinical and regulatory development results to differ materially from those expressed in the forward-looking statements. Many of these risks and uncertainties relate to factors that are beyond Fujifilm's abilities to control or estimate precisely, such as future market conditions, the behaviors of other market participants, the technological success of Fujifilm's Contract Development and Manufacturing Organization services, regulatory authorization or approval of Fujifilm's or its customer's product candidates, and other business effects, including the effects of industry, economic or political conditions, and therefore undue reliance should not be placed on such statements. Examples of forward-looking statements in this presentation include, but are not limited to, statements regarding the potential of Fujifilm's LNP technology to result in one or more competitive products that are authorized or approved by applicable regulatory agencies in one or more countries. Actual results may differ materially from those in the forward-looking statements.

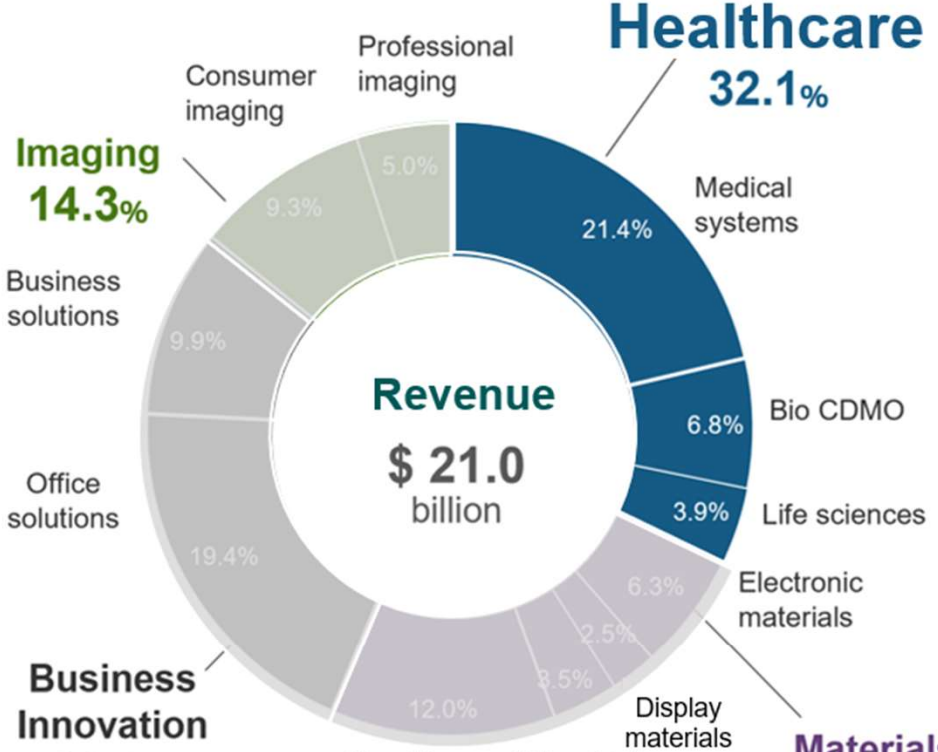
This presentation contains statements related to the biological, chemical, medical, and related characteristics of product candidates under development by Fujifilm and the commercial promotion, distribution, and sale of which will require authorization or approval from regulatory agencies on a country-by-country basis. Positive results of preclinical experiments in nonhuman laboratory models is no guarantee of such results in clinical studies in humans. None of Fujifilm's product candidates described in this presentation have been authorized or approved by any regulatory agencies; and nothing contained in this presentation should be regarded as the promotion or marketing of any such product candidates or any review or decision by any such regulatory agencies as to the safety or effectiveness of such product candidates, or whether such product candidates will be authorized or approved by any such regulatory agencies.

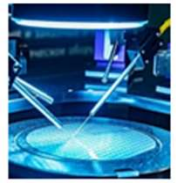
Topics

- ✓ Fujifilm company profile at a glance
- ✓ Expanding field of small molecule development and the power of liposome formulation
- ✓ A liposome application example on Targeted Protein Degrader (TPD)
- ✓ Stability of liposomal is the Critical Quality Attribute : How to achieve?
- ✓ Versability of liposome for different types of API
- ✓ Challenges in liposome CMC development and solutions

Diversify and latest portfolio 2023

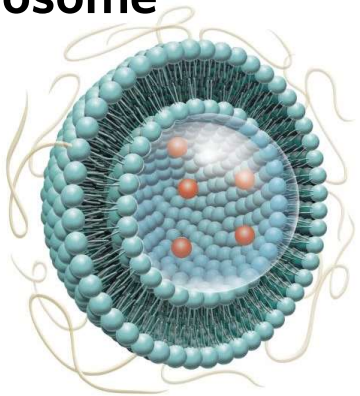
FUJIFILM





Nanoparticle Solutions for Advanced Drug Development

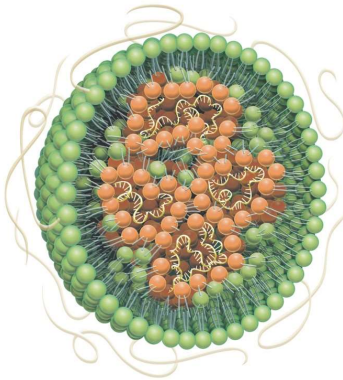
Liposome



Lipid bilayer nano capsule

- ✓ Encapsulating small molecule in the lipid bi-layer capsule
- ✓ Stable capsule accumulates in cancer tissue and releases API in the site
- ✓ The stability of the lipid bilayer is the critical quality attribute (CQA).

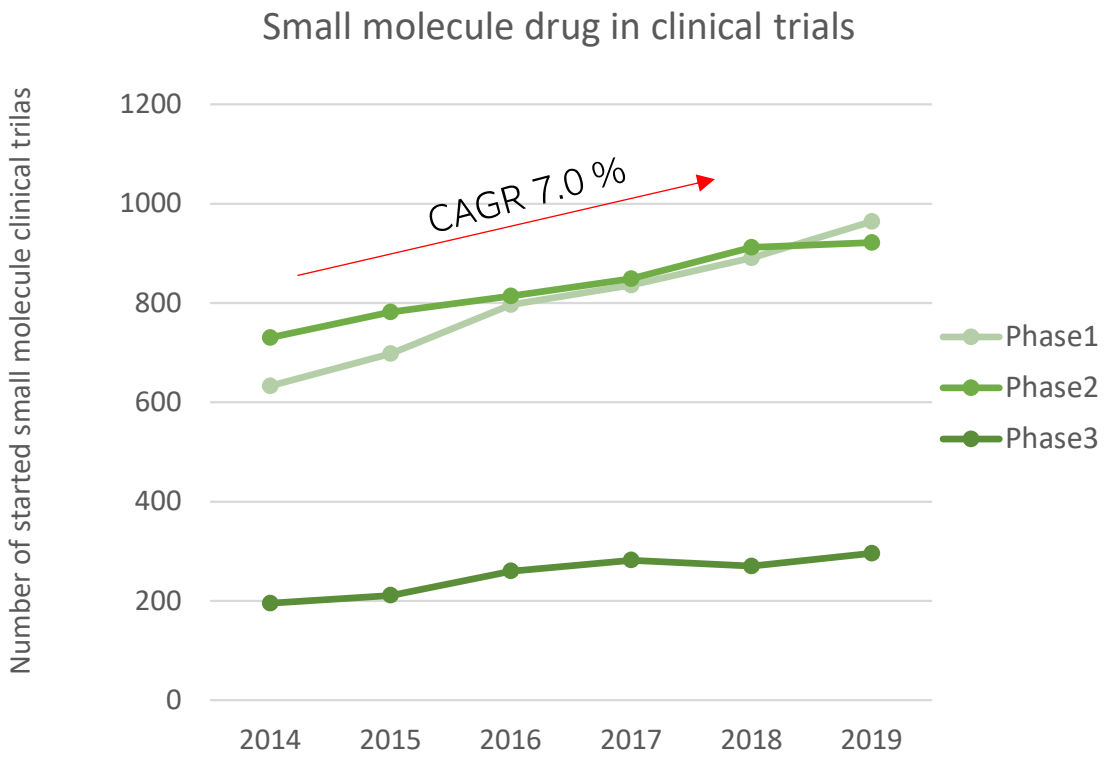
Lipid Nano Particle(LNP)



Ionizable lipid and anionic nucleonic acid nanocomplex particles

- ✓ mRNA delivery for vaccines, therapeutics *in vivo* and *ex vivo* cell therapies
- ✓ Ionizable lipid is the key essential material for efficacy and safety.

Increasing Number of Small Molecule Development

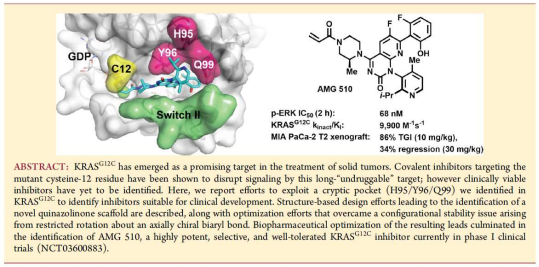


Prepared by author based on the data from https://themedicinemaker.com/manufacture/small-molecules-sizable-market-opportunities

Development of small molecule anti-cancer drugs is expanding in advanced drug discovery fields.

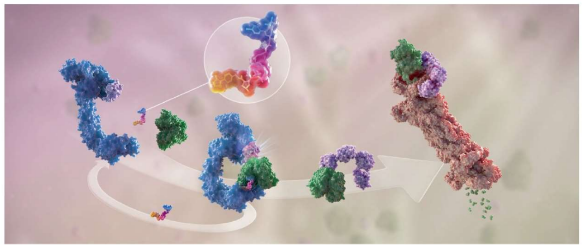
Efforts and Increasing Difficulties of Small Molecule Design

Molecular targeted therapy
Molecules design for hard-to-druggable targets



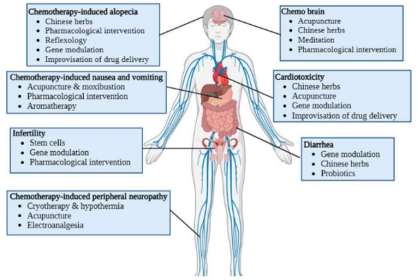
Brian A et al. J. Med. Chem. 2020, 63, 52−65

Targeted protein Degradation (TPD)
Two targeting moieties in one compound



Miklós Békés et al. Nature Reviews. 2022, 21, 181

Chemo with Immune therapy
Combination with Immune check point inhibitors to increase response rate



Uttpal Anand et al. Genes & Diseases, 2023, 10, 1367

The complicated design of molecules potentially have inherited significant difficulties in ADMET.

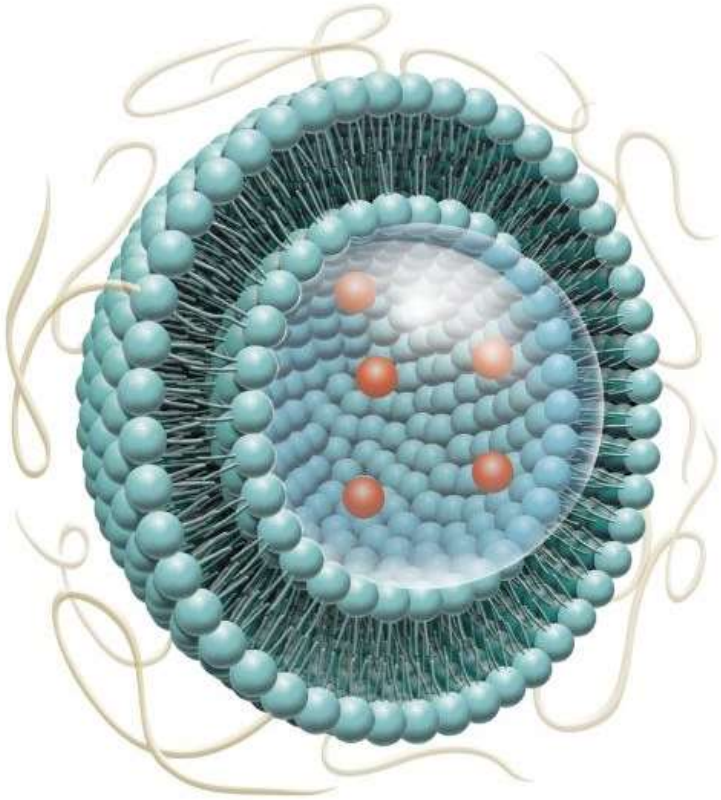
Chemo have challenges on off target side effect

Liposome formulation can potentially help improve pharmacokinetics and safety instead of additional fine-tuning on the chemical structure of the APIs.

Why liposome formulations?

Basic Liposome Structure

100 nm



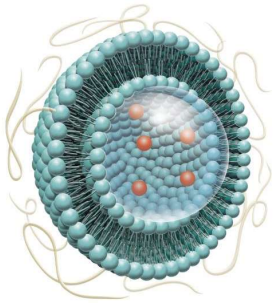
Lipid bilayer
- Internal aqueous phase

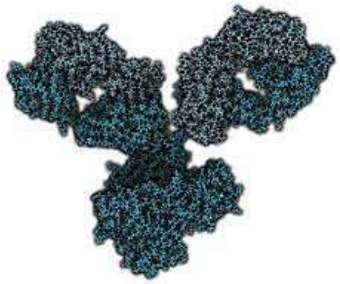
DDS carrier
- Water-soluble drug in inner aqueous phase
- Lipid soluble drug within lipid bilayer

PEG hydration layer
- Liposome stabilization in circulation

Containing small molecule: API in stable carriers
- ✓ Prolongs plasma half-life
- ✓ Improves biodistribution in tumor

Features of Liposome: in Comparison to ADC

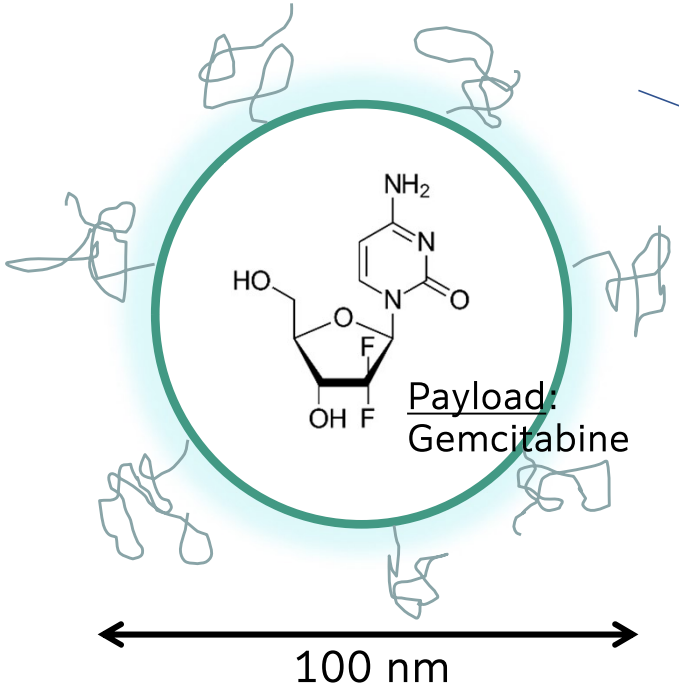


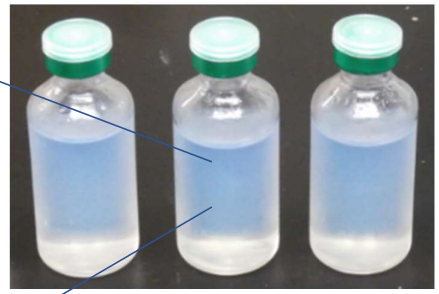


	Liposome	ADC
Distribution mode	Accumulating in tumor by EPR effect	Specific bind to antigen expression cell
Loading in cargo (modification on compound)	Encapsulating into internal volume (No modifcation on API)	Connecting to antibody via linker (Linker modification on API)
Payload capacity	Unlimited Approx. 10,000 per particle	Limited Less than 10 per antibody
Versatility on API	Unlimited Adaptable for wide range of APIs	Limited Adaptable for ultra high potent API

An Example of Liposome Encapsulating Small Molecule; Liposomal Gemcitabine (FF-10832)*

*Designed and manufactured by Fujifilm

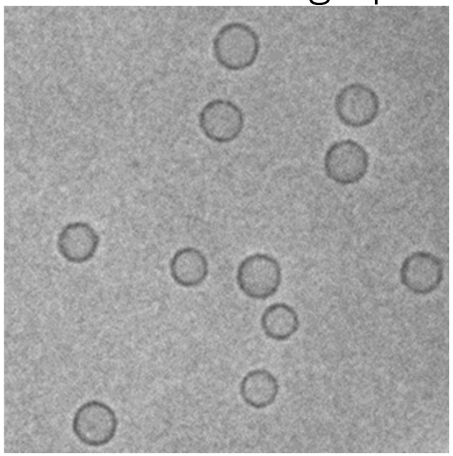




Stability ≥ 3 years under refrigeration conditions

INVESTIGATIONAL USE ONLY:
NOT FOR SALE IN THE US

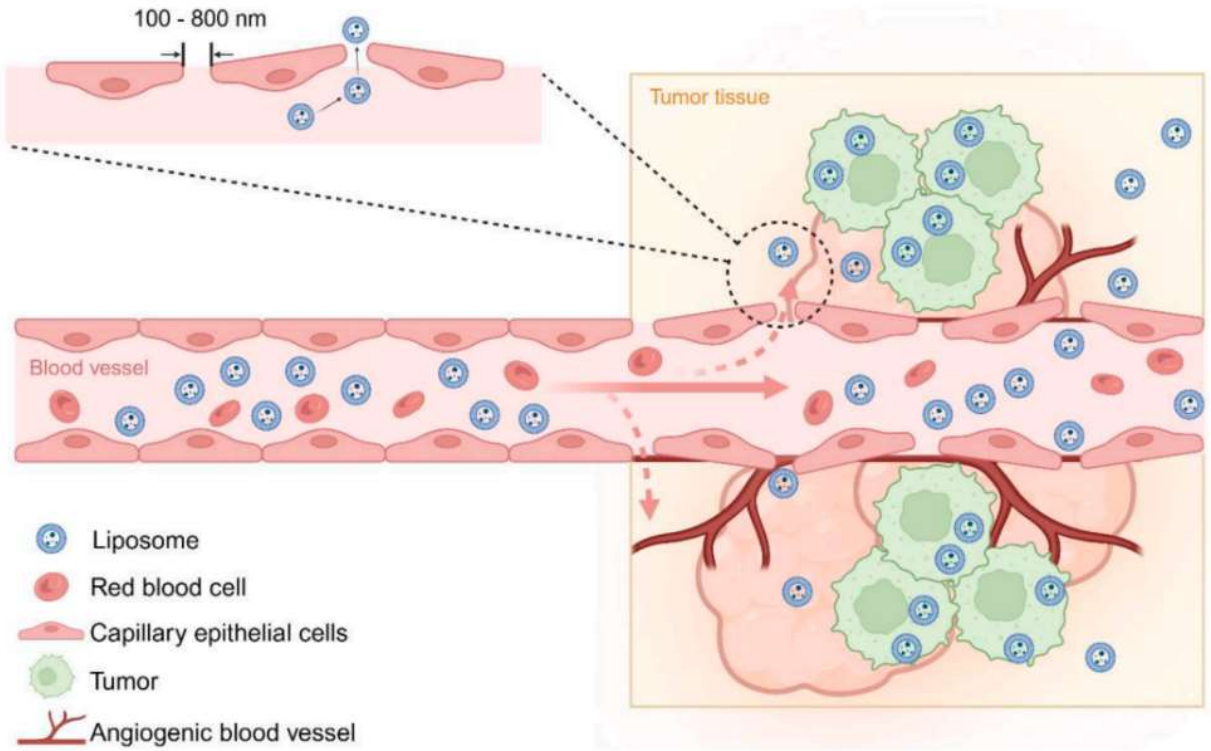
Transmission electron micrograph



100 nm

- Gemcitabine solution is encapsulated in the internal aqueous phase (Gemcitabine hydrochloride was originally synthesized by Eli Lilly & Company in early 1980s and first approved for pancreatic cancer in U.K. in 1995 and U.S. in 1996 for pancreatic cancers.)
- FF-10832 is the first and only gemcitabine-containing liposome formulation in clinical trials in the U.S.

Enhanced Permeability and Retention (EPR) effect



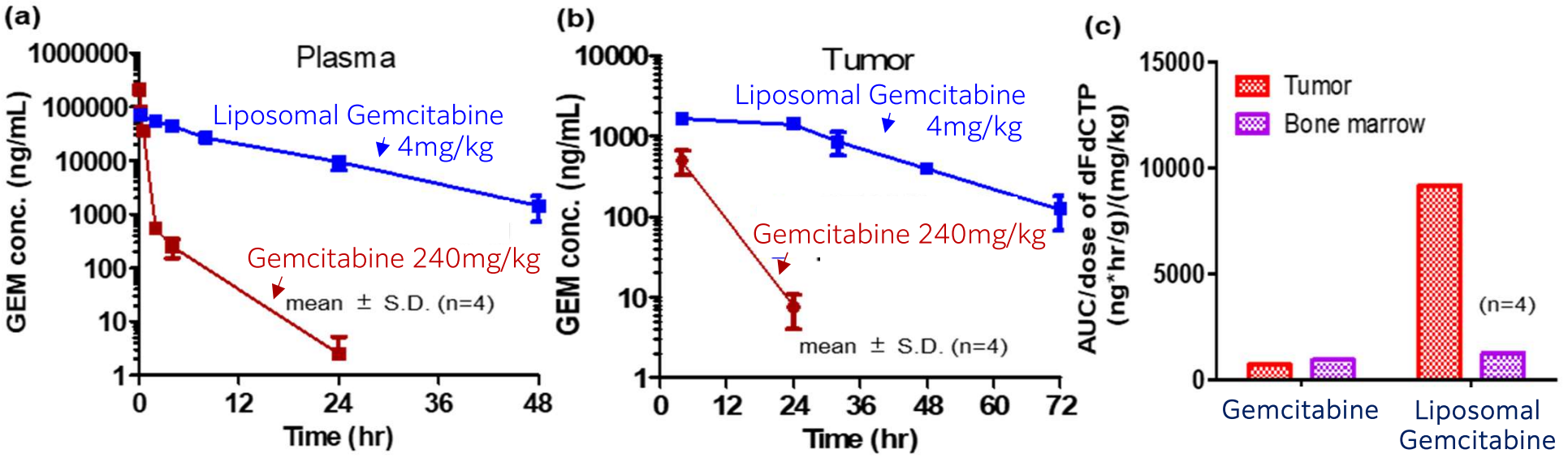
Eur J Pharm Sci. 2024 Feb 1:193:106688. doi: 10.1016/j.ejps.2023.106688.

- Liposome (Φ100 nm) cannot leak out from blood vessels (blood vessels retain particles larger than 6 nm)
- Immature blood vessels in tumors have large pores and liposomes leak out of them.

Liposome Improved the Kinetics of Gemcitabine

(in animal model)

Pharmacokinetics in Mice (i.v.)



Prolonged circulation **Higher accumulation in Tumor** **Improved bio-distribution**

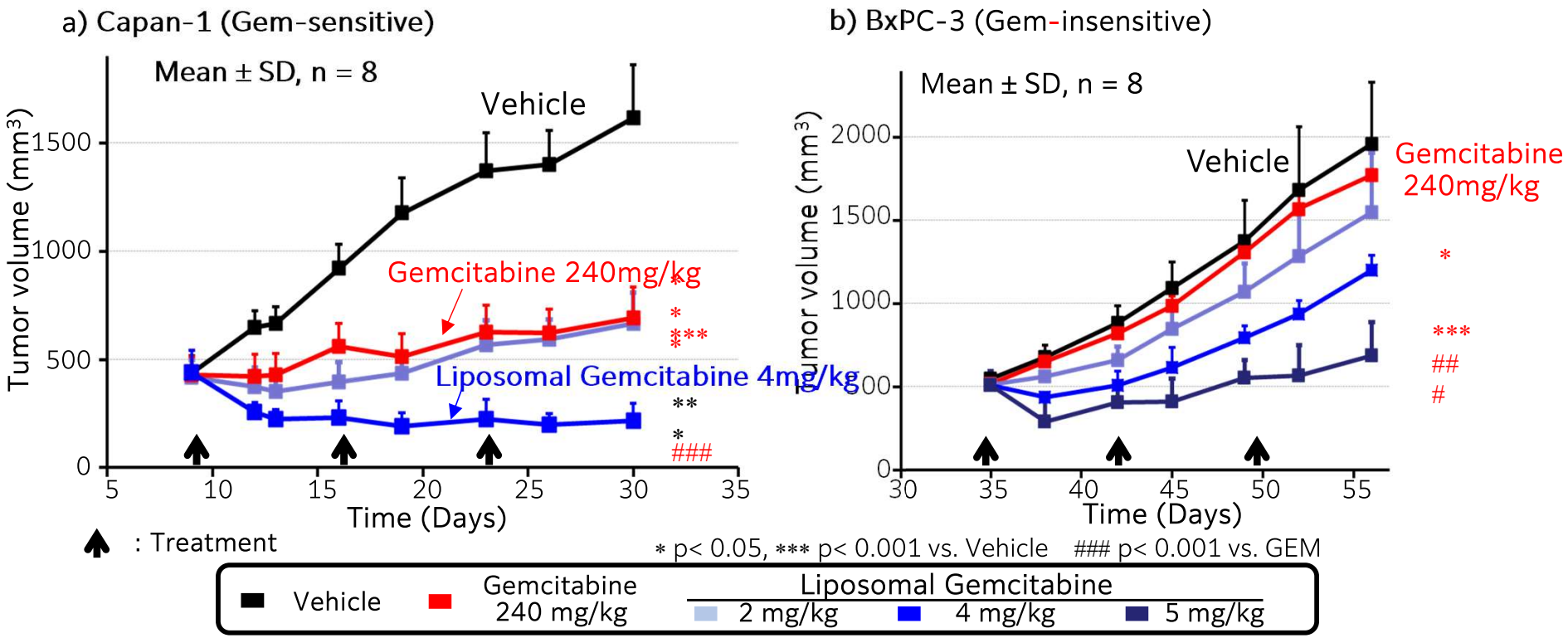
Unpublished data (in-house)

Liposomal formulation has a potential to increase the drug accumulation in tumor

Liposomal Gemcitabine Monotherapy

(in animal model)

Anti-tumor Effects in a Murine Pancreatic Cancer Model (Subcutaneous)



✓ Liposomal Gemcitabine FF-10832 demonstrated superior anti-tumor efficacy to GEM in Capan-1[GEM-sensitive] and BxPC-3[GEM-insensitive] murine tumor models

(Data generated in-house, presented at AACR 2017)

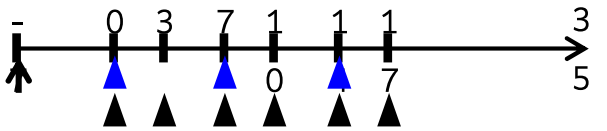
Liposome Increased Response Rate in Combination with ICI

(in animal model)

CTLA-4 Inhibitor and Gemcitabine Combo Efficacy Study w/ & w/o Liposomal Formulation

Study protocol

EMT-6 (GEM insensitive model)



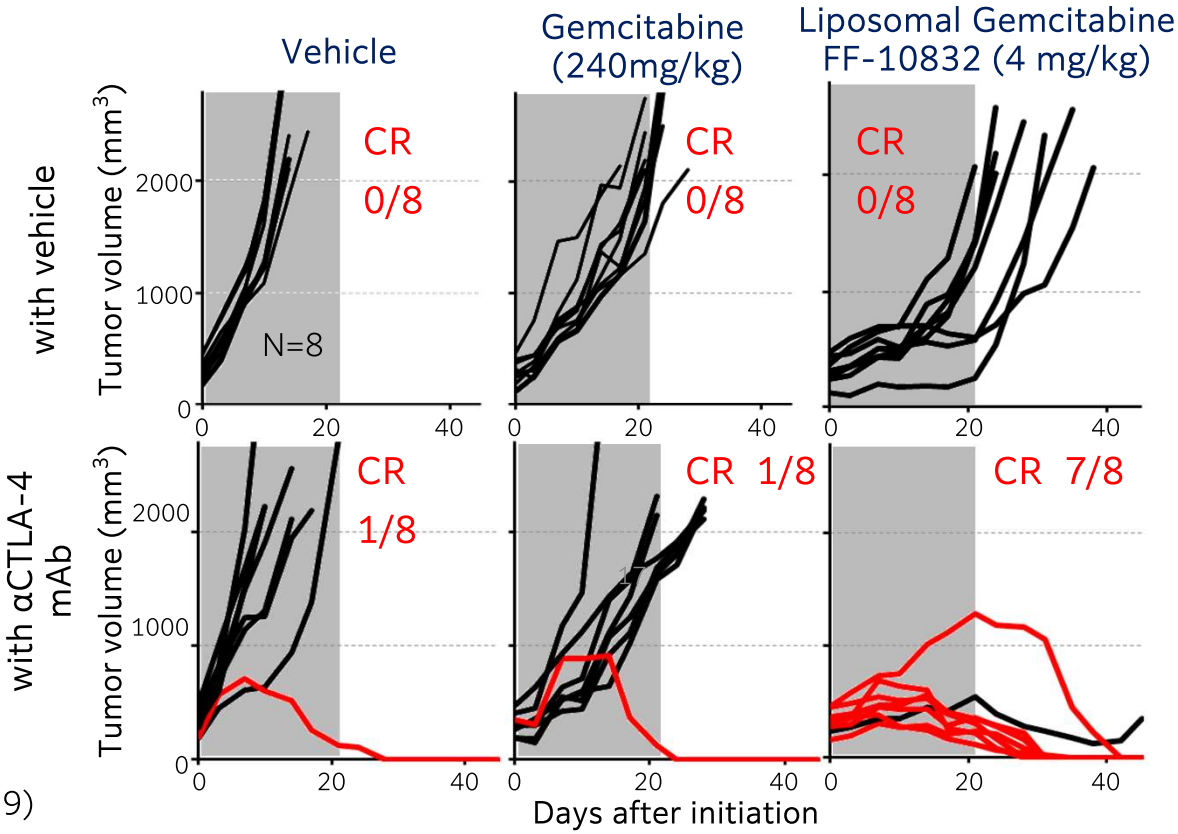
Inoculation (s.c.) to mice (female, 6 weeks)

Gemcitabine or FF-10832 (i.v.)

ICI; 10 mg/kg of αCTLA-4 mAb

Treatment period

CR: Complete remission



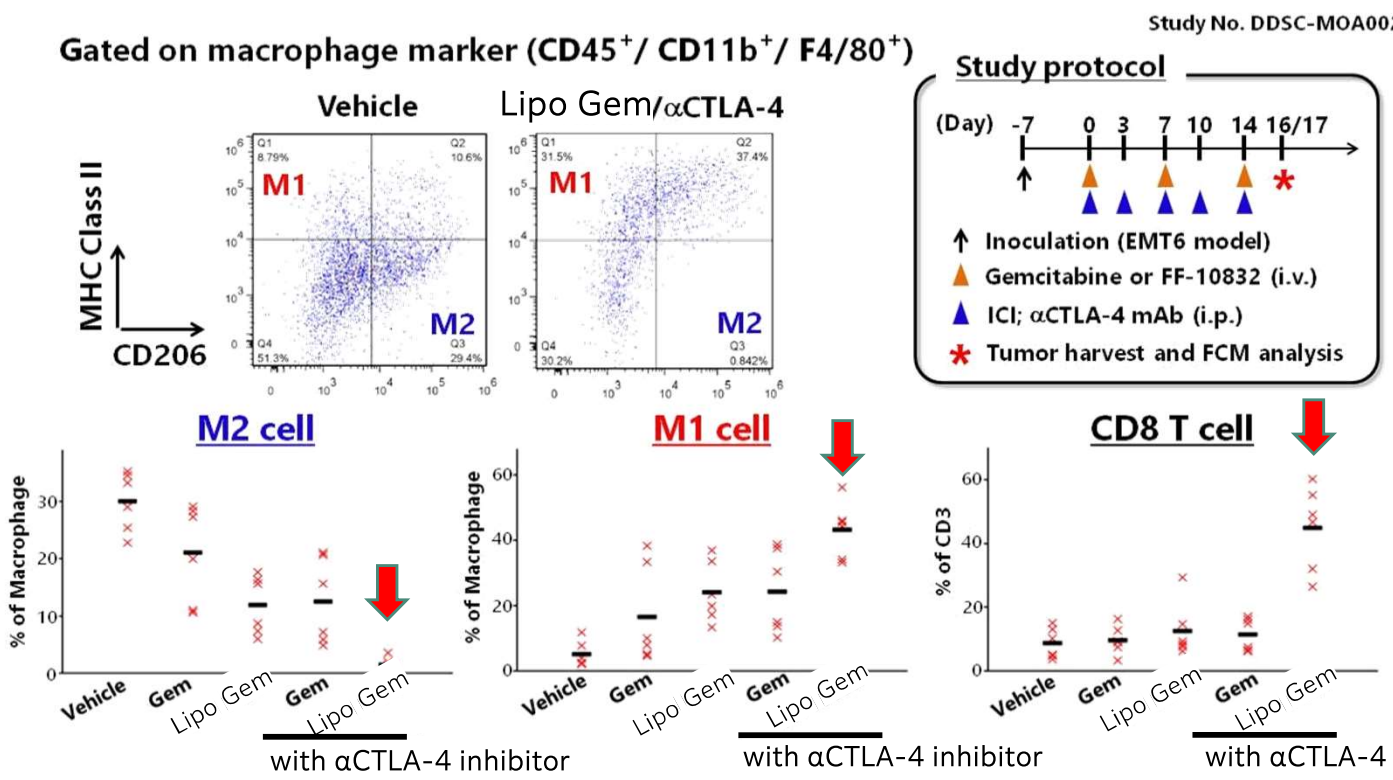
(Data generated in-house, presented at AACR 2019)

In the ICI plus gemcitabine group, 1 of 8 animals experienced Complete remission; in the ICI plus Liposomal gemcitabine group, this number increased to 7 of 8 animals.

Liposome Converted Tumor Immune Environment

FCM Analysis of Tumor Microenvironment in mice (FF-10832 CTLA-4 inhibitor combo)

(in animal model)

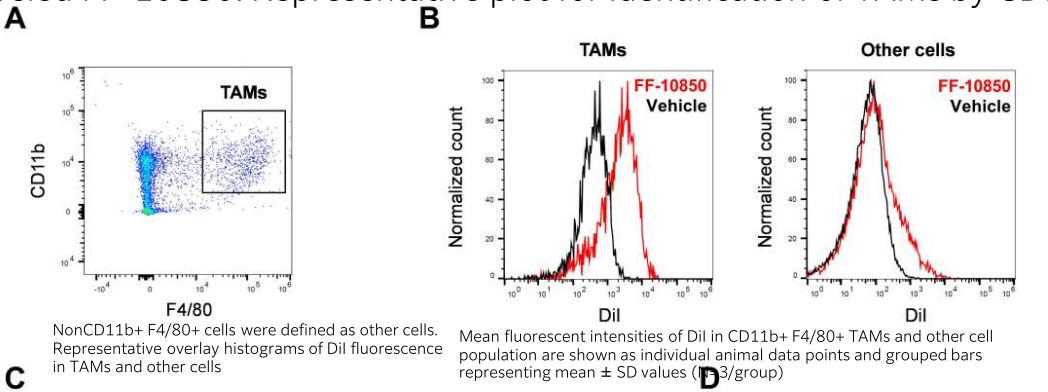


(Data generated in-house, presented at AACR 2019)

Liposome formulation enhanced conversion of tumor immune environment from COLD to HOT

Tumor Associate Macrophages uptake Liposome and released API

Cellular distribution of Dilfluorescence in ES-2 tumor tissue after intravenous administration of vehicle or DiI-labeled FF-10850. Representative plot for identification of TAMs by CD11b and F4/80 staining (in animal model)



NonCD11b+ F4/80+ cells were defined as other cells. Representative overlay histograms of DiI fluorescence in TAMs and other cells

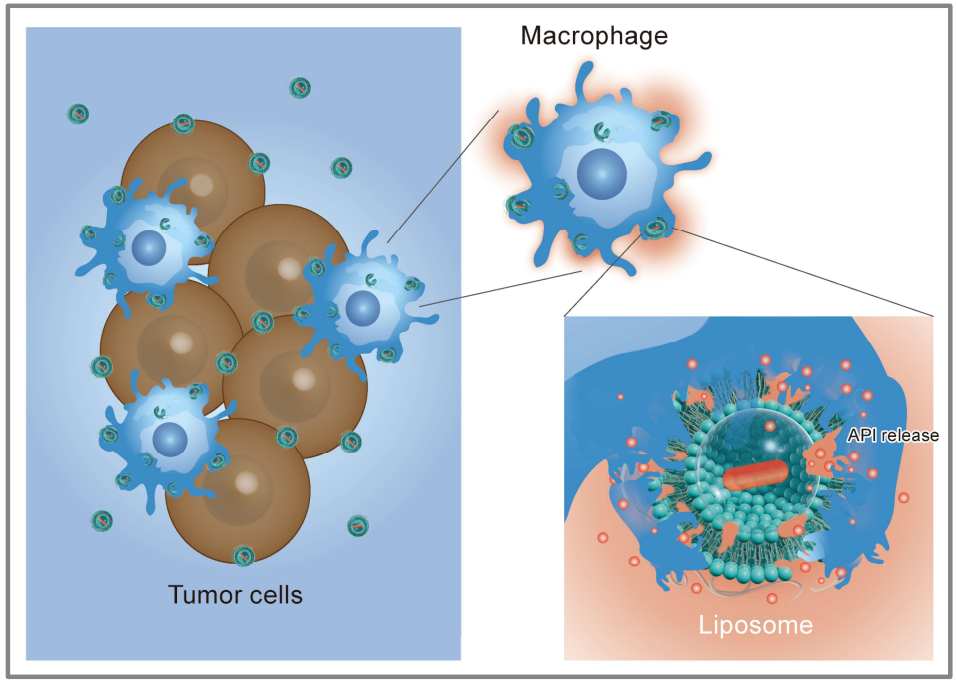
Mean fluorescent intensities of DiI in CD11b+ F4/80+ TAMs and other cell population are shown as individual animal data points and grouped bars representing mean ± SD values (n=3/group)

Liposomes (FF-10850) is untaken by TAMs

Payload release from bone marrow-derived macrophag

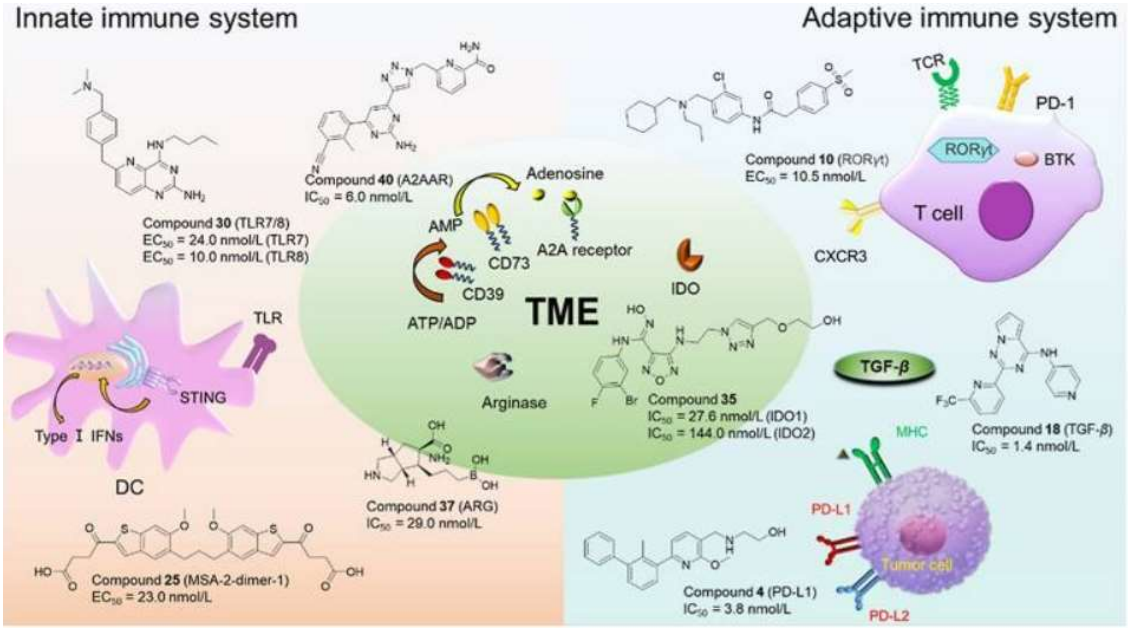
In house data: Susumu Shimoyama et al, *Mol Cancer Ther* 2023

Illustrated model



Liposome was uptaken and payload was released by macrophage in tumor tissue

Potential Utility of Liposome in the Context of Macrophage Targeting

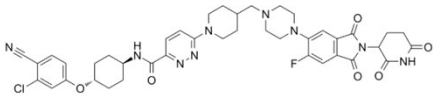


Yinrong et al. Acta Pharm Sin B. 2022 Dec; 12(12): 4287–4308.

- PD-L1 inhibitors
- PD-L1 targeting PROTACs
- RORγt agonists
- CXCR4 antagonists
- CCR2/4/5 antagonists
- TGF-β inhibitors
- SHIP1 inhibitors
- STING agonists
- TLR agonists
- IDO inhibitors
- A2A adenosine receptors
- CD39/73 inhibitors
- PI3K-δ inhibitors
- BTK inhibitors and PROTAC

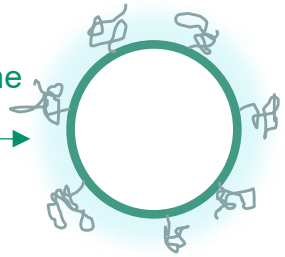
Liposome potentially contribute to the efficient delivery of API to the microphase in cancer tissues

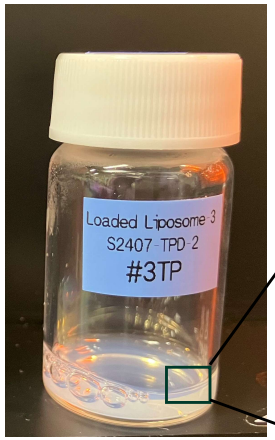
Application example: Targeted Protein Degrader (TPD)



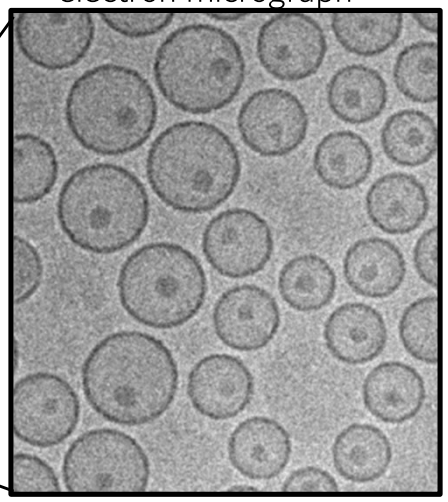
Compound : ARV-110
Molecular weight: 812.3 g/mol
Solubility : Insoluble in water

Tried to
load into liposome



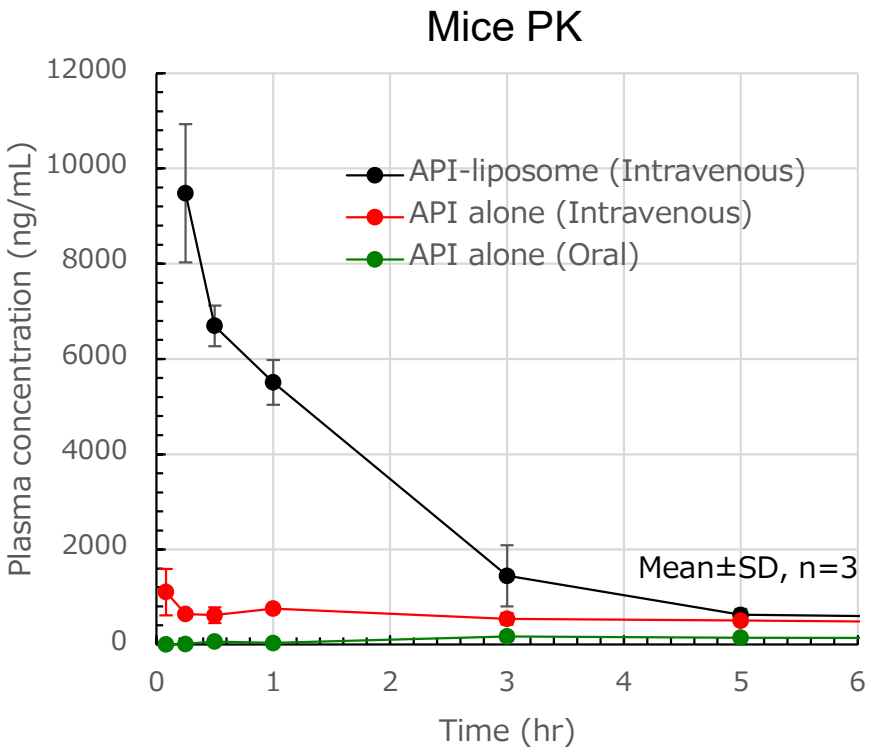


Transmission
electron micrograph



Appearance: clear suspension form
without precipitation
Particle size: 109 nm
Encapsulation efficiency: > 80%

preliminary data



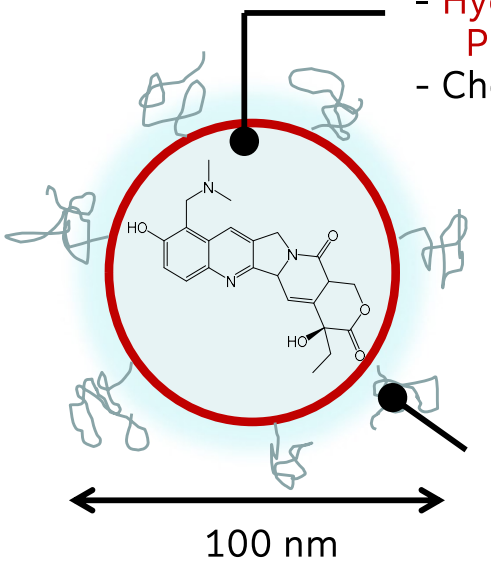
Stability of liposomal is the Critical Quality Attribute (CQA)

- Lasting retention in blood improves drug delivery to tumor
- Stability during refrigerated storage is required in commercial use
  >2 years storage period is desirable for logistics and application considerations

How to achieve?

High-stability Liposome Development

Conventional HSPC Liposome

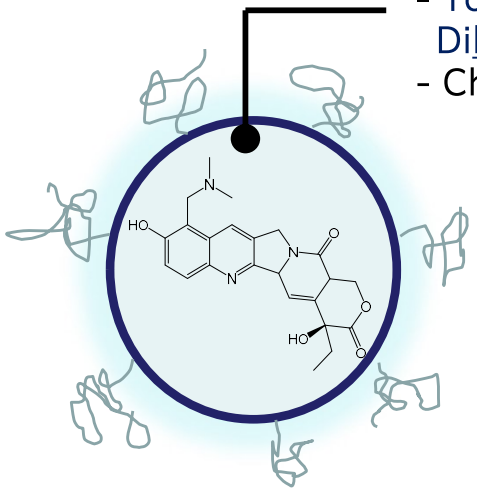


Liposomal shell:
- Hydrogenated Soy Phosphatidyl Choline (HSPC)
- Cholesterol

Stealth coating:
Polyethylene glycol (PEG)

100 nm

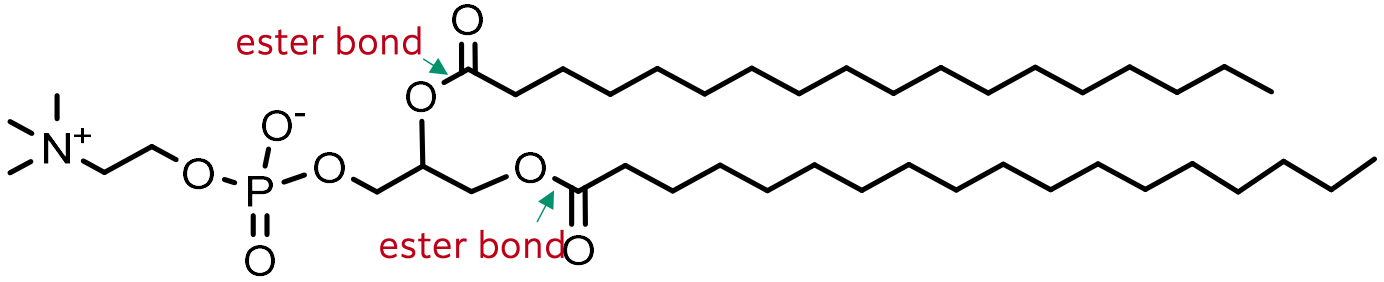
DHSM Liposome



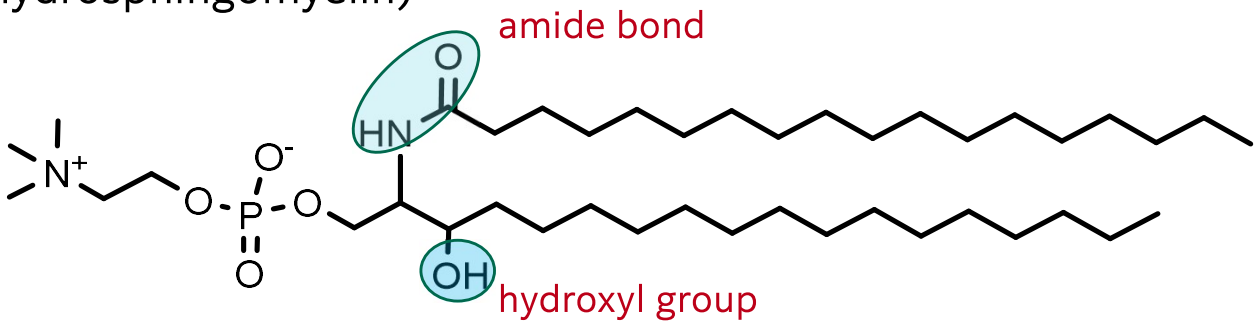
Liposomal shell:
- Totally synthetic Dihydrosphingomyelin (DHSM)
- Cholesterol

Structural Comparison of DHSM and HSPC

HSPC (Hydrogenated Soy Phosphatidyl Choline): Conventional liposomal lipid



DHSM (Dihydrosphingomyelin)

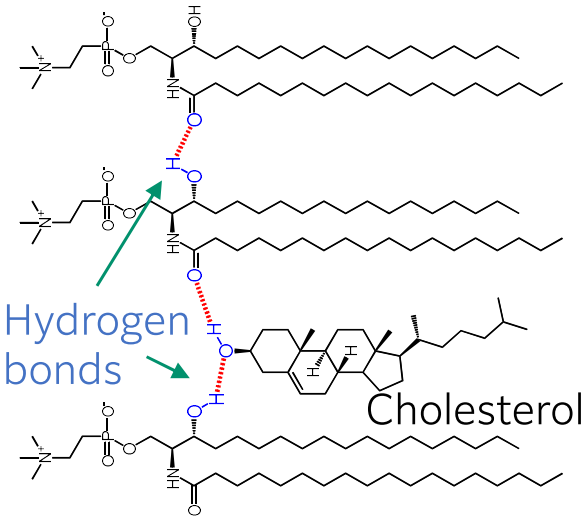


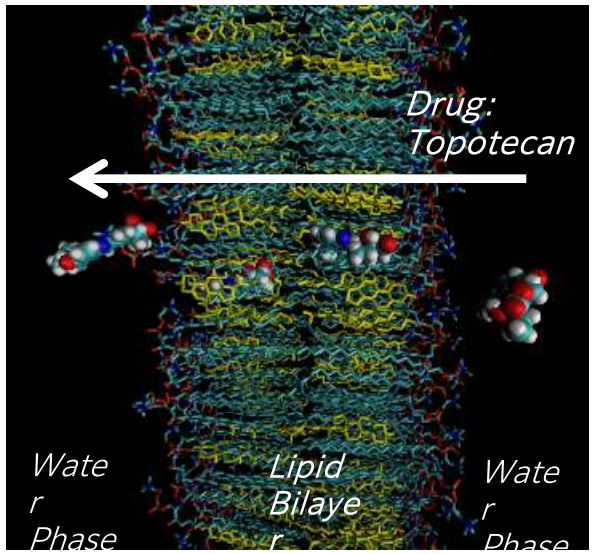
- ✓ No hydrolysis site (ester bond):
  High stability during long-term storage of liposomes in liquid suspension
- ✓ Amide bond and hydroxyl groups:
  Formation of tight lipid bilayer membranes by intermolecular hydrogen bonding

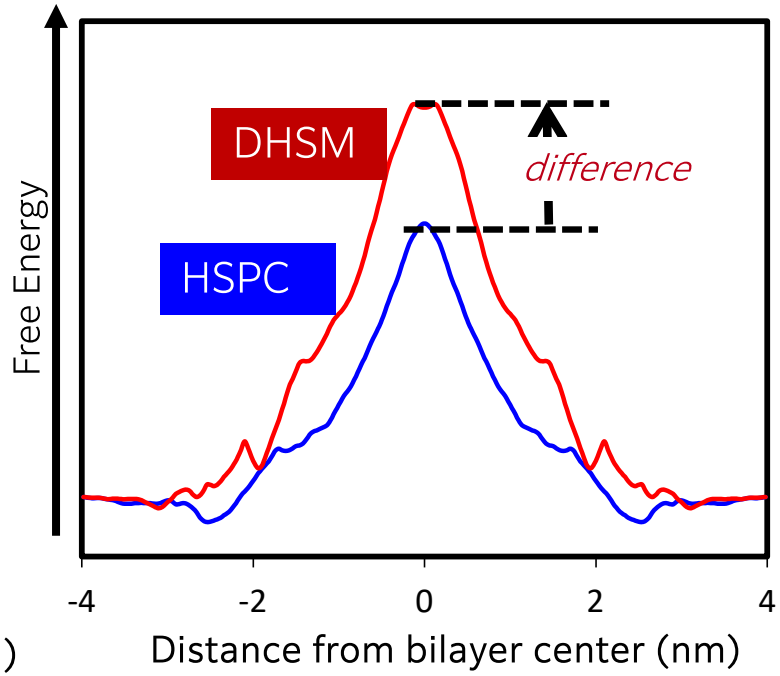
Superior Property of DHSM in Liposome Lipid bi-layer

Estimation of free energy barriers of lipid bilayers for API by Molecular Dynamics simulation

DHSM liposome bilayer





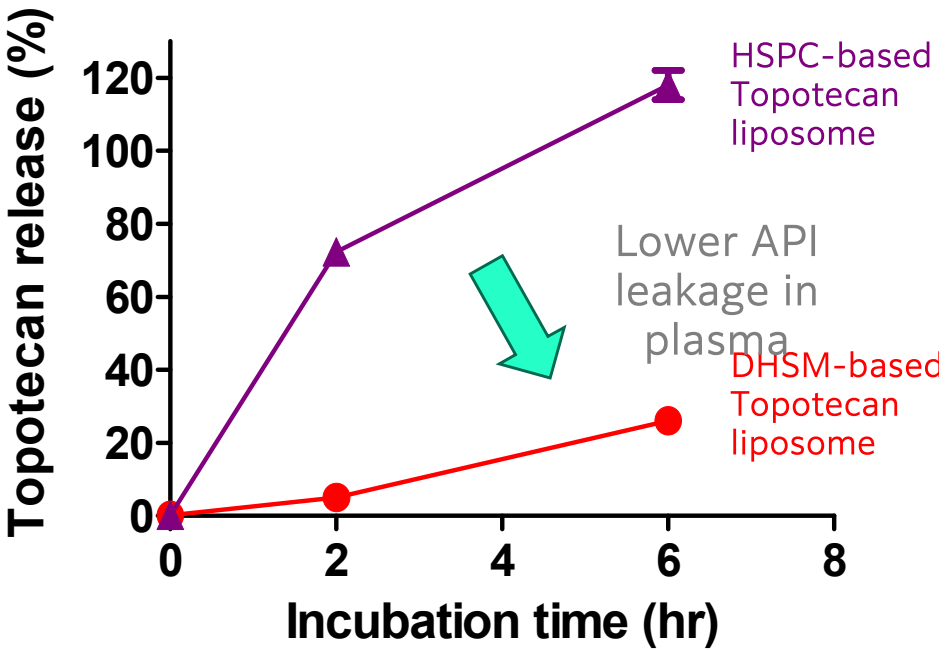


Unpublished data (in-house)

Free energy barrier of drug penetration through DHSM bilayer is higher than that of HSPC due to formation of stronger hydrogen bonds between DHSM and cholesterol.

Property of DHSM Liposome in vitro/ in vivo

In vitro payload release
in mouse plasma



*HSPC (Hydrogenated Soy Phosphatidylcholine):
Conventional lipid used in marketed liposomal drugs

(in animal model)

PK profile of Topotecan with and without liposome in mice

	API solution	Liposome formulation	
	Topotecan 2 mg/kg	FF-10850 0.5 mg/kg	FF-10850 2 mg/kg
C_0 (ng/mL)	758	8028	29166
$T_{1/2}$ (h)	0.42	6.66	6.88
$AUC_{0\text{-}inf}$ (h*ng/mL)	190	74836	325440
Vd_{ss} (mL/kg)	3615	64	61
CL (mL/h/kg)	10550	6.68	6.15

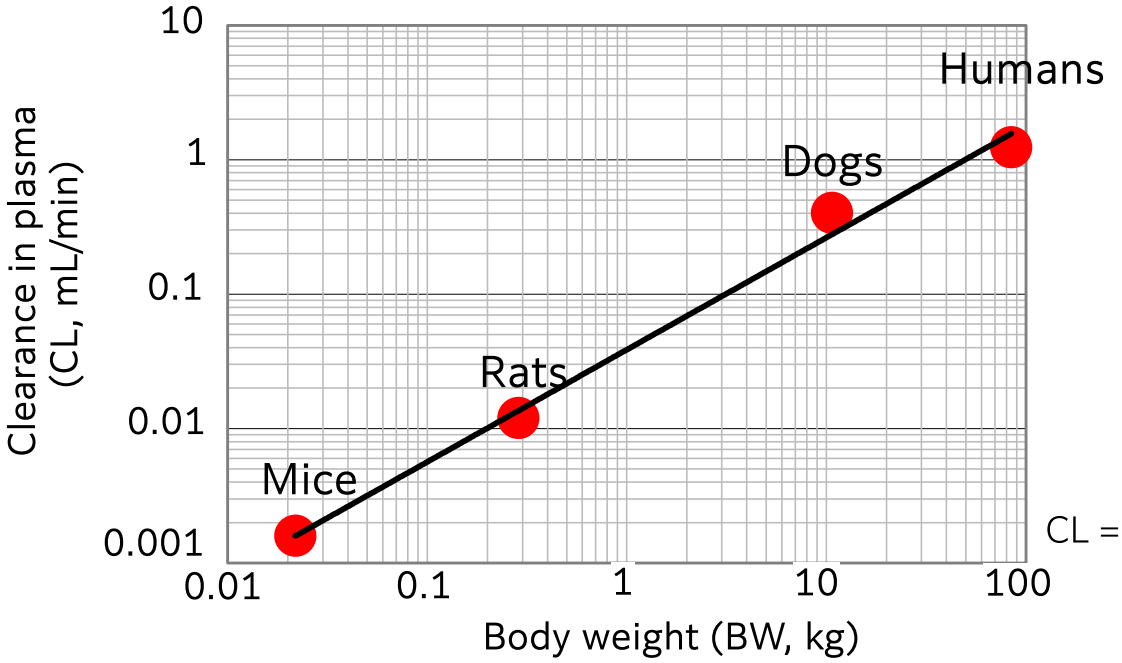
X 1, 700 times

In house data: Susumu Shimoyama et al, *Mol Cancer Ther* 2023

DHSM-based liposome showed slower API release and prolonged plasma circulation

Species Differences of Liposome in PK Profiles

Allometric association between clearance and body weight
[DHSM-based liposomal Topotecan]

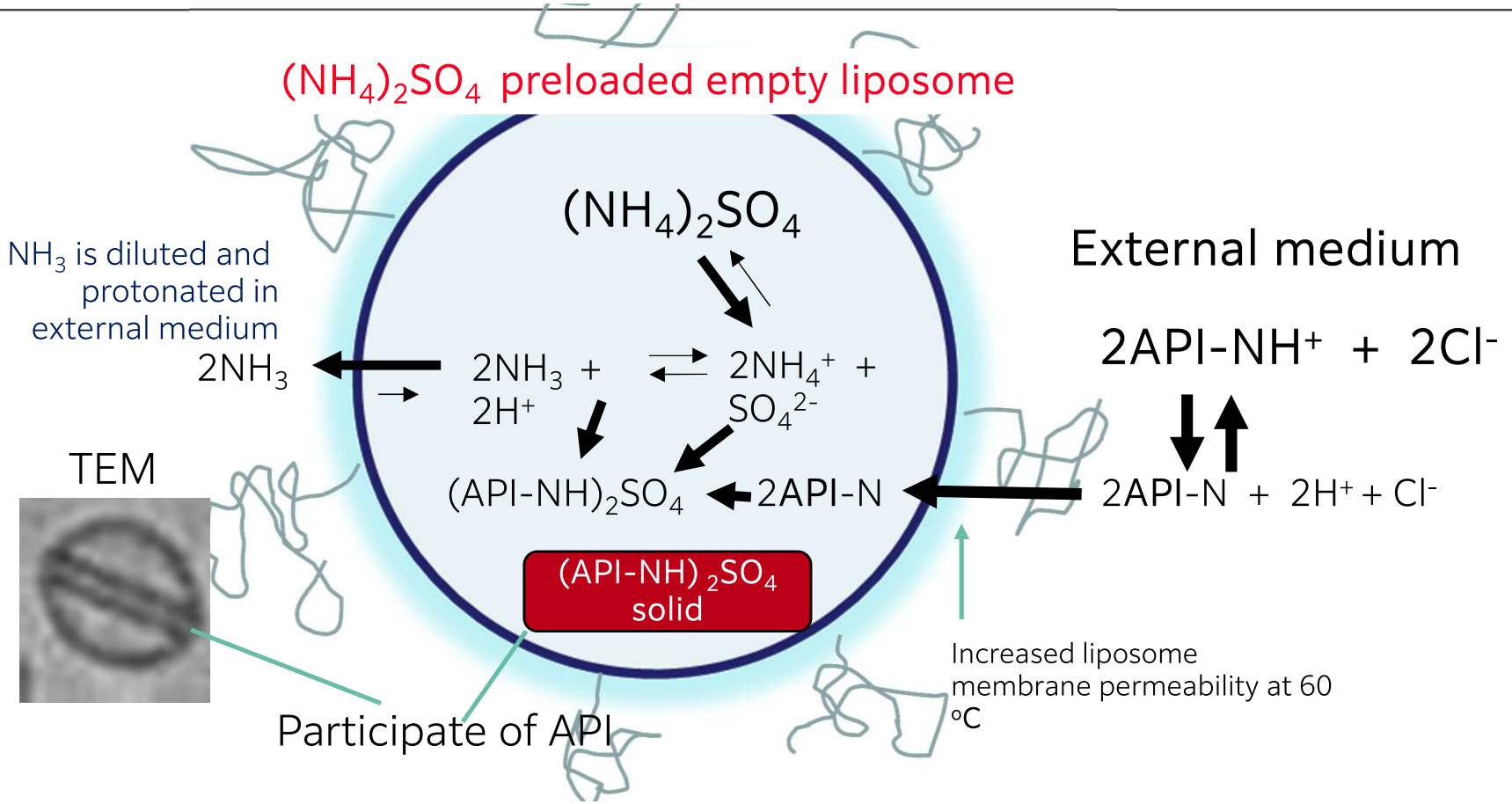


Unpublished data (in-house)

Stable liposomal formulation showed robust PK extrapolation
from preclinical animal studies to human clinical trials.

Versatility of liposomes

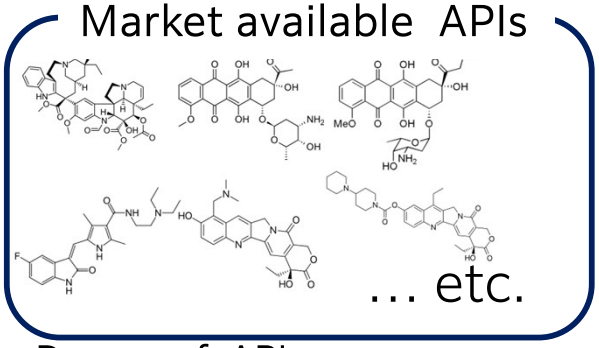
API Loading Method: API Precipitated by Sulfate Ion in the Liposome



- ✓ Protonation of API in liposome formulate insoluble precipitate of sulfate and API precipitate.
- ✓ This process is widely applicable to APIs with weak basic groups

Increased AUC of DHSM Liposome in Variety of API

(in animal model)



Mice pk study i.v.

Liposome Formulation		T1/2	AUC∞(1mpk)	
API	Lipid	hr	ng/mL*h	DHSM/HSPC
A	**HSPC**/Chol/mPEG-DSPE	15.2	433691	x 1.2
	DHSM/Chol/mPEG-DSPE	17.4	506246	
B	**HSPC**/Chol/mPEG-DSPE	1.0	24695	x 3.3
	DHSM/Chol/mPEG-DSPE	3.3	82122	
C	**HSPC**/Chol/mPEG-DSPE	3.5	89195	x 1.8
	DHSM/Chol/mPEG-DSPE	9.9	148189	
D	**HSPC**/Chol/mPEG-DSPE	0.5	6453	x 5.8
	DHSM/Chol/mPEG-DSPE	0.7	37661	
E	**HSPC**/Chol/mPEG-DSPE	2.4	86692	x 2.6
	DHSM/Chol/mPEG-DSPE	9.0	227177	

Unpublished data (in-house)

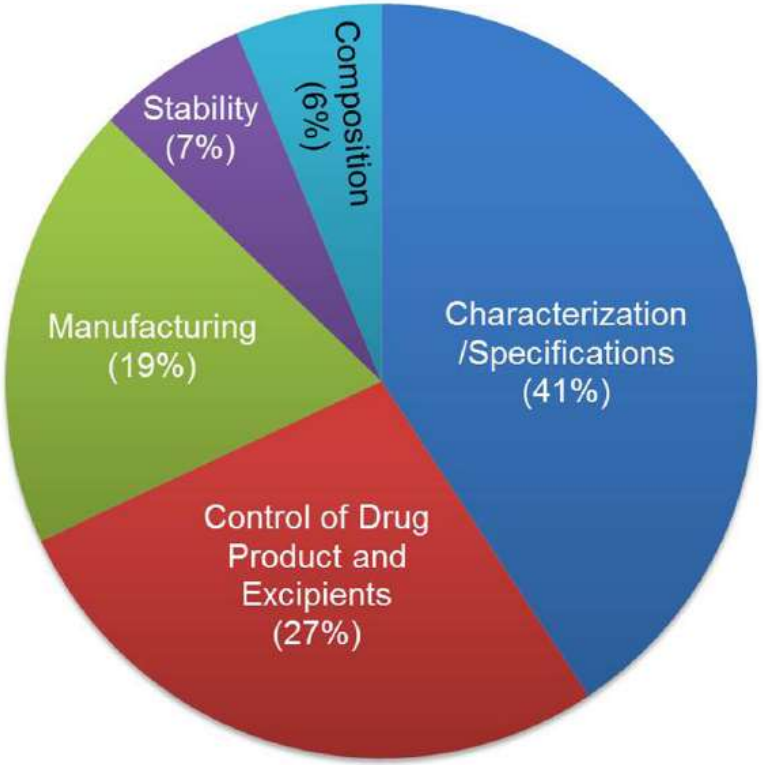
DHSM-based liposomes showed higher AUC compared to HSPC-based liposomes

Topics

- ✓ Fujifilm company profile at glance
- ✓ Expanding field of small molecule development, and the power of liposome formulation
- ✓ Liposome application example on Targeted Protein Degrader (TPD)
- ✓ Stability of liposomal is the Critical Quality Attribute : How to achieve?
- ✓ Versability of liposome for different type of API
- ✓ **Challenges in liposome CMC development and solutions**

Quality Issues in Liposome Development

Quality issues that are liposome-specific



CMC Challenges

- Characterization/Specifications
- Control of Drug Product and Excipients
- Manufacturing
- Stability

Liposomal Drug Product Development and Quality: Current US Experience and Perspective. Kapoor Met al, 2017*AAPS J* 19(3):632-641..

Liposome Drug Development at Fujifilm

Gemcitabine liposome combination with Pembrolizumab

A Study to Evaluate Safety, Efficacy of FF-10832 in Combination With Pembrolizumab in Solid Tumors - ClinicalTrials.gov

This is a Phase 2a, open label clinical trial evaluating FF-10832 in combination with pembrolizumab and as monotherapy. The trial will begin with a safety run-in phase of 10 patients receiving combination therapy with pembrolizumab; FF 10832 will be dosed at 40 mg/m2 with a fixed dose of pembrolizumab (200 mg).

NCT03440450

DHSM-based liposome encapsulation of Topotecan

A Study of FF-10850 Topotecan Liposome Injection in Advanced Solid Tumors - Full Text View - ClinicalTrials.gov

Inclusion Criteria: Patients must meet all the following criteria to participate in the study: Males and females ≥ 18 years of age; Dose-escalation phase: Histologically or cytologically confirmed metastatic and/or unresectable solid tumor, relapsed or refractory to standard therapy, or for which no standard therapy is available that is expected to improve survival by at least 3 months

NCT04047251

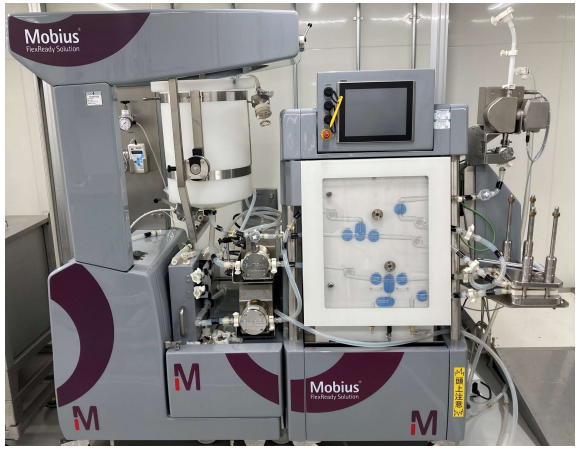
INVESTIGATIONAL USE ONLY:
NOT FOR SALE IN THE US

- ✓ Liposomal formulation design, preclinical toxicity, PK, and efficacy studies, CTM manufacturing, and phase 2 clinical trials in U.S. were fully conducted by Fujifilm.
- ✓ Fujifilm possesses the hands-on knowledge required for liposome formulation and development

Liposome GMP Manufacturing Facility at Fujifilm

- GMP production of LNPs and liposomes
- 35L and 350L scale FUJIFILM-liposome manufacturing equipment
- Highly potent APIs handling area
- KrosFlo® KMPi and Mobius Flex Ready® from mL scale to ~100 L
- Vial filling system 3000 vials/hour

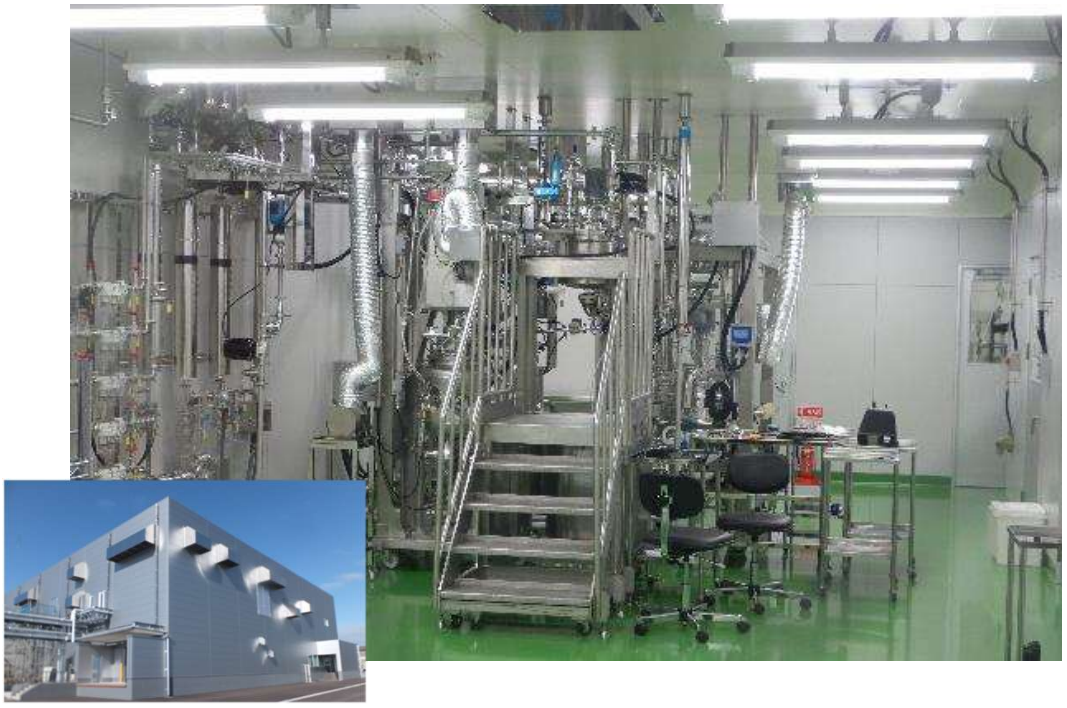
Liposome MFG system

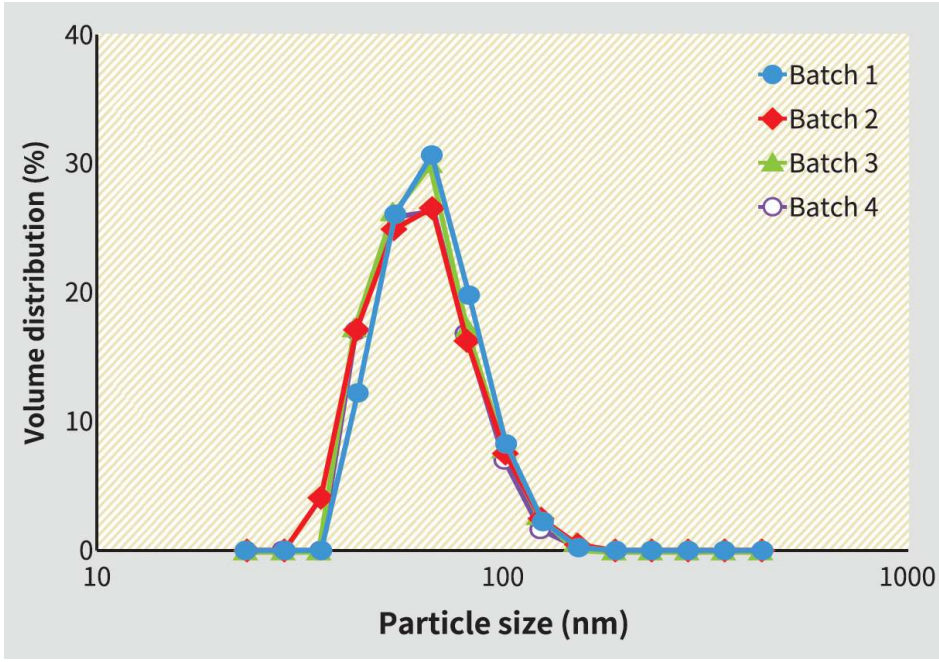


TFF

Vial filling system

DHSM-based Liposomes Manufacturability





Unpublished data (in-house)

Fujifilm produces empty liposomes using a homogenization system with high reproducibility. The system was designed by Fujifilm.

Requirements for Characterization and Specification setting

Examples of Specifications for liposome product

Appearance	Particulate matter (in injections)
Identification test (API)	Insoluble foreign matter
Identification test (lipid)	In-vitro release
pH	Mean particle size
Osmolality	Particle size distribution
API-related impurities	Container content
Lipid-related impurities	Total API content
Residual solvents	Free API content
Elemental impurities	Lipid content
Endotoxin	Lipid composition
Sterility	

Blue：Liposome-specific test items

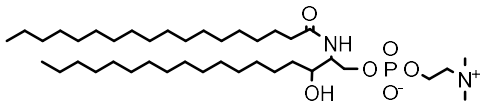
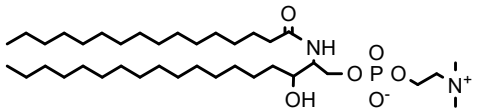
Guidance for Industry, Liposome drug products (April 2018, FDA)
Drug Products, Including Biological Products, that Contain Nanomaterials, Guidance for Industry (April 2022, FDA)
ICH Q6A, Specifications

Analytical devices at Fujifilm

- HPLC-UC/CAD for lipids and API
- LC-MS
- Size and distribution by DLS
- Zeta potential, pH, osmolality, Particle matter
- Residual solvent analysis by GC (FID)
- Sterility
- Spectroscopy (UV, IR) and others
- Capillary electrophoresis
- Fluorescence microplate reader

Fujifilm can provide a full range of analytical services to support drug development

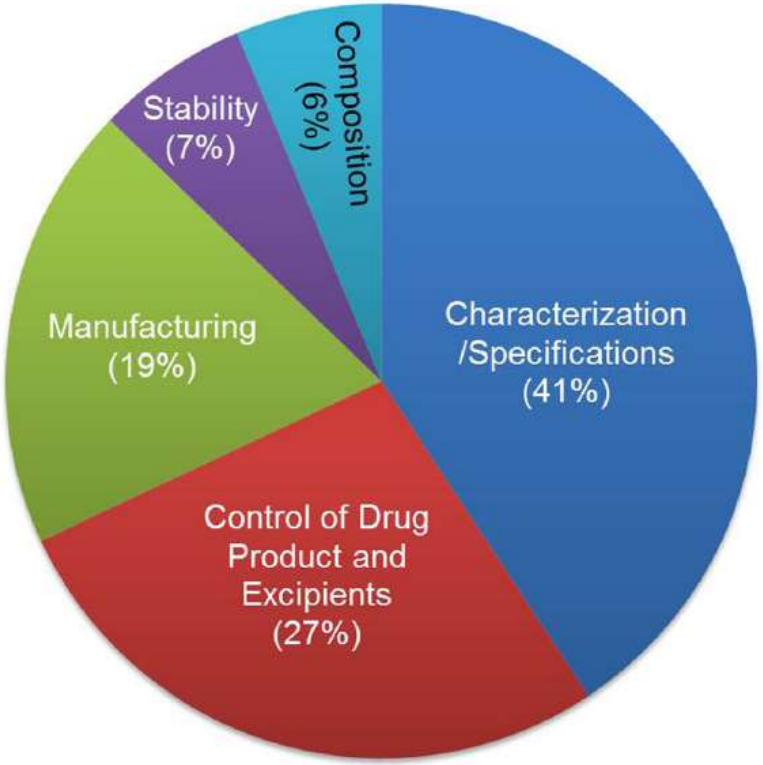
Established DHSM Supply Chain

	Total synthesis DHSM	DHSM synthesized from SM
Source	Totally synthetic material	Egg-derived sphingomyelin (SM)
Structure	C18:100% Purity: more than 97% 	C16:75% C18:6% Other:9% 
Stability of supply chain	No hard-to-find raw materials	Poor- SM is very rare in egg, so supply is limited; few manufacturers.
Quality control	Easy- Single substance	Difficult- Naturally-sourced mixture of lipids with variable chain lengths
Regulatory (Virus safety)	No concerns	Need to meet standards for biological raw material (i.e., virus-free)
Supply	GMP Supply from Nippon Fine Chemical (NFC):, a FUJIFILM collaborator	Need to establish and maintain supply chain

Fujifilm and the partner have established a GMP supply chain for-DHSM

Quality Issues in Liposome Development

Quality issues that are liposome-specific:

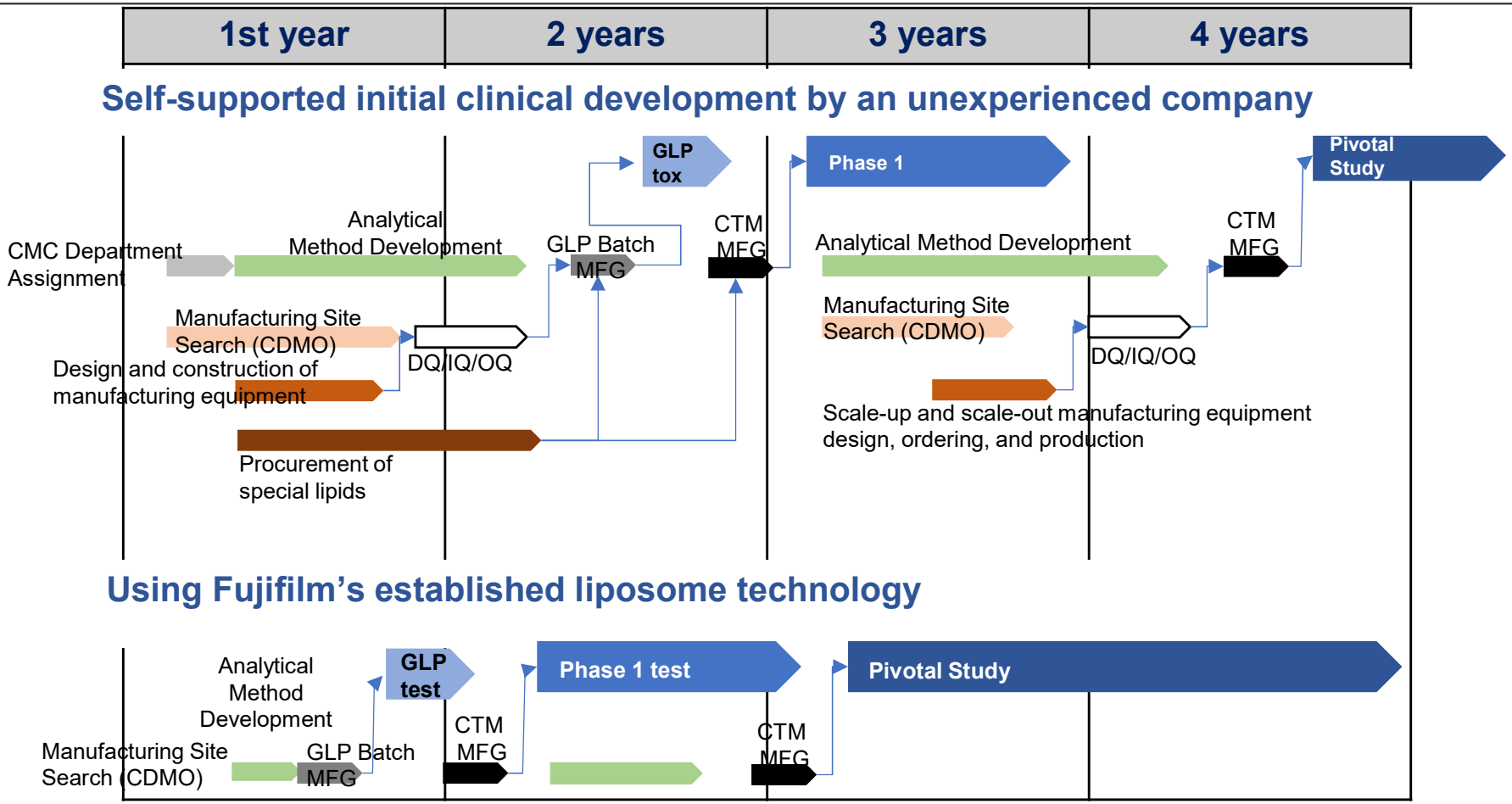


CDMO support to overcome the CMC Challenges

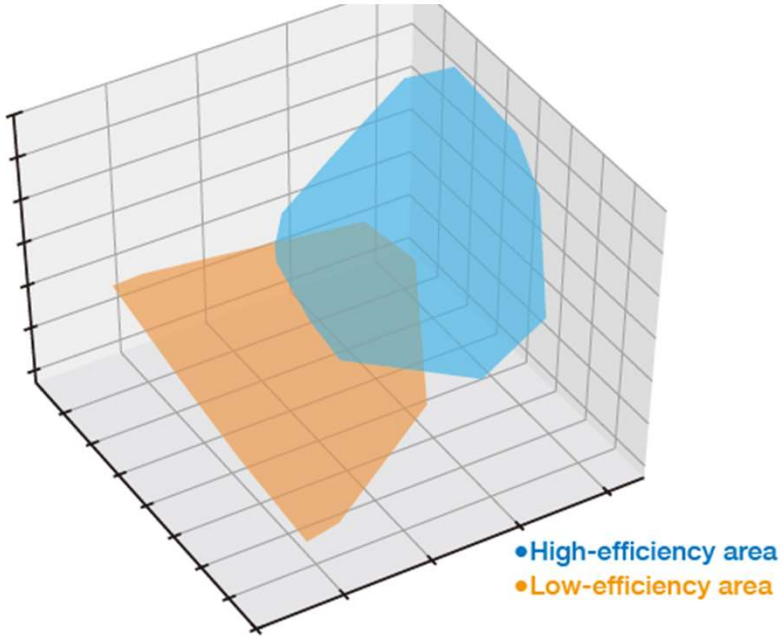
- ✓ Characterization/Specifications
- ✓ Control of Drug Product and Excipients
- ✓ Manufacturing
- ✓ Stability

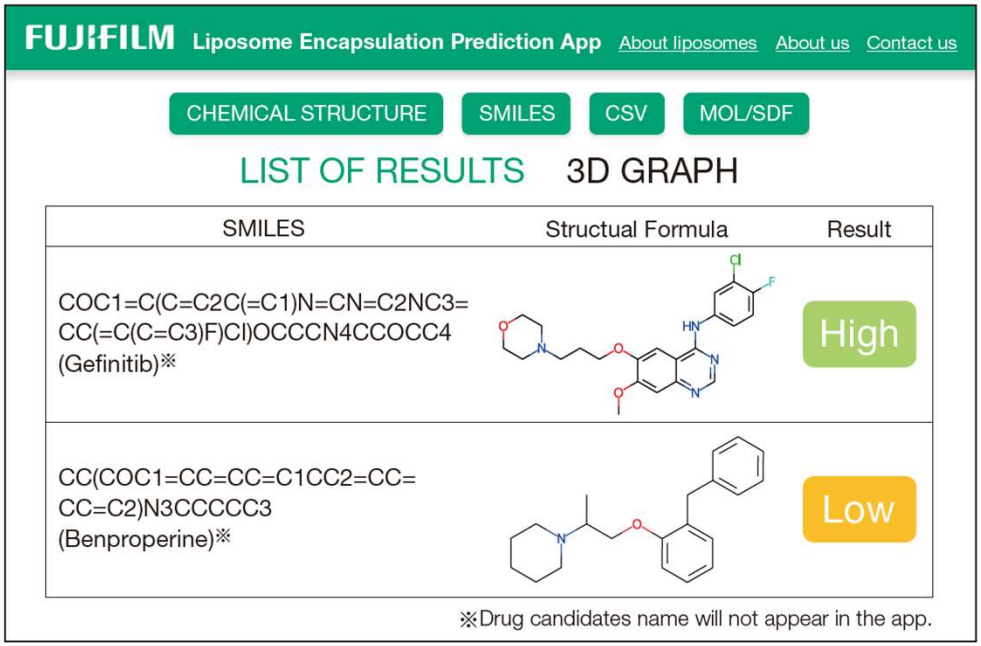
Liposomal Drug Product Development and Quality: Current US Experience and Perspective. Kapoor Met al, 2017*AAPS J* 19(3):632-641..

Faster Liposome Development with CDMO Services



An APP for the prediction of the success of liposomal encapsulation of candidate compounds is available as a free trial





Get Access to The APP 2D Barcode ▶

URL ▶ https://liposome-tools.fujifilm.com/

Summary

Booth #2506

- ✓ Liposome formulation potentially contributes to advanced anti-cancer drug development by improving pharmacokinetics and biodistribution. No chemical modification of the API is required.
- ✓ Liposome is applicable for a wide range of API species, not only chemotherapy but also molecular targeting drugs such as Targeted Protein Degraders(TPD).
- ✓ Stability of liposome is the critical quality attribute. DiHydroSphingoMyelin (DHSM) based liposome is one of the options.
- ✓ To overcome the challenges in the development of liposome CMC , Fujifilm stands ready to provide its liposome platform to collaborators.

FUJIFILM

Value from Innovation