PEGylated Liposomes in the Clinic and Clinical Trials

Polyethylene glycol (PEG) is widely used for surface modification of liposomes and other nanocarriers due to its superior performance. Currently, there are some PEGylated liposomes approved by FDA and many other in clinical trials. Here, we will briefly introduce the PEGylated liposomes and drugs in clinic and clinical trials.

Introduction of Liposomes

Liposomes are self-assembled (phospho)lipid-based drug vesicles that form a bilayer (uni-lamellar) and/or a concentric series of multiple bilayers (multilamellar) enclosing a central aqueous compartment. In 1964, Bangham first reported the bilayered vesicle structure of liposomes. Since then, liposomes have gained much attention in the biomedical and pharmacological fields as a drug delivery system with many advantages.

As drug vehicles, liposomes exhibit outstanding properties, such as protecting the encapsulated substances from physiological degradation, extending the half-life of the drug, controlling the release of drug molecules, and excellent biocompatibility and safety. Furthermore, liposomes can selectively deliver their payload to the diseased site through passive and/or active targeting, thus decreasing the systemic side-effect, elevating the maximum-tolerated dose, and improving therapeutic benefits. **But the rapid phagocytosis and clearance of ordinary liposomes by the mononuclear phagocyte system (MPS) after they enter the blood circulation has somewhat limited their widespread use in the clinic. In order to solve these problems, Blume and Klibanov successfully prepared PEGylated liposomes by applying PEG to the surface of liposomes for the first time in 1990.**

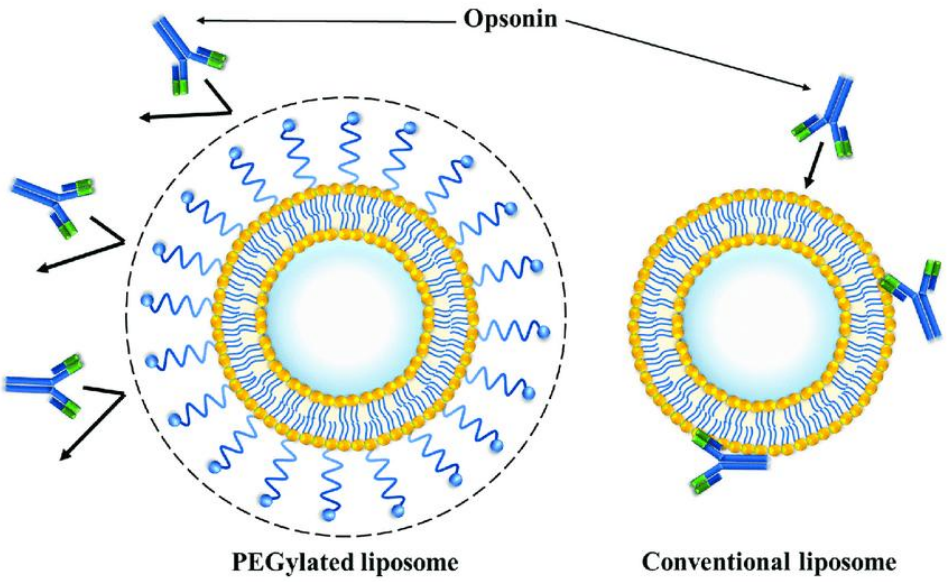

Figure 1. PEGylated Liposome and Conventional Liposome, source: DOI:10.3390/ijms20194706

PEGylation, the covalent linking of PEG chains, changes the absorption of the protein corona on the liposome surface, significantly prolongs the circulation time of liposomes, improves the biodistribution of drugs, and achieves enhanced passive targeting of tumors by exploiting the enhanced permeability and retention (EPR) effect at the tumor site. In addition, PEG chains can generate spatial site resistance on the liposome surface, overcoming the van der Waals force of mutual attraction between liposomes, hindering the aggregation between liposomes and improving formulation stability. **In 1995, the first PEGylated nanoformulation, Doxil®, a PEGylated doxorubicin liposome, was approved for marketing in the U.S.** With great success. Inspired by Doxil®, various PEGylated liposomes entered the clinical trial.

The Marketed PEGylated Liposomal Products

For more than 30 years, liposomes have been blossoming in clinical applications. We searched the approved drug database published on the website of the FDA and EMA, and

found that the following liposomal products have been authorized (Table 1). **Among them, 2 drugs are PEGylated liposomes, Doxil® and Onivyde®**.

Product Name	API	Approved Year/Area	Liposome Components	Indication
Doxil	Doxorubicin hydrochloride (DOX·HCl)	1995, US	HSPC:cholesterol:DSPE-PEG	Ovarian cancer, Kaposi's sarcoma, myeloid melanoma
Caelyx		1996, EU		
DaunoXome	Daunorubicin	1996, US	DSPC: Cholesterol	Kaposi's sarcoma
AmBisome	Amphotericin B (AmpB)	1997, US	HSPC:DSPG, cholesterol	Systemic fungal infection
DepoCyt	Cytarabine	1999, US	DOPC:DPPG	Lymphomatous meningitis
DepoCyte		2001, EU		
Myocet	DOX·HCl	2000, EU	EPC: Cholesterol	Breast cancer
Visudyne	Verteporfin	2000, US	EPG:DMPC	Wet AMD
		2000, EU		
DepoDur	Morphine	2004, US	DOPC:DPPG	Postoperative pain
Mepact	MTP-PE	2009, EU	DOPC:DOPS	Osteosarcoma
Exparel	Bupivacaine	2011, US	DEPC: DPPG: Cholesterol:Tricaprylin	Post-surgical analgesia
		2020, EU		
Marqibo	Vincristine Sulfate	2012, US	SPH: Cholesterol	Leukemia
Onivyde	Irinotecan hydrochloride trihydrate	2015, US	DSPC: Cholesterol:DSPE-PEG	Pancreatic adenocarcinoma
		2016, EU		
Vyxeos	Daunorubicin, cytarabine	2017, US	DSPC: DSPG:Cholesterol	Leukemia
		2018, EU		
Shingrix	Recombinant varicella-zoster virus glycoprotein E	2018, EU	DOPC:Cholesterol	Against shingles and post-herpetic neuralgia
Arikayce	Amikacin sulfate	2018, US	DPPC: Cholesterol	Lung disease
		2020, EU		

Table 1. Summary of liposomal products approved by FDA and EMA. This list is only for liposomal forms approved by FDA and EMA, excludes generics (e.g., doxorubicin hydrochloride (liposomal), lipid complexes (e.g., Abelcet, Amphotec, and Onpattro), and also excludes the nationally authorized liposomal products in Europe.

PEGylated Liposomes: Doxil® and Onivyde®

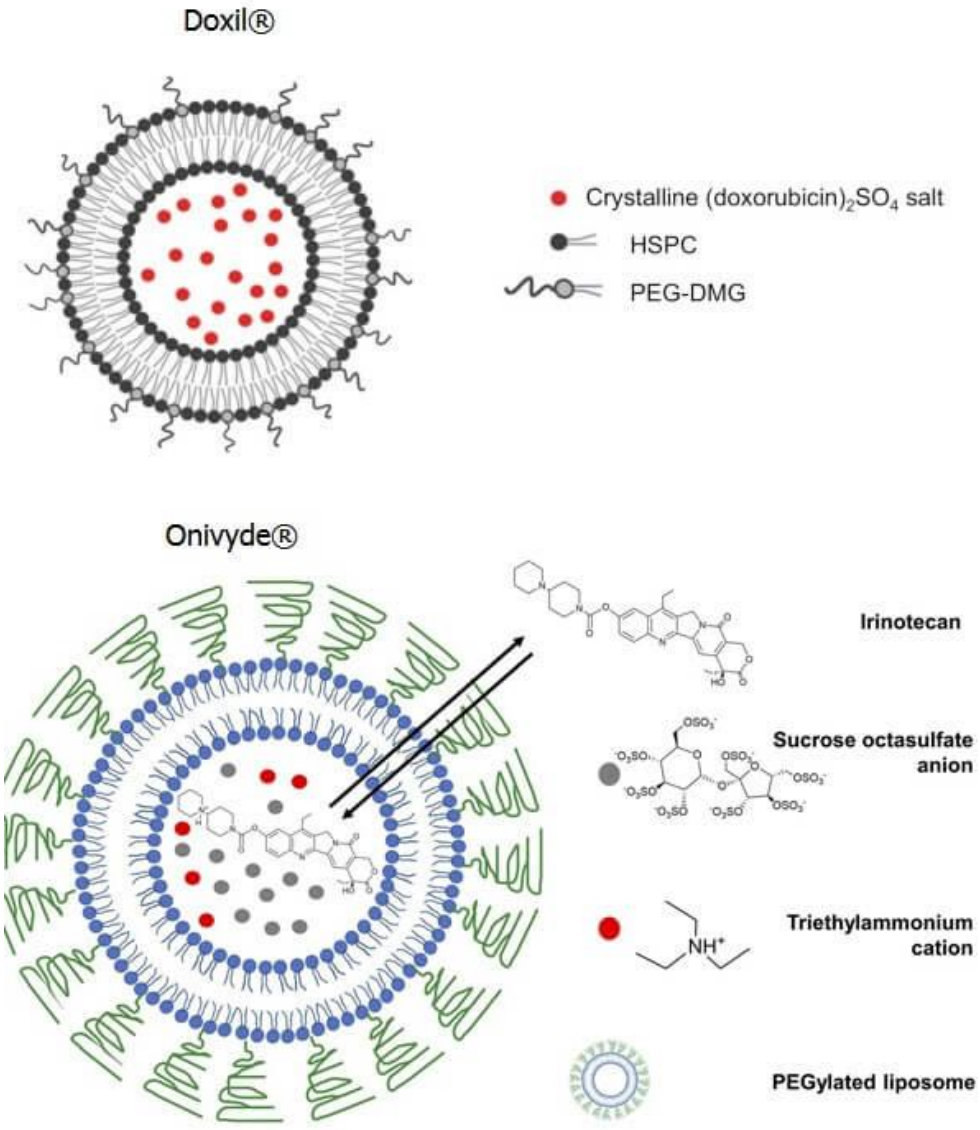

Figure 2. Structure of Doxil® and Onivyde®

Doxil® was the first PEGylated doxorubicin liposome approved in the United States by Sequus Pharmaceuticals, Inc. in 1995, and was first approved for the treatment of AIDS-related Kaposi's sarcoma, and subsequently approved for metastatic breast cancer and multiple myeloma in 2003 and 2007, respectively. The liposomal formulation of Doxil® consists of HSPC, cholesterol (cholesterol) and mPEG2000-DSPE, in the ratio of 56:38:5 on a molar basis, with a PEG modification density of 5%.

Onivyde is a low-density PEGylated irinotecan liposome from Merrimack Pharmaceuticals approved in 2015 for the treatment of patients with metastatic pancreatic cancer with a history of gemcitabine use in combination with calcium folinate and fluorouracil. Onivyde has a liposomal formulation of distearoyl phosphatidylcholine (DSPC), CHOL, and mPEG2000-DSPE in a ratio of 3:2:0.15 on a molar basis, with a PEG-modified density of 0.3%. The drug was present as sucrose octosulfate precipitate in the aqueous phase within the liposomes, with an encapsulation rate of more than 90% and was very stable.

MPEG2000-DSPE used in Doxil and Onivyde, provides "stealth" and sterically stabilized liposomes. The molecular weight of PEG and the mole percentage of PEG-DSPE in lipid composition play important roles on the bilayer packing, circulation time, and thermodynamic stability. The high molecular weight of PEG (>2000 Da) grafted to the lipid headgroup exhibits repulsive forces from the liposome surface, as well as protects liposomes from binding serum proteins and avoids further clearance by the mononuclear phagocytic system (MPS), but also decreases the interaction and endocytosis of liposomes by targeted cells.

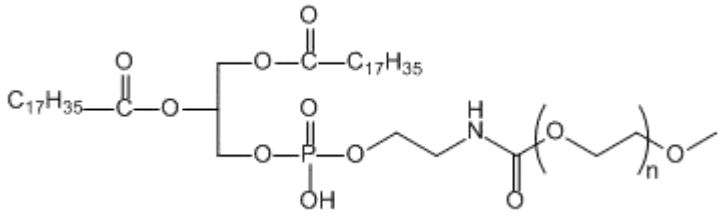

Figure 3. Structure of mPEG-DSPE

PEGylated Liposomes for Tumor Targeting

PEGylated liposomes take advantage of the EPR effect of tumors to increase the accumulation of drugs at the tumor site with their own long circulation properties, achieving passive targeting of tumors. The EPR effect was proposed by Maeda et al. in 1986, and its basic physiological characteristics are increased vascular permeability,

blocked lymphatic return, and infiltration of macromolecules from the blood circulation into the tumor tissue and long-term retention.

The rapid growth of tumors leads to an increased distance between capillaries and the tumor center and a restricted supply of nutrients to the tumor center, resulting in a hypoxic inflammatory environment that promotes the secretion of angiogenic factors such as vascular endothelial growth factor (VEGF), platelet derived gr owth factor (PDGF) and tumor necrosis factor-a (TNF-a), and inhibits the secretion of platelet response factor-1 (TSP-1), resulting in a rapid increase in neovascularization. The irregular structures of tumor neovascularization, heterogeneous spatial distribution, increased blood flow resistance and poor perfusion exacerbate the hypoxic environment at the tumor site. Degradation of the basement membrane (BM) and extracellular matrix (ECM) by matrix metalloproteinase (MMP), and the absence of smooth muscle cells and pericytes necessary for vasoconstriction significantly increase vascular permeability. Blockage of lymphatic vessels in the center of the tumor leads to obstruction of lymphatic fluid return and increases the retention of carriers such as liposomes or large molecule drugs.

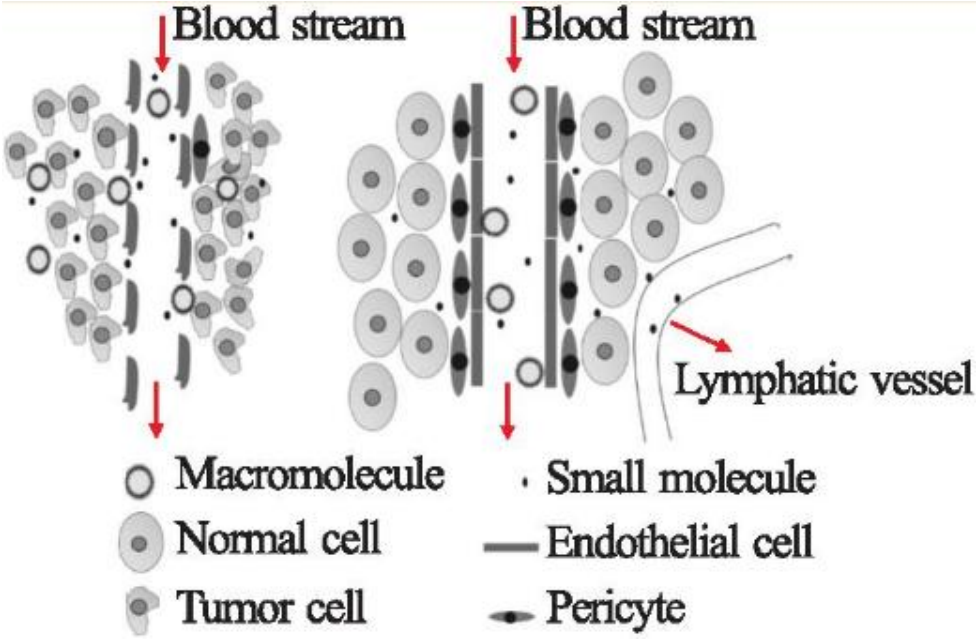

Figure 4.PEGylated Liposomes for Tumor Targeting

PEGylated Liposome in Clinical Trials

Products	Active Agent	Lipid Composition	Indication	Company	Clinical Trial
ThermoDox	Doxorubicin	DPPC, Myristoyl stearyl phosphatidylcholine and DSPE-N-[amino(polyethylene glycol)-2000]	Hepatocellular carcinoma and also recurring chest wall breast cancer	Celsion	Phase III
SPI-077	Cisplatin	Soybean phosphatidylcholine, cholesterol	Lung, head and neck cancer	Alza Corporation	Phase II
MM-302	Doxorubicin	Phase 1/2	Breast cancer	Merrimack	Phase I/II (Terminated)
2B3-101 (2X-111)	Doxorubicin	Glutathione PEGylated liposomes	brain metastases and recurrent malignant glioma	2-BBB therapeutic	Phase I/IIa
2B3-102 (ENX-201)	methylprednisolone	Glutathione PEGylated liposomes	acute relapses of multiple sclerosis	2-BBB therapeutic	Phase I
S-CKD602	Potent topoisomerase I inhibitor	Phospholipids covalently bound to mPEG	Cancer	Alza Corporation	Phase I

Table 2. PEGylated liposomal formulations in clinical trials.

ThermoDox

ThermoDox® is a heat-sensitive PEGylated doxorubicin liposome developed by Celsion and Duke University based on lysolipid thermally sensitive liposome (LTSL) technology for the treatment of hepatocellular carcinoma. The heat-sensitive membrane rapidly changes structure when heated to 40ºC-45ºC, creating openings in the membrane that release doxorubicin directly into and around the targeted tumor.

Although four commercialized liposomes loading doxorubicin (Doxil®, Myocet®, Lipodox®, and Liposomal doxorubicin) were already launched into the market successfully, ThermoDox® is a new product with advanced characteristics, that showed a 5-fold release in doxorubicin concentration at the tumor site when compared to Doxil®. Phase III clinical trials of ThermoDox® in combination with standardized radiofrequency ablation (NCT02112656) have been completed.

MM-302

MM-302 of Merrimack Pharmaceuticals, is a PEGylated liposome modified with antibodies targeting the human epidermal growth factor receptor 2 (HER2) and loaded with doxorubicin, which has applied for phase 1 clinical trial in 2011. MM-302 is designed to overcome the limitations of doxorubicin associated with cardiotoxicity and ineffective targeting of cancer cells. MM-302 was evaluated for the treatment of advanced HER2-positive breast cancer in combination with trastuzumab or trastuzumab plus cyclophosphamide. Promising data from the Phase I clinical trial inspired MM-302 to enter Phase II clinical trials. However, the efficacy results did not show a significant benefit over comparator treatments, which led Merrimack to stop further trials of MM-302 in 2016.

SPI-77

SPI-77 developed by Sequus Pharmaceuticals (Johnson & Johnson) is a liposomal PEGylated formulation of cisplatin, developed for the treatment of recurrent ovarian cancer and stage IV non-small cell lung cancer (NSCLC). It was hypothesized that SPI-77 could mitigate the systemic toxicity of cisplatin and achieve high delivery capacity. However, due to a lack of significant data, in Phase 1 and Phase 2 clinical trials, the manufacturer decided to stop further trials.

S-CKD602

S-CKD602 is a pegylated liposomal formulation of CKD-602, a semi-synthetic camptothecin analogue, with liposomal formulation consisting of N-(carbonyl-methoxypolyethylene glycol 2000)-DSPE and DSPC.

2B3-101/ 2B3-201

2B3-101 is being developed for patients suffering from multiple brain cancer indications, with an initial focus on patients with brain metastases of breast cancer and patients with glioma. 2B3-101 is based on the existing chemotherapeutic agent doxorubicin.

G-Technology® is a technology developed by 2-BBB to couple PEGylated liposomes with glutathione. Two clinical trials have been conducted based on G-Technology®: 2B3-101, a glutathione-PEGylated doxorubicin liposome, which completed a Phase I/IIa clinical trial for brain metastases and recurrent malignant glioma; and 2B3-102, a glutathione-PEGylated methylprednisolone liposome for acute relapses of multiple sclerosis, which completed a Phase I clinical trial in healthy volunteers.

Immunogenicity of PEGylated liposomes

Anti-PEG antibodies were first identified in rabbits in 1983 by Richter et al. Later studies revealed that anti-PEG antibodies can bind to the surface of PEGylated liposomes, alter the composition of the protein corona, activate the complement system and elicit an immune response, becoming a serious problem for all PEGylated agents, including PEGylated liposomes.

Accelerated Blood Clearance (ABC) Phenomenon

In 2000, Dams et al. discovered the ABC phenomenon in rats. After the first injection of normal liposomes or PEGylated liposomes, a second injection of PEGylated liposomes a few days apart resulted in rapid clearance and increased accumulation in the liver and

spleen. This change in the pharmacokinetic properties of liposomes would be a major drawback in their clinical application. If the liposome-encapsulated drug is cytotoxic, the accumulation of liposomes in hepatic Kupffer cells could lead to apoptosis and necrosis of these cells, and recovery of Kupfer cells would take 2 weeks, during which time the development of bacteremia would be fatal for cancer patients.

There are more factors affecting the ABC phenomenon, such as the time interval of repeated injections of PEGylated liposomes, with the strongest ABC phenomenon at an interval of 7 d. In addition, the density and molecular weight of PEGs as well as the end groups can affect the intensity of ABC phenomenon. The dose of the drug can also affect the ABC phenomenon. For example, in mice, macaca fascicularis, rats and minipigs, no ABC phenomenon occurred when Doxil® was injected at a dose of 20 mg-m2; however, ABC phenomenon occurred at an injected dose of 0.2 mg-m2.

Complement Activation-Related Pseudoallergy (CARPA)

In recent years, the use of PEGylated liposomes, represented by Doxil®, has become more and more widespread, and the incidence of metabolic reactions in clinical practice has increased. Patients mostly present with symptoms such as dyspnea, facial swelling, chest pain, flushing, rash, hypotension or hypertension, and cardiac pain, which may be life-threatening. Allergic reactions occur in up to 45% of patients treated directly with Doxil® without steroids and antihistamines, and still occur in 4% to 7.1% of patients treated earlier with steroids and antihistamines. The allergic reactions to Doxil® are caused by its PEGylated liposomes, and doxorubicin alone does not cause these reactions.

Studies have shown that CARPA is closely related to complement activation, and the higher the level of the soluble non-functional form of complement activation end product C5b-9, SC5b-9, the more severe the reaction. PEGylated liposomes can both trigger an innate immune response to activate complement and induce anti-PEGIgM by triggering an

acquired immune response, which activates complement through the classical pathway and triggers severe CARPA.

Not only that, PEGylated liposomes can activate complement through alternative pathways, enhancing the classical activation pathway of complement in the form of positive feedback. Patients with severe reactions to PEGylated liposomes have been reported to have anti-PEGIgG titers approximately six times higher than those of non-reactive patients, and patients with anti-PEGIgG titers three times higher than the detection limit are approximately three times more likely to have more severe allergic reactions than those with anti-PEGIgG titers only one time higher than the detection limit.

Conclusion

In view of the serious problems of PEGylation, researchers have been searching for PEG substitutes such as polyinosinic acid, polyglycidol, polyhydroxyethyl-L-asparagine, polysialic acid, and lipid derivatives containing sialic acid fragments. Unfortunately, no ideal PEG substitutes have been found so far. Therefore, the interrelationship between liposomes and the immune system should be taken seriously without deliberately pursuing a "long acting".

Biopharma PEG has been focusing on the development of a full range of PEG lipids for nanocarrier systems (including various types of nanoparticles, liposomes, micelles, etc.), and has accumulated a large number of data models and rich research experience in the construction and optimization of nanocarriers for gene vaccines and protein drugs.

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