Liposome-based diagnostic and therapeutic applications for pancreatic cancer

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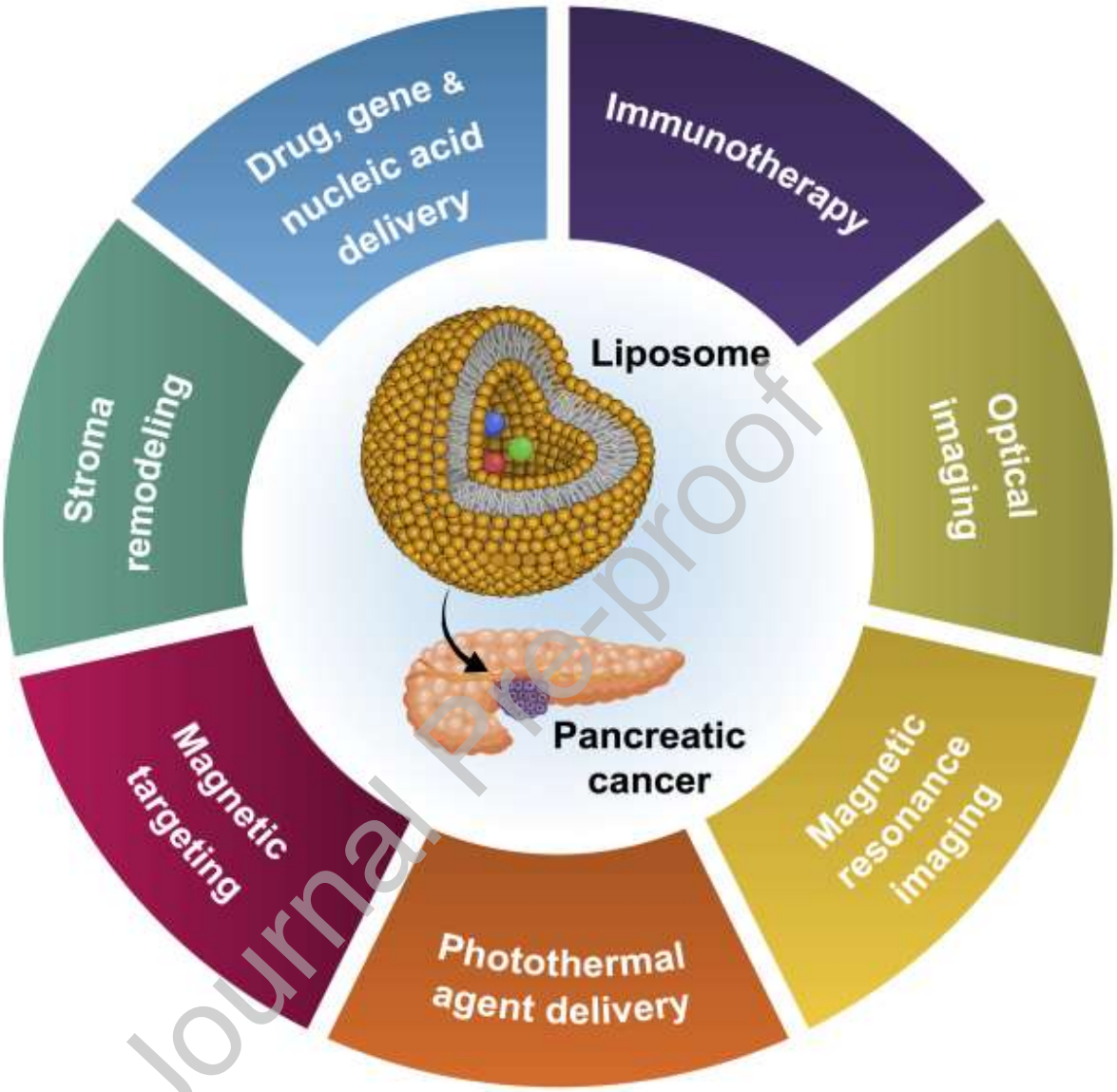
Graphical Abstract



Abstract

Pancreatic cancer is one of the harshest and most challenging cancers to treat, often labeled as incurable. Chemotherapy continues to be the most popular treatment yet yields a very poor prognosis. The main barriers such as inefficient drug penetration and drug resistance, have led to the development of drug carrier systems. The benefits, ease of fabrication and modification of liposomes render them as ideal future drug delivery systems. This review delves into the versatility of liposomes to achieve various mechanisms of treatment for pancreatic cancer. Not only are there benefits of loading chemotherapy drugs and targeting agents onto liposomes, as well as mRNA combined therapy, but liposomes have also been exploited for immunotherapy and can be programmed to respond to photothermal therapy. Multifunctional liposomal formulations have demonstrated significant pre-clinical success. Functionalising drug-encapsulated liposomes has resulted in triggered drug release, specific targeting, and remodeling of the tumor environment. Suppressing tumor progression has been achieved, due to their ability to more efficiently and precisely deliver chemotherapy. Currently, no multifunctional surface-modified liposomes are clinically approved for pancreatic cancer thus we aim to shed light on the trials and tribulations and progress so far, with the hope for liposomal therapy in the future and improved patient outcomes.

Keywords: liposomes; drug delivery; controlled release; nanocarrier; stroma remodeling; cancer; intratumor

1. Introduction

Pancreatic cancer (PC) survival is one of the lowest, with mortality rates gradually rising, making it one of the top three leading causes of cancer death. Statistical models have predicted that PC will have over 60,000 new diagnosis and almost 50,000 deaths in the US in 2022 [1], which unfortunately is an increase from 2020 [2]. Pancreatic cancer is one of the common tumors of the alimentary tract [3] and is characterized by early metastatic spread [4]. Despite the continual intensive progress in treatment strategies [5], long-term survival is poor [4], with a global average 5-year survival rate of 6% [6]. Unsatisfactory results with standard treatment of FOLFIRINOX® have made it necessary to continue efforts in search of new drugs and novel treatment methods for PC [7].

Nano-sized drug delivery systems such as liposomes (LPs), have been extensively used in PC treatment. They can form lipoplexes with small interfering RNAs (siRNAs), evade the reticuloendothelial system (RES), (hence, providing longer circulation times), as well as being capable of encapsulation of amphiphilic drugs simultaneously. Their ease of surface functionalization, targeted delivery, and stabilizing drugs *in vivo* [8, 9] have made them successful in chemotherapy. To date, LPs have been applied as delivery systems of several anticancer agents (e.g. nucleoside analogs, mitosis inhibitors, enzyme modulators) and gene/nucleic acid (TR3 siRNA, siRNA of NGF) in PC [10], either alone or in combination with multiple targeting strategies. Liposomal surface functionalization with, e.g., antibody fragment conjugates [11] have also been used for targeted delivery of numerous compounds, including chemotherapeutics [12-15] and insulin [16]. Physicochemical and biological signals such as temperature [17], pH [18], magnetic field [19], redox potential [20] and photodynamic sensitivity [15] have been utilized for controlled targeting of nucleic acids [21] and chemotherapeutic agents [22, 23]. Above all, the most successful results have been obtained by combining the strategies above with anticancer drugs [24].

This review provides an update on the current research of liposome-based PC therapies including chemotherapeutics and nucleic acid delivery, co delivery of different drugs, stroma remodeling therapy, immunotherapy and stimuli responsive LPs. Furthermore, this review also highlights the major challenges and hurdles for successful delivery and clinical translation of anti-cancer therapeutics for PC treatment. The overall benefits, challenges and future perspective of using liposome for PC treatment have also been discussed.

2. Hurdles and current challenges in pancreatic cancer therapy

With a dismal 5-year survival rate, PC remains lethal worldwide [25, 26]. Despite all advances in cancer therapy, there has yet to be an effective treatment for PC, even in patients with resectable surgery, the survival rate remains low because of drug resistance in cancer cells and tumor recurrence [27]. The high mortality rates of PC can be attributed to challenges such as limited diagnostic methods, especially in the early stages, the aggressive nature of this cancer, and its resistance to therapeutic agents. The drug resistance is a result of its tumor microenvironment which is highly immunosuppressive with a desmoplastic stromal reaction. The immunosuppressive profile is due to increased activity of regulatory T cells (Treg), myeloid-derived suppressor cells (MDSCs), and programmed cell death ligand-1 (PD-L1) up-regulation which inhibits the normal cluster of differentiation 8^+ ($CD8^+$) T cells' function in PC [28]. Desmoplasia leads to extracellular matrix (ECM) hyper density, hypoxia, attenuated vascularization, and finally restriction of drug delivery to the tumor site [26, 29]. An additional challenge facing the success rate of treatments is the genetically heterogeneous feature of PC. Mutations are a hallmark of cancer. The most common signaling pathways which go under mutations in PC are the Kirsten Rat Sarcoma (KRAS), STAT3, and Sonic Hedgehog (SHh) pathways plus tumor suppressor genes including TP 53, P16/CDKN2A and SMAD4 [28, 30, 31]. With various genomic mutations across patients, therapeutic approaches which target specific genomic features can work for some and fail for others. There is no 'one size fits all' effective treatment.

Single-drug therapies by gemcitabine (GEM), cisplatin, oxaliplatin, paclitaxel (PTX), albumin-bound PTX, and combination therapies including FOLFIRINOX (combination of folinic acid, 5-fluorouracil, irinotecan, and oxaliplatin) and nab PTX-GEM have been used in PC patients. Despite best efforts with chemotherapeutic strategies, there have been no significant changes in treatment outcomes over the past decades [32] mainly because drug resistance is inevitable [33-35]. Radiotherapy is another treatment option, especially before surgery and for local tumors. However, radiotherapy mostly relieves the symptoms and tumor recurrence stays a problem even after radiotherapy [26].

Though chemotherapy and radiotherapy remain the popular treatment options, there is broad recognition that innovative and more specific targeted therapies are needed to overcome resistance and increase treatment efficacy have been brought to light.

Several studies have been performed to target the mutated genes, signaling pathways, and tumor microenvironment. Clinical investigations to target mutated KRAS pathways have yet to be successful rapid drug resistance is again hindering the success rate [28, 36]. In a study conducted by Olive *et al.*, it was demonstrated that inhibiting the SHh-associated desmoplasia is effective for GEM delivery, whilst other researchers demonstrated that SHh inhibition could prevent the stromal formation and results in poor survival [37-39]. To diminish the ECM density and destroy the rigid barrier for drug delivery, hyaluronidase was utilized, but its efficacy in cancer therapy was denied because of the risk of thrombosis formation [40].

Immunotherapy is a novel approach to cancer therapy in which the patients' activated T cells are used to destroy their tumors. Even though various immunotherapy strategies including immune checkpoint mono and combination therapy, chimeric antigen receptor (CAR) T-cell therapy, checkpoint inhibition, and monoclonal antibodies have been applied in PC patients, the results are not satisfying, mainly due to the immunosuppressive property of tumor microenvironment which prevents drug delivery and T cell transmission to the targeted site [28, 41].

To overcome the treatment hurdles of PC and to make an effective targeted therapy, various kinds of delivery systems, including gelatin-based nanoparticles (NPs), polymer-based nanocarriers, inorganic, and lipid-based NPs, have been formulated for delivering therapeutic agents like chemotherapy drugs and oncogene repressor siRNAs [35, 42-49].

Nanosystems are of peak interest as delivery vehicles, Meyer *et al.* provide a detailed review of the benefits of particle delivery systems owing to their particular success in their size, shape and their suitability for surface modifications. [50] Lipid-based NPs such as LPs are of specific benefit due to their high biocompatibility, ability to encapsulate drugs and capacity to carry them to the tumor microenvironment and their ease of modification, i.e., attachment of targeting agents. Therefore, it is no surprise that liposomal formulations are being vastly investigated for cancer therapies.

3. Liposome-based pancreatic cancer therapies

LPs are bilayer vesicles made by phospholipids enclosing aqueous core. LPs have been extensively studied as nanocarriers of choice for the delivery of a wide range therapeutic agents from last few decades as they are suitable to encapsulate several types of drugs which may be hydrophilic as well as lipophilic in nature. Liposomal formulations have shown promising results in drug delivery for several types of cancer, including treatment Onivyde™, Marqibo®, Doxil®, Visudyne® and Depocyt® [51, 52]. Currently, the nanoliposomal irinotecan (Onivyde) is the only liposomal formulation approved for the treatment of metastatic pancreatic cancer [53]. Different therapeutic agent-loaded LPs have been utilized in PC, such as small molecules (chemotherapeutics) and large molecules (nucleic acid and proteins) to name a few. Advancements in liposomal delivery systems have achieved both targeted delivery and controlled release of anticancer drugs, an essential property in cancer treatment [54].

LPs hold key characteristics which allow them to be tuned and modified to be beneficial in various types of therapy. **Figure 1** highlights the vast capabilities LPs can attain, lending them as promising multifunctional delivery agents.

Various anticancer drugs have been delivered using the targeting property of LPs in PC [55, 56]. Among different anticancer drugs, LPs have been used extensively for targeted delivery of GEM, antimetabolites class. GemLip®, a liposomal GEM formulation, showed better pharmacokinetics, pharmacodynamics, and anti-tumoral activity than conventional GEM [57, 58]. Additionally, ease of surface modifications allowed increased circulation time in body, as a PEGylated liposomal formulation of GEM was prepared which showed great entrapment efficacy and drug loading, high stability and improvement in cytotoxicity to GEM–resistant PC cells [59]. Multifunctional liposomal formulations are found throughout literature, typically consisting of liposomal formulations functionalized with targeting ligands (i.e. monoclonal antibodies, proteins, small molecules, aptamers, and peptides) to reduce off-target binding of the drugs in healthy tissues and increased on-target binding of the drug to cancer cells, since the targeting ligands will only bind to specific receptors expressed on these cells. Due to this increased on-target binding there is a decreased risk of drug toxicity and increased efficacy of the treatment.

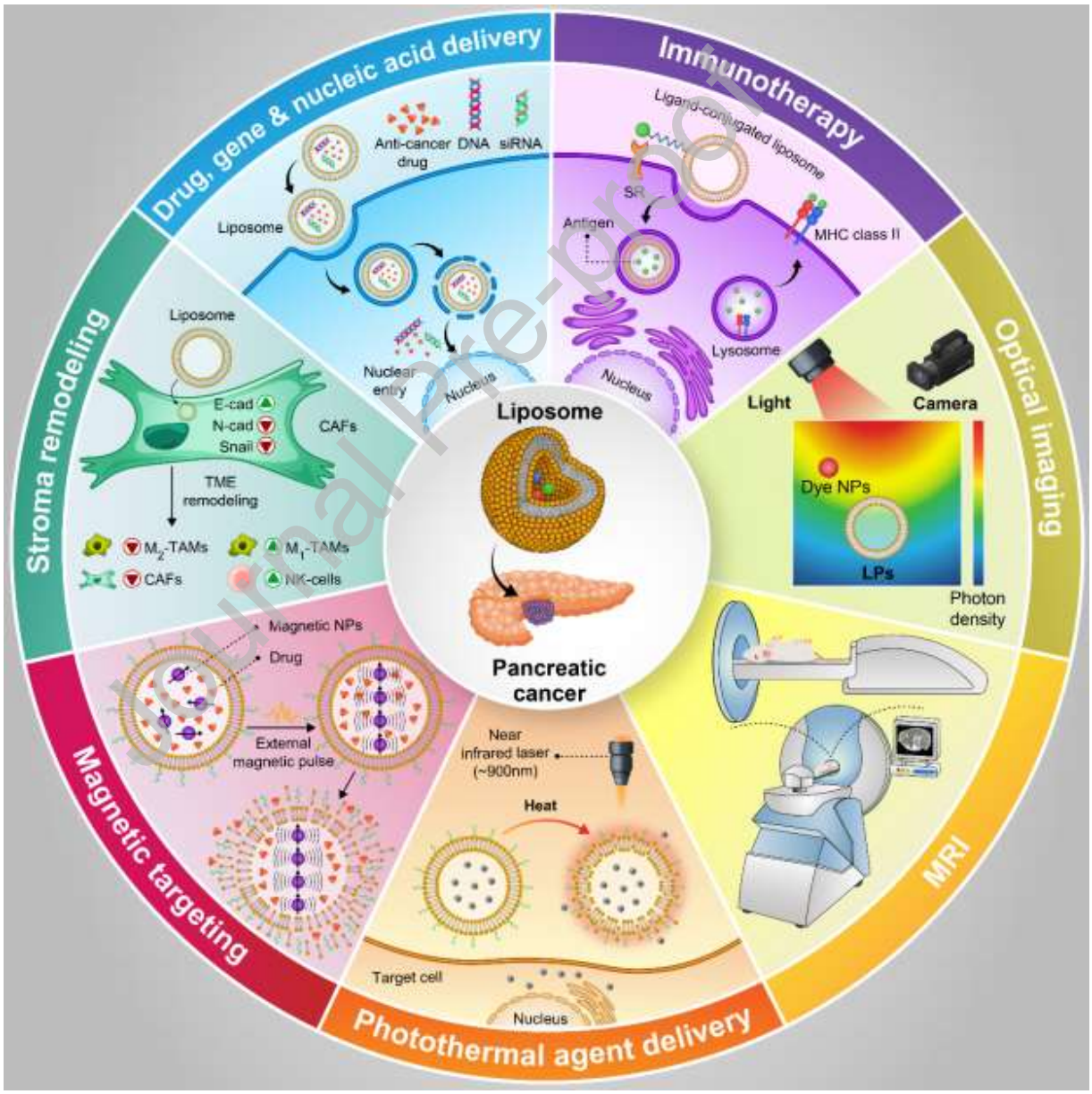


Figure 1. Schematic illustration of liposome-based drug delivery systems in PC diagnosis and treatment. Liposomal delivery systems are capable of being applied for early detection of the PC through MRI and optical imaging technologies plus delivering various therapeutic agents including genes and anti-cancer drugs to the exact tumor site for an effective targeted therapy.

With the capability to load various agents onto LPs, the mechanisms of therapy can be tailored. The possibility/ potential of pharmaceutical delivery alongside targeting agents, gene therapy, and photothermal therapy using liposomes is examined and discussed. **Table 1** gives a summary of some of the liposomal delivery systems already exploited in the treatment of cancer.

Table 1: Various nano-systems for different therapeutic strategies.

Composition	Size/loading/encapsulation	*In vivo* models	Findings	Therapeutic Strategy	Ref
Lipid encapsulated gemcitabine	Size 79 ± 2 nm Encapsulation >96%	Mice with Capan-1 or BxPC-3 tumors	Suppression of tumor growth	Chemotherapeutic loaded liposomal therapy	[60]
GSH surface modified liposome with encapsulated doxorubicin.	Size 65.2 ± 5.7 nm DOX encapsulation > 95% and a DOX loading content ~10%	Mice bearing subcutaneous Huh7 tumors and pancreatic ductal adenocarcinoma (PDA) BxPC3 cell line	Inhibited tumor growth	Liposome with targeting agent and cytotoxic agent	[61]
TR-PTX/HCQ-Lip	Size 135.47 ± 2.85 nm loading PTX 83.72 ± 1.96% HCQ 80.96 ± 2.38%	BxPC-3 orthotopic pancreatic cancer model	Suppression of tumor growth and inhibition of autophagy and stroma fibrosis	Liposome with agent to modify stroma pathways	[62]

HSA-BMS@CAP-ILTSL	Size 121.5 ± 2.8 nm loading efficacy of BMS-HSA in CAP-ILTSL was 10.75 ± 1.7%	Pan 02 subcutaneous mouse model	Suppression of tumor growth	Immunotherapy and photothermal	[63]
CpG-DNA-peptide-liposome complex		TM4SF5-expressing mouse PDAC cells (PANC02-hTM4SF5)	Suppression of tumor growth	Gene Therapy	[64]
TLR7 agonist, conjugated with cholesterol prepared into liposomes	Size 110 nm	CT26 colorectal cancer, 4T1 breast cancer, and Pan02 pancreatic ductal cancer models.	Suppression of tumor growth and metastasis	Lymphatic Targeting	[65]

Numerous studies have overcome various barriers such as poor penetration due to the dense stroma and complex tumor microenvironment, chemo-resistance and undesirable systemic effects of chemotherapy. LPs offer improved biocompatibility and the ability to encapsulate drug loads, carry them to the tumor site, and enhance cellular uptake. The extensive modifications available to LPs lend them to being used in multiple therapeutic strategies for PC therapy.

3.1. Chemotherapy delivery

The application of LPs for chemotherapeutic agent delivery presents the potential to play a vital role in PC therapy, such as the delivery of chemotherapy drugs. Recently, Matsumoto *et al.* demonstrated that treating mouse xenograft PC tumor models with FF-10832, novel GEM-loaded liposome, augments the plasma stability antitumor properties of GEM while reducing

systemic toxicity [66]. Loading of chemotherapy drugs to LPs that can release upon a stimulus's action is widely evaluated. We provide examples of successful delivery and release of chemotherapy agents using LPs responding to pH and heat and how targeting agents can be incorporated into LPs to achieve effective delivery. Studies have described drug delivery systems that release their drug load in response to a stimulus to overcome the challenge of hindered drug penetration into the tumor microenvironment. These are being developed with PC characteristics, e.g., enzyme and pH triggered systems that respond mainly to the acidic tumor microenvironment conditions to aid penetration into poorly permeable tumors. One study developed GEM-loaded LPs with a 'charge exchange' capability which allowed for active transportation *via* transcytosis by exploiting the acidic tumor microenvironment of PC aided by ultrasound technology. [60] *In vivo* testing found that these clever nano-systems could penetrate the tumor and hinder tumor growth much better than GEM alone (and control nanodroplets).

A further nanocarrier utilizing tunable charged moieties for active targeting is outlined in a different study conducted by Wang *et al.* in which positive surface charges encourage fast active transportation into cells. This strategy allowed the targeted delivery of anticancer agents into the tumor by passing unwanted systemic effects. Similarly, these anticancer-loaded LPs show promise as a new treatment for PC as *in vivo* evaluation found tumor regression in those treated with these [61]. From **Figure 2**, the doxorubicin (DOX)-encapsulated liposome surface modified with GSH (GCSDL) can be seen to show improved tumor growth suppression.

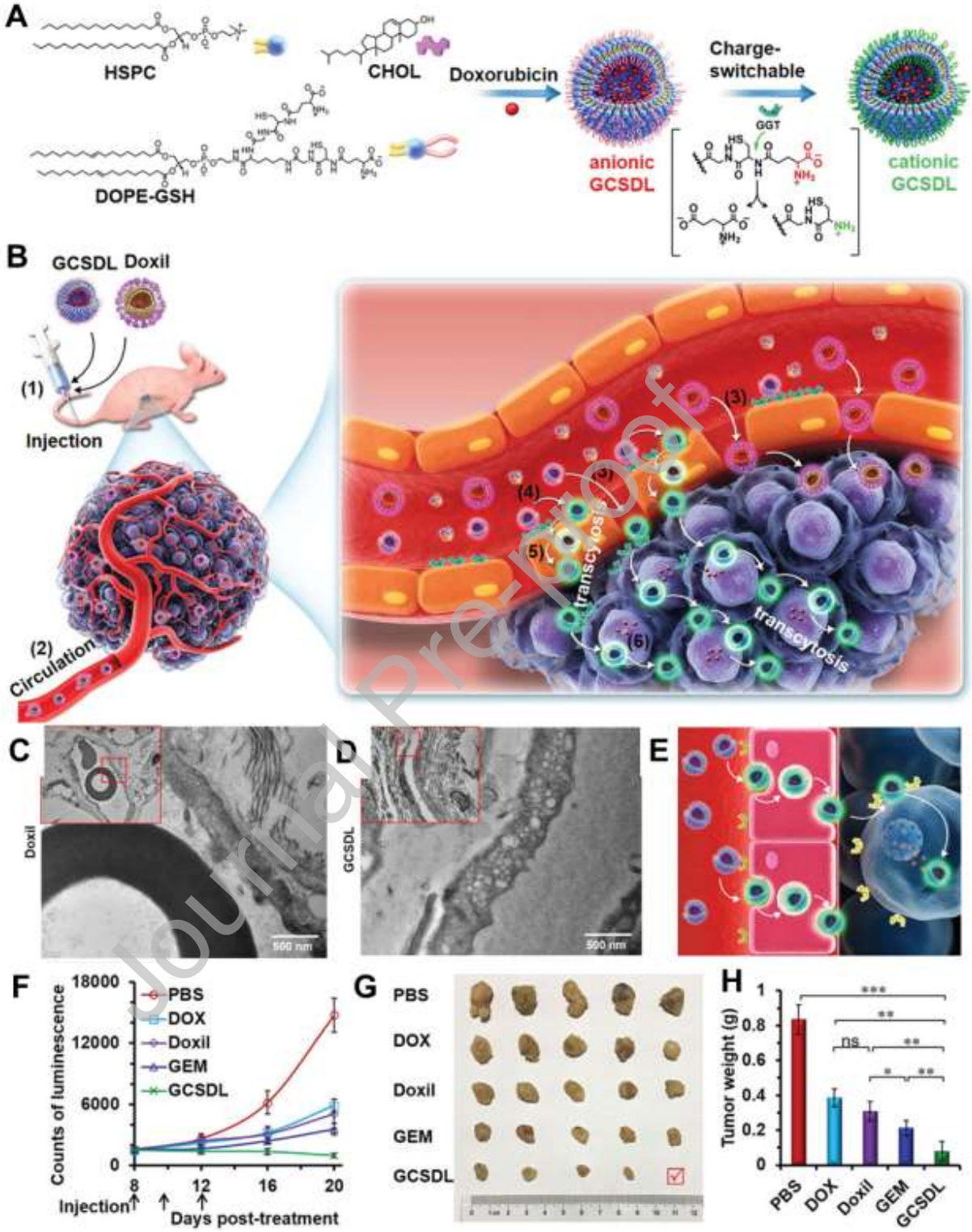


Figure 2. Schematic illustration of GCSDL (composed of HSPC, CHOL, DOPE-GSH, and embedded DOX) application in which the GGT enzyme catalyzes the γ-glutamyl transfer reactions of GSH moiety

that results in cationic primary amines generation and the anionic GCSDL conversion into the cationic form **(A)**. Following intravenous injection (1) and circulation in the bloodstream (2), a few of GCSDL or Doxil diffuse into the tumor periphery through extravasation of the leaky blood vessels (3); GCSDL / TVEC contact and GGT catalyzation, leads to the conversion of the anionic GCSDL into cationic form (4); The caveolae-mediated endocytosis is activated due to the cationization and proceeds the vesicle-mediated trans-cytosis, resulting in the increased tumor accumulation and deep penetration into interior parenchyma (5) **(B)**. Tumor blood vessels' ultrastructures captured by TEM **(C, D)**. GCSDL transcytosis suggested by TEM **(E)**. Luminescence intensity of BxPC3-Luci tumors-bearing mice during the experiment **(F)**. Dissected tumors images and the tumor weight average at the end of the experiment **(G, H)**. Adapted with permission from reference [61], copyright Small (2020).

Xu *et al.* studied pH-sensitive LPs of GEM to mitigate multi-drug resistance (MDR) associated with the use of GEM as first-line therapy. Results depicted 4.2 fold increase in half-life (t1/2) and restoration of sensitivity of PC cells to GEM [18]. Similarly, using biologic signals to stimulate drug cargo release at PC cells, Wei *et al.* formulated thermosensitive LPs for co-delivery of human serum albumin (HSA)-PTX (mitotic inhibitor) and HAS-Ellagic acid (enzyme modulator) that showed robust tumor growth inhibition, apoptosis *in vivo* and overcame the drawback of poor blood retention associated with HSA LPs of PTX [17].

Additionally, a LP delivery system responsive to redox reactions was evaluated. This system improved the drug internalization, that is, more irinotecan (IR) could be loaded onto/into the liposome thus higher drug into the cell. Drug release was activated once in the cell by a GSH-induced redox reaction which causes liposomal collapse [20]. So far, some examples of LPs programmed to make use of internal stimuli within the tumor environment have been discussed, it is also possible to utilize external stimuli to exploit drug release. The cavitation effects of ultrasound can also be a powerful tool to enhance targeted chemotherapy delivery to PC by promoting site-specific drug release under a focused ultrasound beam. For instance, ultrasound-sensitive DOX-loaded LPs (L-DOX) showed improved tumor volume reduction compared to free DOX and L-DOX [67].

More recently, Dwivedi and Kiran *et al*. showed that utilizing ultrasound pulses with (DOX)-loaded magneto-liposomes resulted in apoptosis and greater anti-cancer effects in of Panc-2 and BXPC-3 cell lines where ultrasonication gave rise to increased permeability and distribution of

drug. *In vivo* experimentation revealed that the magnetic nature allowed for localized accumulation, therefore along the ultrasound waves resulted in targeted and controlled treatment, leading to reduction in tumor growth in Balb/c nude mice (pancreatic xenograft model) [68].

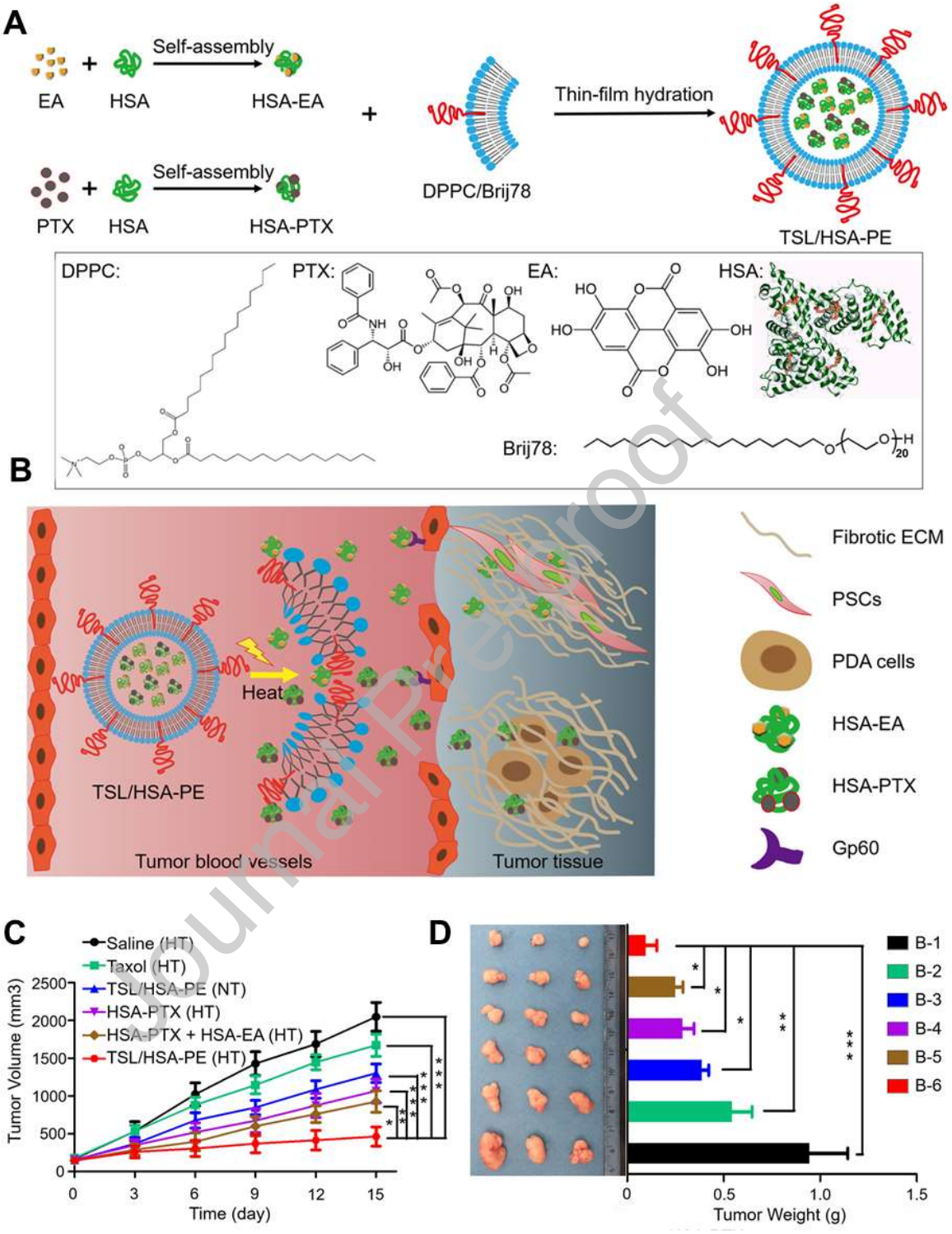


Figure 3. An illustration representing the preparation and *in vivo* application of TSL/has-PE nanocarriers in nude mice bearing BxPC-3 and HPaSteC, treated with intravenously injection of these formulations, at doses of PTX 5 mg/kg and EA 4 mg/kg, for about 2 weeks **(A, B)**. Tumor volume curves during the

experiment (The suffixes “HT” and “NT” in the curves indicate various heat treatments of the tumors) **(C)**. The tumor xenograft images and tumor weight (B-1: Saline (HT); B-2: Taxol (HT); B-3hasSL/HSA-PE (Nhas B-4: HSA–PTXhasT); has: HSA–PTX + HSA–has (HT); B-6: TSL/HSA-PE (HT) **(D)**. Adapted with permission from reference [69], copyright Clinics and research in hepatology and gastroenterology (2019).

Ultrasound-mediated drug delivery was also used with a microbubble-liposome complex carrying irinotecan and oxaliplatin. The efficacy of the drug combination was increased as part of this system. Tumors treated with ultrasound and drug-loaded microbubble-liposome were claimed to be 136% smaller than those treated with drugs alone [70]. A chemotherapy-microbubble formulation in combination with ultrasound has been evaluated in a Phase I clinical trial, with promising outcomes [69]. Additionally, to enhance the delivery of drugs, an array of targeting agents attached to liposomes are being widely employed. Liposomal drug delivery to PC cells presents a superior platform through passive or active targeting. GEM liposomes conjugated with hyaluronic acid (HA) were developed to target CD44 receptors. Studies presented the highest sensitivity of CD44 receptors expressing PC cell lines towards the developed formulation and higher cytotoxic activity than non-targeted liposomes [71]. Other examples include β-cyclodextrin matrix metalloproteinase-2 (MMP-2)-responsive liposomal formulation of Pirfenidone (anti-fibrotic) with GEM [12], $ATB^{0,+}$(SLC6A14)-targeted liposomes [13], MT1-MMP activated liposomes [14], Glypican-1-targeted liposomes [61], etc. To investigate the efficacy of intracellular drug delivery to overcome GEM resistance in PC, HA-coupled-pH-sensitive liposomes showed enhanced internalization *via* CD44-mediated endocytosis and significant reduction of tumor volume in both Mia PaCa-2 and Gr2000 PC models [72].

The vital effect of incorporating an antibody fragment conjugation within a liposomal drug delivery system was evaluated with various *in vitro* assays. GEM and PTX were loaded onto a targeted liposome system. Greater cell internalization in BxPC-3, PC cell line, was observed with the targeted system additionally, for the targeted liposome system, IC_{50} value is around 4 times lower concentration of carrier with targeting agent to reach the same cytotoxic effect c.f. non-targeted, with drug alone requiring an even higher concentration to have the same effect. The therapeutic potential was assessed by looking at signaling pathways related to the regulation of

cell apoptosis with the targeted liposome system with a higher cell survival than the non-targeted system [11].

Other active targeting strategies have been successfully developed to endow liposomes with enhanced targeting features, such as glypican-1-targeted liposomes [61]. Glypican-1 is a predominant feature of PC cells and is under-expressed in healthy cells. When coupled with liposomes and GEM, orthotopic PDAC mouse models revealed the most significant reduction in tumor size [61]. Kimura *et al*. discuss a further agent to promote targeted drug delivery; rBC2LCN lectin is a protein that will specifically bind to fucosylated glycans found on pancreatic tumor cells. Liposomes carrying DOX, surface modified with rBC2LCN lectin, were evaluated *in vivo*, exploiting protein-specific binding to improve drug delivery to tumor cells. Kimura *et al.* report a decrease in tumor weight (xenograft Capan-1 mice) upon treatment with the targeted formulation. They argue that using this small molecular weight protein will be advantageous over traditional antibody-coated liposomes and should be further evaluated for targeted drug delivery to PC cells [73]. Further, the use of smaller molecules such as peptides has also been shown. Sounni *et al*. represent the benefits of a liposome with cRGDfK peptide (a αVβ3 inhibitor) spacer, which responds to the enzyme MT1-MMP present on tumor cells and will release its drug load upon heat activation, giving a system that provides controlled drug release. This system performed well *in vivo*, hindering tumor growth as shown in **Figure 4** [74].

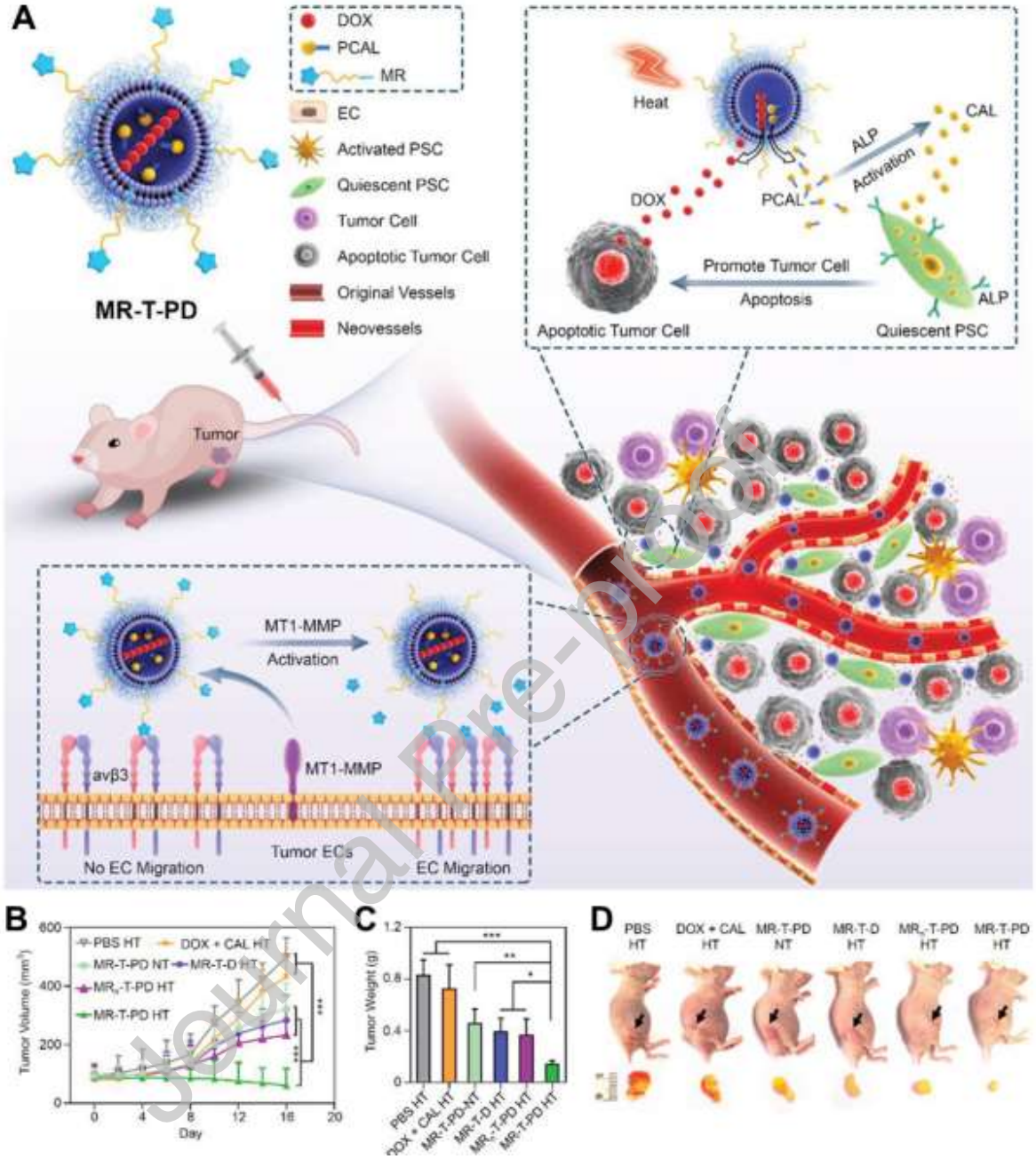


Figure 4. Schematic illustration showing the potential therapeutic mechanisms of MR-T-PD (composed of DOX, PCAL, and MR)-loaded thermosensitive liposomes in a PC mouse model bearing BxPC-3 and HPaSteC xenografts. The MT1-MMP on the surface of tumor endothelial cells (ECs) activates MR-T-PD to release cRGDfK which promotes MR-T-PD accumulation in the tumors. Additionally, under heat treatment, MR-T-PD releases PCAL and DOX into the interstitium. The released DOX induces apoptosis in the tumor cells whereas the PCAL prodrug is converted to CAL and CAL promotes the antitumor effects of DOX **(A)**. Tumor volume curve during the treatment **(B)**. Tumor weight curve **(C)** and

representative images of tumor-bearing mice and tumor tissues at the end of the treatment (the black arrows indicate the tumors) **(D)**. Adapted with permission from reference [74], copyright Advanced Functional Materials (2021).

Multi-functional liposomes, developed for diagnosis and therapy of disease simultaneously, are considered the next generation of nano-therapeutics, theranostics. Such liposomes have been explored and are at an early research stage for PC treatment [19, 75] and will become a standard practice in the future. Many developed formulations have passed pre-clinical studies and are employed in different stages of clinical trials to assess survival rates. For instance, GEM in combination with nab-PTX and the multi-drug FOLFIRINOX (composed of folinic acid (leucovorin), 5-fluorouracil, irinotecan, and oxaliplatin) exhibited improved therapeutic activity in contrast to GEM monotherapy in randomized clinical trials, being approved as first-line treatment for advanced PC management [32]. Another example, is the novel formulation of nano-sized liposomal encapsulated irinotecan (Nal-IRI), which was developed to improve drug delivery, effectiveness, and limiting toxicity featured by conventional chemotherapy. In combination with leucovorin-modulated fluorouracil (5-FU/LV), Nal-IRI was found to significantly improve overall survival in patients who had been previously on GEM therapy in Phase III clinical trials (NAPOLI-1), being later approved as second-line treatment for PC [76]. EndoTAG™-1, another PTX embedded liposomal formulation, showed well tolerability and efficient efficacy and survival among 212 PC patients enrolled in a randomized, Phase II clinical trial [77].

Liposomal formulations present many advantages over current first-line treatments. Numerous studies improve the pharmacokinetics of drugs by using liposomes. Liposomes provide the additional benefit of improved pharmacokinetics; numerous studies have shown liposomes loaded with various anticancer drugs. A PEGylated liposomal formulation, GEM with Cromolyn (anti-inflammatory) displayed prolonged circulation and enhanced cytotoxic efficacy in BxPC-3 PC cell lines and BxPC-3 tumor-bearing nude mice [23]. In addition, Onivyde®, which consists of long-circulating liposomes composed of Irinotecan (a topoisomerase I inhibitor) combined with Fluorouracil (nucleoside metabolic inhibitor) and Leucovorin (folic acid antagonist), has been approved by FDA for PC treatment [78]. A similar liposomal formulation was also prepared [22], combining the chemotherapeutic drug Irinotecan and the alkaloid berberine,

which can be isolated from a variety of plants. *In vivo* and *in vitro* studies using PC models, demonstrated that the co-delivery of Irinotecan and berberine from liposomes results in improved efficacy and reduced intestinal toxicity compared with Onivyde® [79].

However, it does not come without the trials and tribulations experienced by any new formulations. Liposomes do exhibit some downfalls in their formulation development as an anticancer drug carrier. A drawback facing GEM loading into liposomes is the low drug loading efficiency of the process. The low pKa of GEM limits its influx through remote loading, which undermines the overall success of PC therapy. Several studies have explored alternative ways to improve the loading efficiency of GEM into liposomes, such as combining traditional remote loading with hypertonic loading and small volume loading [80]. To improve GEM loading into thermosensitive liposomes, GEM was complexed to copper (II) gluconate, assembling stable copper:GEM (1:4) complexes capable of superior GEM solubilization [81]. Despite their challenges, liposomes are paving the way for more efficient therapy. Their active targeting ability, responsiveness to stimuli, and controlled release make them a great contender for treating PC. These characteristics serve them well for chemotherapy delivery, there is also evidence of successful delivery of other important molecules, such as nucleic acids.

3.2. Nucleic acid delivery

Nucleic acids such as anti-microRNA (anti-miR), antisense oligodeoxynucleotides (oligos), and siRNA are used as RNA interfering molecules/drugs to repress gene expression. These molecules have the therapeutic capability of repressing disease-causing or disease-associated genes that do not respond to conventional therapeutics, i.e., monoclonal antibodies or small molecules [82]. Nucleic acids outfit therapeutic synergy plays an important tole to surpass compensatory effects observed in cancer cells following the knockdown of a target. Though RNA interference therapy is considered an alternative to chemotherapy in PC, several challenges are yet to be overcome, including lack of stability due to degradation by nucleases, low potency and poor cellular internalization at their targets, and off-target effects [83].

Although many drug delivery systems have been utilized for the successful delivery of nucleic acids, liposomes are most widely tested and applied to deliver nucleic acids. Despite the excellent transfection efficiency of liposomes to form complexes with siRNA, cationic liposomes

remain toxic due to the generation of reactive oxygen species (ROS) [84, 85]. On the other hand, nanoparticles of poly (ethylene glycol)-block-poly (l-lysine) (PEG-PLL) constructed for delivery of mutant K-ras siRNA *in vivo* and *in vitro* showed increased inhibition, migration and invasion of PC cells. Results also depicted an increase in PC cells in the G0/G1 phase rather than the S phase [86]. Similarly, the neutral DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) liposomes deliver nucleic acids in PC. DOPC is used to overcome toxicity issues associated with cationic nanoparticles. DOPC is natural, non-immunogenic, highly versatile phosphatidylcholine and physiologically more stable than DOPE liposomes. Apart from this, liposomes composed of phosphatidylcholine have demonstrated the capacity to efficiently transport drugs to target cells [82].

Atu027 is a siRNA lipoplex with the ability to knock down protein kinase N3 (PKN3) expression. Silencing PKN3 expression leads to inhibition of tumor metastasis and angiogenesis. AtuPLEX, a cationic lipoplex, was developed to carry Phase I clinical trial studies for ATU027. ATU027 response was substantially more significant in patients with PC [87]. Therefore, a Phase II clinical trial was conducted for ATU027 combined with GEM [88]. DCR-MYC or DCR-M1711 is a Dicer siRNA developed by Dicerna Pharmaceuticals. DCR-MYC targets c-Myc cells overexpressed in cancer. It causes silencing of c-Myc expression, inhibition of tumor metastasis and growth in different types of cancers, including PC. In Phase I clinical trial, treatment of DCR-MYC once a week for two weeks followed by a drug-free week was tested. Study results showed a significant safety profile and promising siRNA-based c-Myc targeting. The same was tested in the Phase II trial for Advanced Hepatocellular Carcinoma (HCC). Despite being the first siRNA targeting c-Myc that was evaluated clinically, the results did not meet the researcher's expectations, hence, the trial was terminated [89]. Xie *et al.* developed nanoparticles of miRNA/siRNA as a novel strategy to improve PC therapy by targeting both cancer cells and cellular interactions simultaneously within the tumor stroma as shown in **Figure 5**. These nanoparticles were developed to overcome the compromised EPR effect in PC [90]. To date, liposomes for co-delivery of miRNA and siRNA have not been developed.

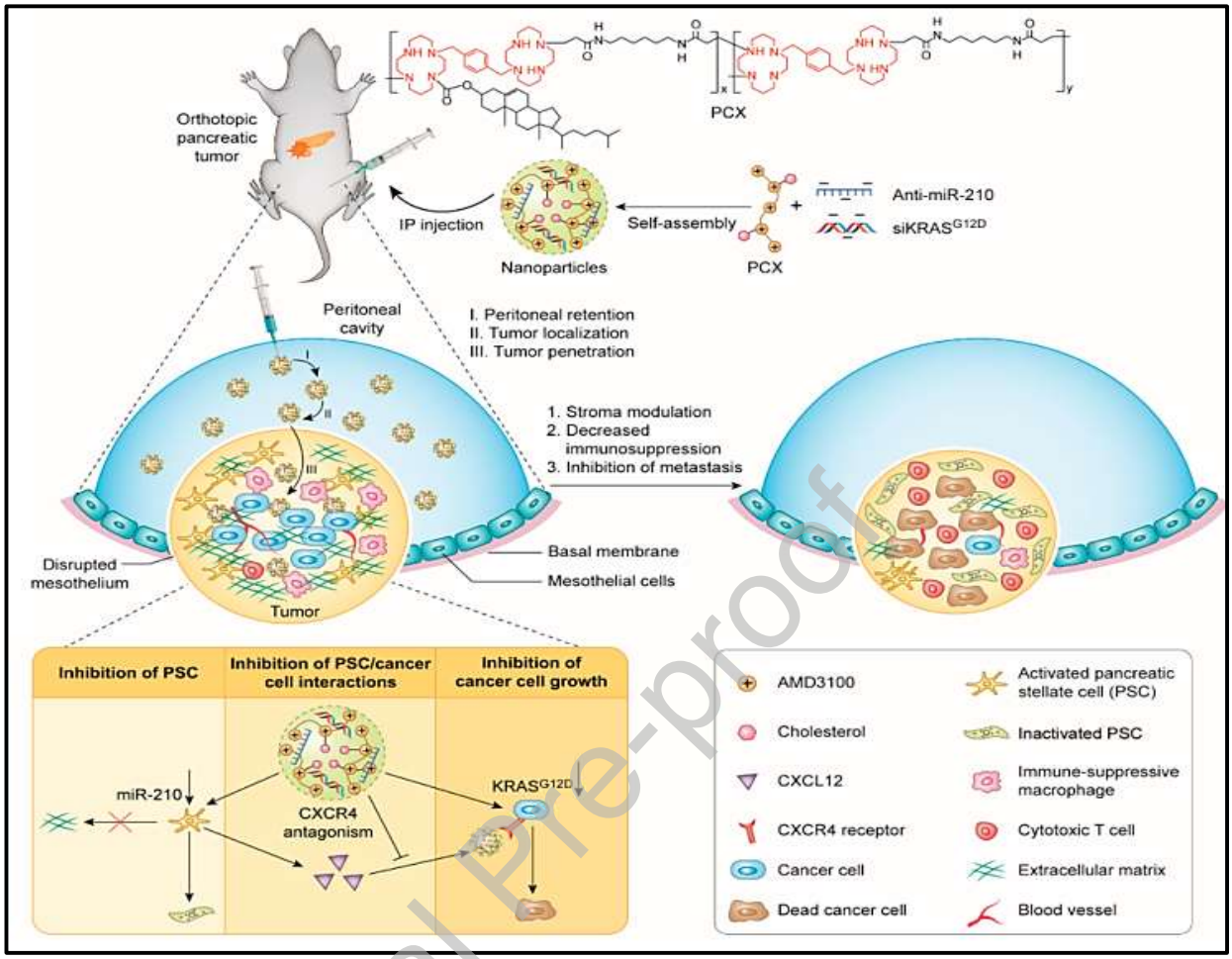


Figure 5. EPR-independent delivery of miRNA/siRNA in PC treatment. Herein, anti-miR-210 and siKRAS^{G12D} – loaded PCX nanoparticles were injected intraperitoneally in an orthotopic pancreatic tumor. Following injection, the PCX nanoparticles internalized deeply into the tumor and resulted in metastasis blockade, immunosuppression attenuation, and desmoplastic stroma modulation *via* cancer-stroma interaction inhibition and pancreatic stellate cells inactivation. Adapted with permission from reference [90], copyright ACS nano (2020).

One strategy to defeat the PC drug resistance and to be successful in targeted therapy is the application of noncoding RNAs especially siRNAs loading NPs including a liposomal system to inhibit the expression of oncogenes, regarding the fact that cationic liposomes are highly toxic and they produce a high level of ROS [91, 92]. In one study in 2019, a formulation of low-molecular-weight heparin-coated lipid-siRNA, aiming to inhibit Bcl-2 (LH-Lip/siBcl-2) was utilized in BXPC-3 cell lines and PC mouse models. To improve the NPs delivery, low dose

PTX-encapsulating PEGylated liposomes (PTX-Lip) have been used before the treatment, and in the end, remarkable inhibitory effects on tumor proliferation and metastasis have been observed [91].

Pathogenic activation of different signaling pathways, especially the KRAS pathway, is a reason for the growth of PC cells, metastasis, and low survival rate. Hence, new approaches are made to target these mutated pathways through applying the biological inhibitors by NPs [93]. In a recent study, Yu *et al*. utilized size-adjustable Thermo and fibrotic matrix-sensitive liposomes (HSA-BMS@CAP-ILTsL) encapsulating BMS-202 loaded albumin NPs (HSA-BMS) and mild hyperthermia in female C57BL/6 mice and Panc-2 cell lines. The study aimed to block immune checkpoints and the results represented hypoxia and metastasis attenuation, enhanced T-cells' activity plus interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) secretion [65]. Moreover, the application of PTX-loaded lipid-based CRISPR/Cas 9 (short guide RNA-sgRNA) cationic liposomes, functionalized by R8-dGR, on BxPC-3 cell lines and Ba1b/c nude mice resulted in hypoxia-inducible factor-1α (HIF-1α) suppression in addition to vascular endothelial growth factor (VEGF) and MMP-2/9 inhibition due to increased PTX efficacy [94].

3.3. Co-delivery of anti-cancer agents

Liposome nanocarriers, as mentioned, are desirable delivery vehicles for anti-cancer agents due to their passive and active tumor targeting abilities, resulting in enhanced therapeutic efficacy, reduced systemic toxicity, and circumventing drug resistance. However, passive or active targeting abilities alone are insufficient for accumulating high quantities of drugs at the tumor site. Nucleic acids inhibit or silence specific RNA gene expression. Nucleic acids conjugated with anti-cancer agents or drugs allow a sufficient amount of nucleic acid and drug to be delivered into the same population of cancer cells simultaneously, creating synergistic effects [95]. Nanocarriers supporting the combination of siRNA and anti-cancer therapeutics *i.e.,* chemotherapeutic agents, small molecule inhibitors or photodynamic sensitizers have been developed.

Similarly, another liposome was synthesized with GE-11 peptide antibody to co-deliver H1F1 α-siRNA and GEM. The therapeutic efficacy in PC was enhanced with remarkable apoptosis and reduction in tumor burden induced by GE-11 peptide conjugated GEM-siRNA liposomes [24]. Co-treatment of PTX and gene therapy was proven significant when Wand *et al*. demonstrated

its efficacy in treatment of PC using PTX and PEGylated cationic (PCat) siRNA liposome for targeting Survivin protein overexpressed in PC. It significantly enhanced tumor suppression efficacy and delayed tumor regrowth [96]. Kang *et al.* also reported similar results using MEK inhibitor in combination [97]. Using liposomes, insulin-promoter (IP) thymidine kinase and Ganciclovir (TK/GCV) co-delivery have been investigated for supressing PC cells in mice to overcome the toxicity issue associated with multiple sclerosis doses of TK/GCV. In conclusion, multiple cycles of liposomal IP-TK/GCV to ablate PC cells were achieved with minimal toxicity [16]. To target Myeloid cell leukemia 1 (Mcl-1) overexpression and overcome GEM resistance in PC cells, a combination of GEM and siRNA in liposomes was used as a novel strategy and proved a valuable tool for developing new strategies for PC therapy [21].

Few liposomal formulations have passed from Phase I to Phase II of clinical trials. Yet, only limited liposomal formulations have been used in clinical settings. In light of ATU027 Phase I clinical trial study results, Phase II studies in combination with GEM began in 2013. The study ended in 2016, with an enrolment of 29 subjects. The liposomal formulation of ATU027 with GEM for PC treatment was well tolerated and safe. Results also proved that a twice-weekly administration was better than once a week [88]. Liposomal combination therapy systems have also been utilized in PC. As GEM resistance is a severe obstacle to successfully treating PC, many researchers have emphasized overcoming this issue. Application of PEGylated pH-sensitive liposomes (PSL) carrying curcumin and GEM, in MIAPaCa-2 PDAC cell lines and Sprague-Dawley (SD) rats resulted in higher GEM concentration at the tumor site and more cytotoxicity [98]. In another study, Wang *et al.* investigated the efficacy of GEM-(KRAS-siRNA)-loaded apolipoprotein E3-based liposomes in PANC1 cells and mice models. The study exhibited a suppression in KRAS protein and related oncogenic signaling pathways, apoptosis induction, and attenuated cancer progression [99]. Co-delivery of GEM and siRNAs is also investigated by applying gemcitabine-Mcl1 siRNA encapsulating cationic liposomes *in vitro* and *in vivo*, causing a reduction in GEM resistance and more anticancer effects compared to drugs alone [21]. In addition, GEM combined with phosphatidylserine (PS)-targeting agent, saposin C-dioleoylphosphatidyl serine (SapC-DOPS), carried in lipid-based nanovesicles was studied and this combination delivery system indicated a higher survival rate in the treatment models [100]. In 2019, Chen *et al.* synthesized liposomes functionalized by TR peptide, loading autophagy inhibiting hydroxychloroquine (HCQ)-PTX (TR-PTX/HCQ-Lip). They demonstrated that these

liposomes could internalize and target the tumor site appropriately plus scavenging the autophagy in PC models [62]. Similarly, Madamsetty *et al.* developed PEGylated liposomes encapsulating epidermal growth factor receptor (EGFR) inhibitor and tyrosine-protein kinase Met (cMET) inhibitor (N19), combined with free GEM are formulations applied successfully by in AsPC-1 and Panc-1 cell lines plus female SCID mice models. The results proved that this co-delivery system attenuates cancer cell proliferation and augments the GEM sensitivity [101].

3.4. Cancer immunotherapy

In recent decades, immunotherapy, aiming to fight tumor progression by targeting the tumor microenvironment immune cells, has increased attention [102]. The tumor microenvironment comprises various cells like fibroblasts, extracellular matrix, chemokines, and immune cells, has strong immune-suppressive properties and plays a crucial role in cancer onset and invasiveness. As effective drug penetration in cancer therapy mainly relies on the tumor microenvironment, targeting and altering its components is the main purpose of immunotherapies; a broad term which includes cancer vaccines and the application of monoclonal antibodies (checkpoint inhibitors) [103, 104].Macrophages and dendritic cells which are antigen-presenting cells (APCs), could act as tumorgenic and anti-tumorigenic factors, therefore are also important targets in immunotherapies. Regulatory T cells (Tregs) are blockade effector T-cells, which consist of another immuno-suppressive component of the tumor microenvironment, being a possible target for immunotherapy strategies [104-106]. There are various immunotherapy targets such as chemokines and chemokine receptors, toll-like receptors, and overexpressed proteins which evoke an immunosuppressive response.

Immunotherapy acts like a cancer immunity cycle; APCs, i.e. dendritic cells, catch the cancer antigens (formed following cancer cell death), and stimulate the immature T-cells in lymph nodes. Then, tumor-specific cytotoxic T-cells find the cancer cells ultimately resulting in cancer cells being destroyed by effector T-cells *via* apoptosis. This results in more cancer antigen release and increased immune response [106]. Despite the enhanced popularity of immunotherapy in cancer treatment, these methods still have restrictions like short half-life, a low retention time of therapeutic agents in the tumor microenvironment, and they are not effective for defeating many solid tumors yet [104].

For immunotherapy to be successful, cancer antigens must be delivered to APCs properly and this could be more effective by utilizing nano-delivery systems. Examples of different 'nano-carriers' which have been formulated to deliver the drugs into the specific component of the tumor microenvironment are seen throughout literature for various cancer types, such as lipid-based [107, 108], polymer-based e.g. micelles[109], nanogels [110], and natural nanocarriers, e.g. exosomes. [111] Though each of these nano-systems can be used enhance the effectiveness of drugs with poor pharmacokinetics and the delivery of immunotherapy agents, here, liposomes are discussed and have been considered suitable for immunotherapy due to their tunable surface, safety, varied sizes, and the ability to be used for combinational therapies [103, 112]. The main advantages of liposomal formulations relays on their ability to encapsulate chemotherapy drugs and immunotherapy agents, thus increasing the lifetime / preventing the degradation of the biological materials and due to their chemical makeup, have favorable biocompatibility and physiological stability thus longer circulation time. [113, 114] In a study performed by Wan *et al*. they formulated a liposomal formulation consisting of 1V209, a Toll-like receptor agonist, conjugated to cholesterol covalently (1V209-Cho-Lip) and investigated its effects in Pan02 murine pancreatic ductal cancer cell lines **(Figure 6).** The results demonstrated activation of dendritic cells in the tumor microenvironment and sentinel lymph nodes which strengthens the immune response. Additionally, 1V209-Cho-Lip had a hindering effect on cancer recurrence due to memory $CD8^+$ T-cell generation and more effective therapeutic delivery to lymph nodes than 1V209 [115].

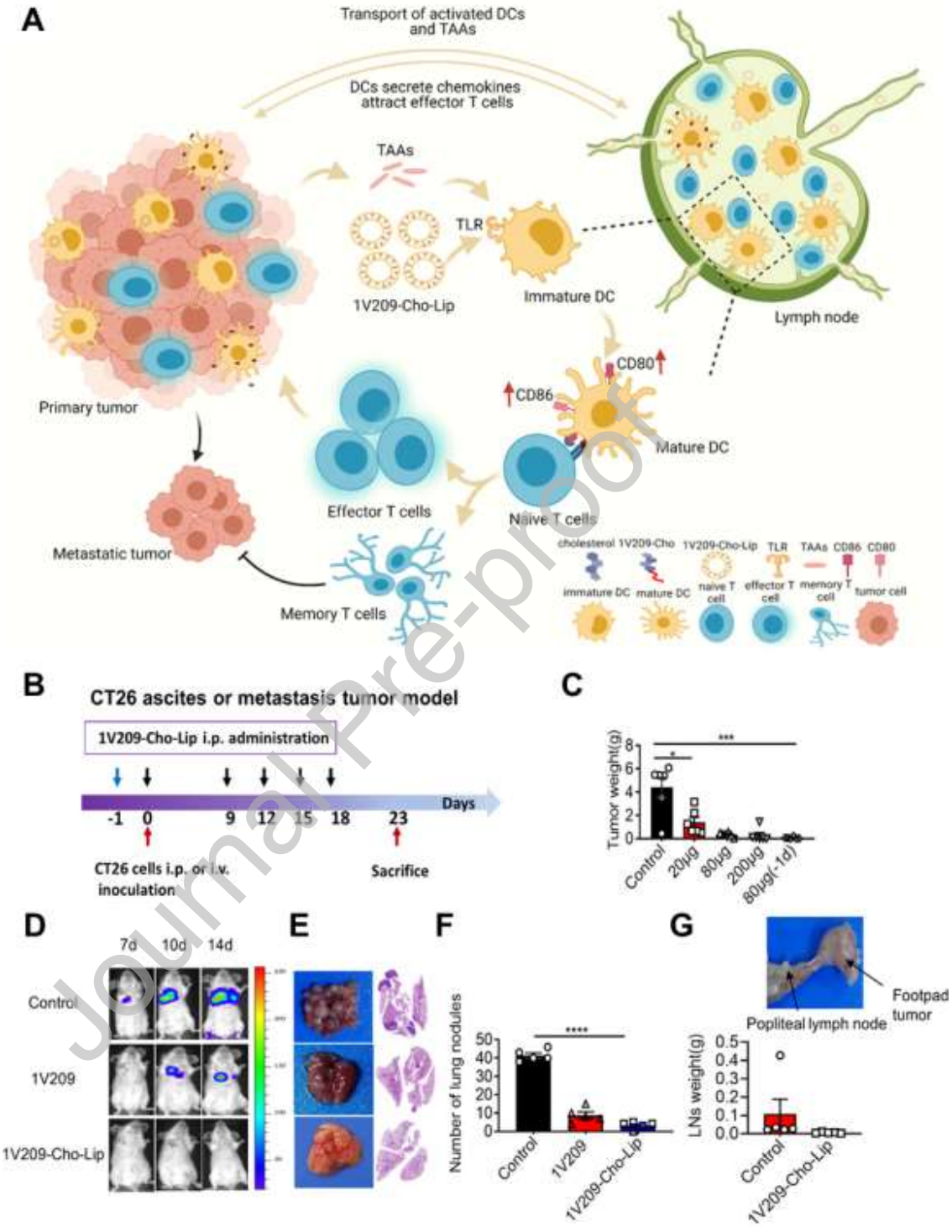


Figure 6. A Schematic illustration indicating the tumor growth and metastasis hindering through innate and adaptive immunity evoke and immune memory effects employing Node-Targeted Cholesterolized TLR7 Agonist Liposomes **(A)**. CT26-bearing Balb/c mice were injected with 4 mg kg^{-1} 1V209-Cho-Lip

on day −1 or 0, 9, 12, 15, 18 and the mice were sacrificed on day 23 **(B)**. Average weight of post-dissection tumors after treatment with PBS control and 1V209-Cho-Lip **(C)**. Representative of lung tumor signals by IVIS on 7, 10, and 14 days after receiving the CT26 cells i.v **(D)**. Image of post-dissection lungs, H&E and the numbers of lung nodules after harvesting lungs on day 23 **(E, F)**. Average popliteal lymph nodes weight at the end of treatment in the CT26 lymphatic metastasis model **(G)**. Adapted with permission from reference [116], copyright Nano Lett (2021).

Targeting tumor-associated macrophages (TAMs) with clodronate liposomes also saw an increase in $CD8^+$ T cell infiltration in tumor models, which corresponds to an anti-tumor affect, resulting in suppressed tumor growth. Macrophages are known to prevent CD8+ T cell infiltration. Though it was found that T cells were activated when PC tumors were treated with clodronate liposomes, the macrophages in the tumor site were not depleted. The authors suggest that incorporating CCR2-neutralizing antibodies alongside targeting macrophages may be required to increase the efficiency of clodronate liposome for targeting proliferating macrophages at the tumor site [117]. Additionally, it is widely recognised that one of the most relevant chemokines/receptor interactions contributing to pancreatic cancer tumour growth is the CXCR4/CXCL12 pathway. Chemokine receptor CXCR4 is highly expressed in pancreatic tumours (produced by B cells) and its chemokine counterpart CXCL12, produced by CAFs, are present on lymph nodes and other places where pancreatic tumor cell metastasizes [118, 119]. These pair also work to maintain the tumour microenvironment to help tumour cells survive, through interactions with other non-malignant cells which lead to immunosuppression [120]. Though CXCL12 has another receptor CXCR7 which plays part in cancer progression, CXCR4 is well-defined to be associated with poor prognosis in PC. The CXCL12/CXCR4 axis brings immune cells to the tumour environment therefore can be seen as contributing to cancer cell metastasis and proliferation [120]. Studies continue to look into the effects of CXCR7 and the CXCL12/CXCR4/CXCR7 axis in cancer [121-123]. Further, Zhang *et al.* confirmed there was an increased expression of CXCR4 in pancreatic tumour environments and also identified a correlation between the expression of CXCR4 and an increased in biomarkers VEGF-C and Ki-67, concluding they most likely contribute to the metastasis and growth of pancreatic cancer [124]. Throughout literature, studies have reported immunotherapies targeting the CXCL12 cytokine or CXCR4 receptor for pancreatic cancer. In fact, Plerixafor is an approved CXCR4 antagonist and currently used throughout clinical trials. Another study concerned with

biomarkers for pancreatic cancer prognosis identified, through transcriptomic analysis, chemokine CXCL10 was an important contributor to pancreatic tumour progression. The analyses gave insight to correlations between CXCL10 , hence pancreatic cancer progression and various components of the immune system [125]. CXCL10 alongside CCL21 chemokines act (with their receptors expressed on tumour cells) as important factors in cancer-associated pain. These facilitate the migration of cancer cells to neurons, resulting in greater pain in patients (with resectable tumours) associated with greater expression of the corresponding receptors, CXCR3 and CCR7 [126]. Therefore, recognising CXCL10 is a valuable marker for pancreatic cancer prognosis.

Arguments have been made throughout literature that these aforementioned chemokines and hence their receptors could be potential targets for managing pancreatic tumour growth, metastasis and pain. There are already approved inhibitors being used widely. Checkpoint inhibitors such as nivolumab, cemiplimab and pembrolizumab and plexifor, a CXR4 inhibitor, are increasingly present throughout current clinical trials for pancreatic cancer treatment and are usually given in combination with a range of chemotherapy drugs, however to the best of our knowledge no liposomal formulated immunotherapy treatments are seen in active clinical trials. A current clinical trial (clinicaltrials.gov, NCT02907099) is investigating the immune response of patients with metastatic pancreatic cancer upon treatment with both monoclonal antibodies and CXCR4 inhibitor. As well as, including Onyvide in combination with these, is seen in clinical trial (clinicaltrials.gov, NCT02826486) with pembrolizumab (PD-1 inhibitor) and Motixafortide (CXCR4 inhibitor) [127]. These immunotherapies are emerging in clinical trials, many in combination with chemotherapy drugs, including chemokine inhibitors, (clinicaltrials.gov NCT05465590,) and anti-PD-1 antibody (clinicaltrials.gov, NCT03989310). Therefore, it will be no surprise to predict that liposomal formulations with immunotherapy components and chemotherapeutics will arise in the near future.

TLR7 also appears as a target in a recently reported study involving both chemo- immuno-therapy ultilizing a ‘silicasome’ (a lipid-bilayer coated silicia nanoparticle) to deliver a toll-like receptor agonist and chemotherapeutic, with favourable outcomes [128]. This was achievable as TLRs are lipid soluble and irinotecan was captured within the pores of the silica, therefore this chemo-immunotherapy ‘co-delivery’ could be feasible with liposomes due to their ‘fatty’

exterior being able to carry proteins and biological matter and ability for drug capsulation interior.

Exosome-liposome hybrid nanoparticles are newly being explored for immunotherapy in other cancers, thus may translate to pancreatic cancer in the near future. Multifunctional nanosystems are becoming more attractive across the various therapies. A liposomal formulation combining immune checkpoint inhibitor alongside photothermal therapy has been cleverly designed to enhance immune responses saw success in reducing tumor growth in pancreatic cancer [65]. Again, photothermal therapy coupled with immunotherapy to treat pancreatic cancer, this time an inorganic nanocarrier achieved promising anticancer immune response [129]. Thus, showing the benefit of incorporating photothermal techniques, which is elaborated in the next section.

3.5. Photothermal and photodynamic therapy

Another promising strategy for cancer-targeted treatment is photothermal therapy (PTT). PTT has been gaining more attention during the past decade due to its lower toxicity and ability to deliver the drugs to the same tumor site more effectively with controlled release into the deeper parts of the tumors *via* exposure to light. Photothermal therapy uses a photothermal therapeutic agent (PTA) and radiation. The release of therapeutic agents encapsulated in different nanocarriers can be affected by/ programmed to respond to a stimulus, e.g., light or temperature.
Light wavelengths across the spectrum, from ultraviolet to visible light and near-infrared (NIR), could be applied in photothermal therapy. Due to less cytotoxicity and the aim of delivering the drugs into deeper sites of the tissues, NIR light (wavelength 650-900 nm) is more appropriate as it can penetrate further. The characteristics of PTAs are also significant when considering accumulation into specific cancerous tissues. The PTAs should be able to absorb the light and convert it into heat. For this reason, PTAs must be non-toxic with high NIR light absorption potency. Various PTAs including small organic molecules and inorganic nanomaterials have been formulated for photothermal therapy, in which the PTAs must be able to augment the cell temperature to 42-45℃ (in 15-60 minutes) to destroy the cancer cells [130, 131].
In photothermal therapy, PTAs enclosed in a nanocarrier, convert the light into heat and induce hyperthermia in lysosomes following exposure to NIR light. This causes cytoplasmic membrane damage, increased influx of Ca^{2+} into the cell, and cell death induction. Researchers have shown that both apoptosis and necrosis could happen due to photothermal therapy, but apoptosis

preferentially occurs after the application of low-energy radiation. The main target for photothermal treatment is usually the tumor microenvironment because of its dense matrix which acts as a barrier against drug penetration. Disruption of the ECM due to hyperthermia, results in the more effective delivery of drug-loaded nanoparticles and enhanced tumor sensitivity to chemotherapy. [130] However, the major drawback of photothermal therapy is the disruption of surrounding healthy tissues from heat escape. To overcome this, photothermal absorbers including indocyanine green dye could localize the produced heat in the tumor site [132].

Until now, different kinds of nanoparticles with high absorption potency in the NIR light wavelength, such as gold nanoparticles, graphene oxide (GO) nanosheets, and carbon nanotubes have been utilized as a photothermal transducers. Liposomes are also suitable for this kind of therapy [131, 132]. In another study, the application of NIR-sensitive dye (IR 780) combined with sunitinib (an anti-angiogenic agent)-encapsulated liposomes (Lip-IR 780-sunitinib) in 4T1 cell lines and mice bearing 4T1 tumors followed by exposure to laser irradiation resulted in effectively controlled release of sunitinib at the expected site and increased anti-angiogenic effects [133]. In the context of PC therapy, Yu *et al*. formulated a liposome-based nanocarrier to overcome the immunotherapy drawbacks by modifying the ECM density. In this study, they loaded a complex of an immune checkpoint blockade (BMS-202) and the human serum albumin (HSA-BMS) into fibroblast activation protein-α (FAP-α) responsive (CAP) and thermosensitive liposomes (TSL) including IR-780 (HSA-BMS.CAP-ITSL) and applied it for mild hyperthermia therapy on pan02 cell lines and mouse pan02 PC models. The results demonstrated that due to the release of HSA-BMS *via* FAP-α activity and NIR laser exposure, there had been increased secretion of TNF-α and IFN-γ followed by improved T-cells' activity which led to cancer cells' proliferation and metastasis attenuation (**Figure 7**) [65]. Again, innovative therapeutic strategies are being employed to combat PC. Photothermal delivery proves successful in animal models, further research for translation into a human is needed.

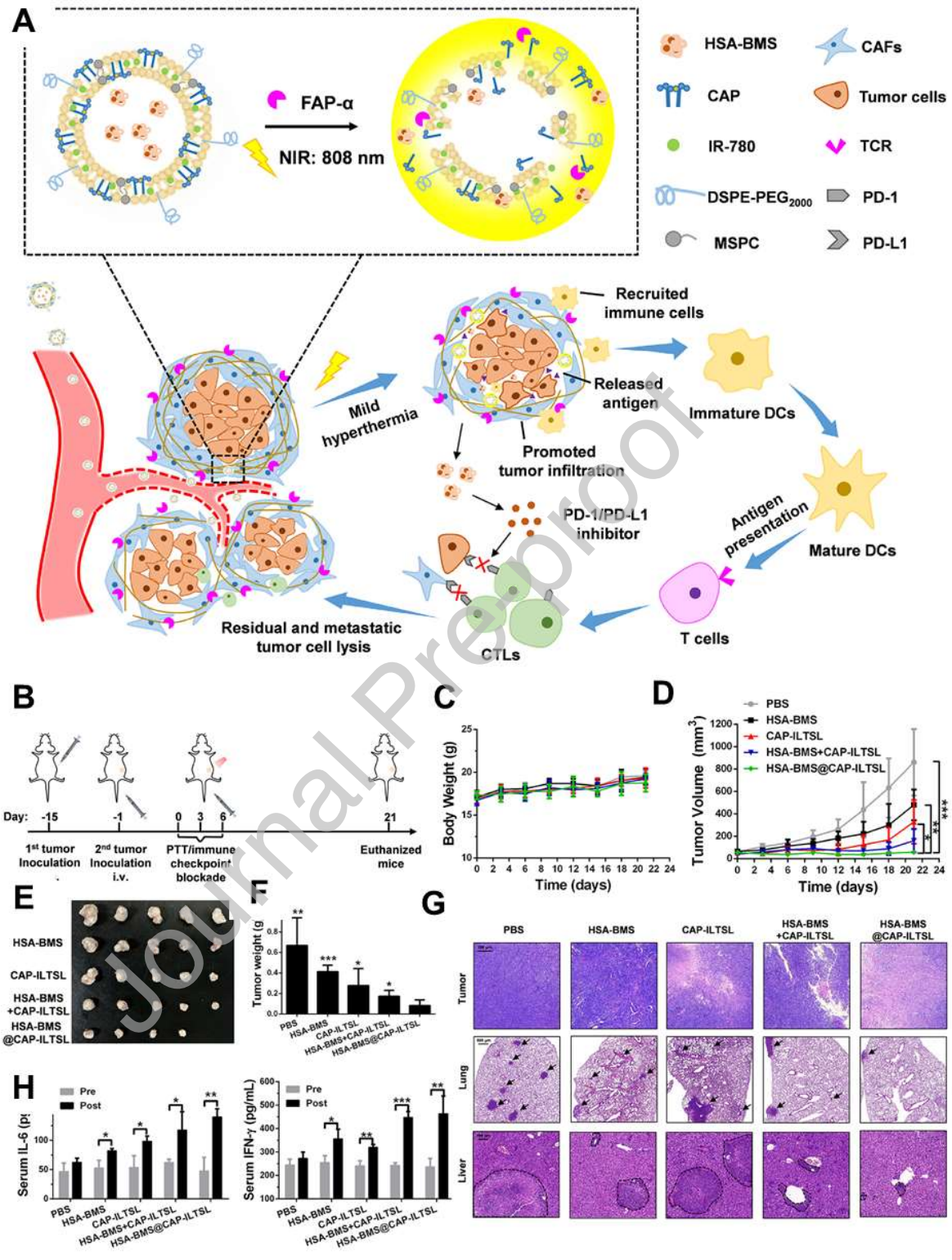


Figure 7. This schematic illustration shows the application of mild hyperthermia plus FAP-α responsive size-adjustable nanoparticles (HSA-BMS@CAP-ILTSL) for combinational treatment of photothermal therapy and immunotherapy **(A)**. Subcutaneous tumor and artificial metastatic induction and treatment in Pan 02 female C57BL/6 mice models which were under NIR laser irradiation after 4 h treatments **(B)**. The curves of mice weight (n = 5) and the tumor growth **(C, D)**. Representative images of tumors **(E)**. Mice tumor weights (n = 5) and H&E staining images of tumors, lungs and livers (arrows and dashed lines indicate the metastatic areas) **(F, G)**. Serum levels of cytokine IL-6 and IFN- γ measured by Elisa kits after the treatment (n = 3) **(H)**. Adapted with permission from reference [65], copyright Acta Biomaterialia (2021).

Photodynamic therapy is a non-toxic and non-invasive strategy, employed for cancer therapy including PC. In PDT, upon light irradiation, the administered photosensitizers (e.g. indocyanine green, riboflavin, curcumin, hematoporphyrin) results in the production of cytotoxic ROS, which which destroys the tumor by acting in three main targets: cancer cells; the tumor microvasculature; and elements of the host immune system [134, 135]. In the context of PC treatment, several studies using PDT have been performed. Particularly, PDT has been previously employed in randomized clinical trials and has been shown to successfully induce necrosis in the irradiated regions of PC tumors [136, 137]. For instance, the intravenous administration of the photosensitizer verteporfin in 15 patients with locally advanced PC on a randomized Phase Ib/II clinical trial, resulted in tumor necrosis induction without adverse effects, after laser irradiation (690 nm) during 60 minutes to 90 minutes [136, 137]. However, the clinical use of photosensitizers is still cumbersome owing to their hydrophobic nature and poor stability in physiological conditions. Therefore, LPs have been formulated for more effective chemophototherapy (combination of chemotherapy with photodynamic therapy) approaches [138, 139]. For instance, the photosensitizer 2-[1-hexyloxyethyl]-2-devinyl pyropheophorbide-a (HPPH) was conjugated with the phospholipid lysophosphatidylcholine to generate a porphyrin-phospholipid (PoP), further incorporated into LPs capable of being permeabilized with near infrared light. The obtained PoP-based l LPs were loaded with DOX, enabling the light-triggered release of this chemotherapeutic drug. During in vivo studies with PC models, DOX-loaded PoP-based LPs demonstrated enhanced liposomal DOX accumulation at tumor site and induced tumor vascular permeability after near-infrared laser irradiation (665 nm). In contrast to stable standard liposomal formulations, the administration of leaky PoP-based LPs resulted in enhanced DOX bioavailability in laser-irradiated tumors. In a different study,

PoP-based LPs s [22, 140-144]. In a different study, PoP-based LPs encapsulating irinotecan showed over 90% drug release after laser (665 nm) irradiation, increased drug influx into the neoplastic tissue, and significant tumor destruction in PC mice models [145]. This antitumor photodynamic was corroborated by *in vitro* and *in vivo* PC models with PoP-based LPs loaded with cabazitaxel combined with light laser irradiation [146].

A recent study reported the development and utility of anti-EGFR monoclonal antibody (cetuximab)-targeted LP, presenting it as a nanocarrier for simultaneously carrying a photosentizer (i.e. lipidated benzoporphyrin derivative) within the lipid bilayer and the chemotherapeutic drug irinotecan in the aqueous core for the concomitant PC treatment and associated desmoplasia mitigation. Moreover, the attenuation of collagen density (by > 90%) and enhanced collagen nonalignment (by > 10^3 fold) observed after the treatment with this targeted photoactivable multi-inhibitor liposomal formulation is a promising result for patients' survival improvement [147].

4. Stroma remodelling therapy

Until now LP-targeted drug delivery systems have been widely discussed. However, manipulation of the tumor microenvironment to aid the efficiency of chemotherapeutics is another therapeutic strategy currently of interest.

Inefficient tumor penetration is a huge challenge for drug delivery, especially for desmoplastic cancers, like PC. By modifying the components of the tumor microenvironment, i.e., disrupting vital cancer-progressing signalling pathways, it is possible to "remodel" the tumors complex makeup to create an environment that aids the enhancement of cancer treatments. PC's characteristically dense stroma comprises ECM and cancer-associated fibroblasts (CAFS). There is evidence of potential benefits to "altering" the tumors "chemical makeup", i.e., targeting these cancer progressing mechanisms alongside the delivery of chemotherapeutics. The small molecule JQ1 inhibits the BET family of proteins and has been used to hinder tumor growth. It was found that when treated with JQI, there was a diminished desmoplasia growth with PC patient-derived tumor xenografts, which would overcome a big challenge for drug penetration. JQ1 was subsequently administered alongside GEM to determine if there was an improved effect due to 'stroma remodeling', which was indeed seen by a decrease in tumor growth c.f. GEM

alone. Therefore, highlighting the synergistic benefit of combination cytotoxic agents and remodeling of tumor environment. [148]

In other research, LPs loading extracellular matrix-degrading enzyme collagenase type-1 (collagozomes) on PC stroma-remodelling and drug delivery capacity was investigated in C57BL/6 mice through the intravenous injection of PTX NPs, 24 hours after collagozomes administration. The results showed that collagosomes enabled a sustained enzyme release at the treatment site and induced significant ECM degradation, thereby improving drug diffusion and increasing PTX uptake and cytotoxic activity on tumor cells [115].

Another attempt of modifying the stroma was targeting the HGF/c-MET pathway. Hepatocyte growth factor (HGF) and its receptor c-MET are significant in PC progression and metastasis[116]. Pothula *et al.* reported that tumor progression was substantially reduced when inhibiting the pathway and incorporating AMG102 (a monoclonal antibody against human HGF) alongside the anticancer agent, GEM, within an orthotopic PC mouse model. Thus, remodeling the stroma led to increased efficacy of the chemotherapeutic [116]. LPs have been tailored to "remodel" other stroma-possessing cancers, to aid the efficacy anticancer drugs. A study attempting to overcome the challenge of poor drug penetration in breast cancer employed surface-modified '4T1 cell membrane protein chimeric LPs' with Neutrophil elastase (NE). NE was chosen because it can interact with elastin and collagen 1, vital to the tumor ECM. The LPs were assessed *in vivo* with a 4T1 orthotopic breast cancer mouse model. Though there was little effect on tumor growth, the authors found that binding NE to liposomes had a notable impact on the ECM than free NE. Therefore, the authors administered their NE LPs with chemotherapeutics to determine if it would enhance the therapeutic effect. Significant findings showed that pre-treating tumor-bearing mice with their NE LP formulation prior to treatment with chemotherapeutics decreased tumor growth compared to chemotherapy alone [149]. Winkler and Chen *et al*. provide excellent detailed overviews of the complex nature of the tumor microenvironment and the various ways in which the ECM can be remodelled to aid therapies [150, 151]. There is also evidence of other nanosystems employed for synergistic remodelling and therapeutic effects. A polymeric micelle was loaded with cyclopamine, an inhibitor targeting CAFs, and anticancer agent, PTX, namely M-CPA/PTX. Targeting the SHh pathway modulates the stroma while applying a cytotoxic agent to hinder tumor growth. These loaded-polymer-micelles were evaluated in orthotopic human PDAC xenograft models; M-CPA/PTX suppressed

tumor growth as did the micelle with PTX alone. Therefore, the inhibitor did not increase the effect of the cytotoxic agent, however a 27% reduction in the deposition of collagen was reported for the combined micelle, owed to the cyclopamine in the formulation. M-CPA/PTX (compared to M-PTX) showed significantly decreased relapse after treatment. HA and LOX were reduced by M-CPA/PTX, which is positive due to their role in stroma formation. [152]

One excellent example of a study that uses multiple strategies to have an enhanced combined effect exhibits chemotherapy delivery with stimulus responsive LPs with bound targeting agents and an inhibitor to remodel the tumor microenvironment. This LP was developed to load and deliver DOX to PC cells. DOX-loaded thermosensitive LPs, functionalized with cyclic RGD pentapeptide and integrin inhibitor cilengitide (MC) (MC-T-DOX) were administered intravenously in males' nude mice bearing BxPC-3 tumors. MC was released after the action of type 1-MMP (MT1-MMP), which is widely expressed on tumor endothelial cells, improving blood profusion and enhancing the accumulation of MC-T-DOX in tumor tissues **(Figure 8)** [14]. This study showed modulation of tumor vasculature together with enhanced chemotherapy delivery *via* a heat-triggered mechanism can act synergistically towards improved chemotherapy delivery to PC. This formulation highlights the importance of not-only targeted drug delivery but creating a more suitable environment to aid therapeutic efficacy.

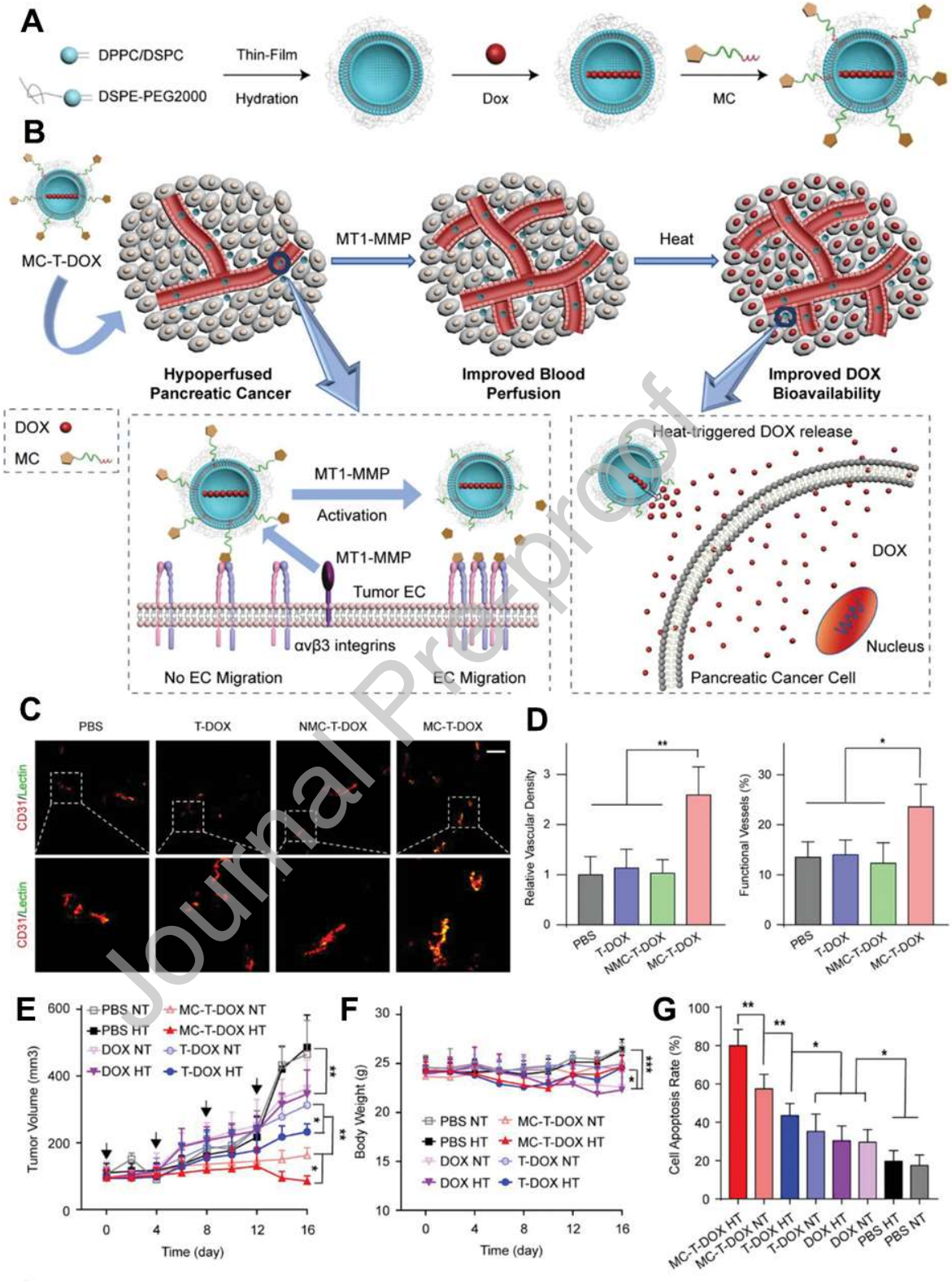


Figure 8. A schematic illustration representing the preparation of MC-T-DOX, a doxorubicin (DOX) loaded smart liposome **(A)**. MC-T-DOX improves the tumor blood perfusion and drug delivery in PC. Low density MT1-MMP-activated cilengitide (MC) is modified onto DOX-loaded thermosensitive liposomes (TSLs), yielding MC-T-DOX. Following IV injection of MC-T-DOX into the hypo perfused pancreatic tumor in BxPC-3 mice models at a low dose of cilengitide, every 4 days for four cycles at an identical DOX dose of 3 mg kg^{-1}, MT1-MMP on tumor ECs could activate MC-T-DOX to release cilengitide, which then promotes ECs migration and angiogenesis, resulting in higher levels of MC-T-DOX accumulation and distribution in the tumor site which would be improved after subsequent heat-triggered DOX release, in the interstitium **(B)**. Representative photos of tumor and functional blood vessels plus blood density quantitative analysis and functional blood vessels percentage **(C, D)**. Tumor growth curves (the arrows indicate the time points for treatment [137]) **(E)**. Body weight changes during the experiment (n = 6) and quantitative analysis of cell apoptosis (n = 6) **(F, G)**. Adapted with permission from reference [14], copyright Advanced Science (2020).

Another system that combines active targeting, stroma remodeling and drug delivery which responds to the acidic tumor microenvironment, is presented by Chen *et al.* This liposomal treatment was designed to have multifunctional capabilities of destructing stroma formation whilst utilizing PTX to have therapeutic anticancer effects. They combine cRGD peptide with the TH peptide, which they refer to as TR peptide, to achieve integrin αvβ3 targetting and pH activation. Together with cytotoxic agents, these are loaded onto the LPs to give a formulation called TR-PTX/HCQ-LP (TR peptide- PTX/ hydroxychloroquine-LP). *In vivo* performance of these highly decorated LPs was assessed using heterogenetic and orthotopic xenograft BxPC-3 tumors; mice treated with TR-PTX/HCQ-Lip exhibited more significant anti-tumor effect significantly decreased tumor mass compared to free drug and variations of drug/hydroxychloroquine / peptide-loaded LP formulations. Additionally, TR-PTX/HCQ-Lip was able to diminish autophagy and stroma fibrosis shown in **Figure 9** [94]. These are only a few examples of formulations designed with components to have a synergistic anticancer effect that promise targeted liposomal therapy for PC.

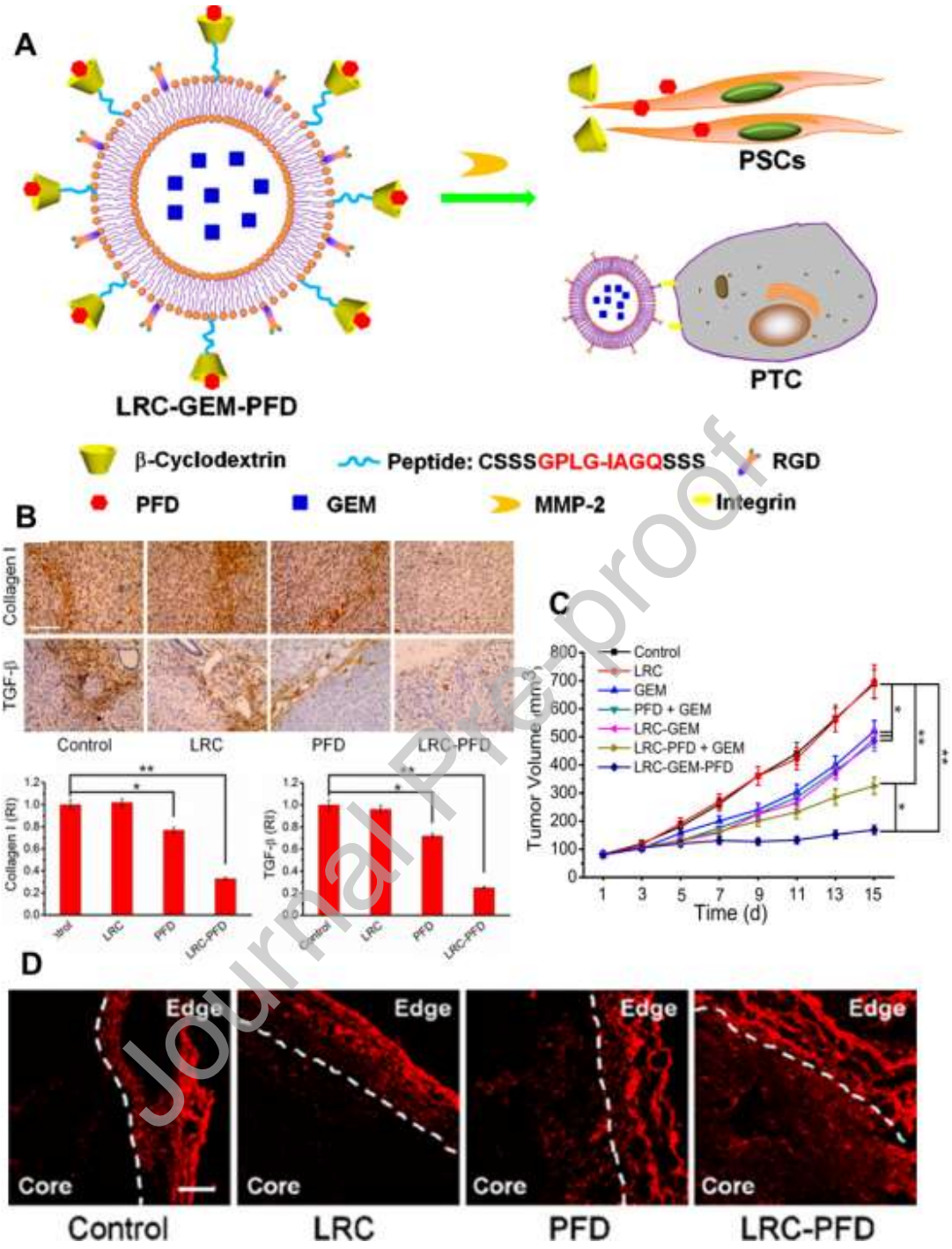


Figure 9. Schematic illustration of the nanocarrier LRC-GEM-PFD in which, PFD is inserted into the hydrophobic chamber of β-CD, and the liposome encapsulates GEM. Cleavage of LRC-GEM-PFD by MMP-2 results in the regulation of the PSCs by PFD and recognition of the PTCs by GEM-loaded liposome **(A)**. Expression levels of Collagen I and TGF-β in tumor tissue after LRC, PFD, and LRC-PFD treatment. (**Up:** IHC stained slices images. **Down:** Statistic quantitative analysis of collagen I and TGF-β

from IHC results) **(B)**. Tumor volume curves of PSCs/Panc-1 pancreatic tumor in mice models treated by various GEM formulations with a GEM dose of 20 mg/kg **(C)**. Rhd penetration into the PC tissues (Panc-1 and PSCs coimplanted) following IV injection of different PFD formulations (Red: Rhd) **(D)**. Adapted with permission from reference [12]**,** copyright ACS applied materials & interfaces (2016).

5. Ferroptosis based pancreatic cancer therapy

Amongst several therapeutic strategies that have been suggested, has been explored is the non-apoptotic cell death. Many studies have discussed the deliberate "inducement" of ferroptosis, as a beneficial cancer cell killing strategy [153, 154]. This is a viable strategy since PC cells are resistant to apoptosis. The majority of patients with PC have KRAS gene mutations that aid ferroptosis [154]. It has been expressed that deliberate inducement of ferroptosis could be exploited as a way to treat PC. Ferroptosis is a type of non-apoptotic cell death governed by iron and works to reduce the protein GPX4 and thus leads to an accumulation of lipid ROS. There are three common ways in which ferroptosis has been activated with the main targets for the induction of ferroptosis are GPX4, Xc system and iron. It has been reported that excess iron ions can trigger the formation of ROS, which can result in cell death. Therefore, excess iron ions can be exploited in the killing of tumor cells. GPX4 is an enzyme protecting cells against peroxidation. Many small molecules can inhibit the function of GPX4 and lead to the accumulation of these ROS [154]. The Xc transporter plays a key role in the uptake of cystine for redox homeostasis in PC tumor cells [155]. Commercial drugs have been utilized to induce ferroptosis in PC. Artesunate, marketed as an anti-malarial, has been shown to promote ROS formation through oxidative degradation of lipids. PC cell lines, BxPC-3 and Panc-1, were used to determine the hallmarks of ferroptosis by artesunate and found it was dependent on iron and induced cell death through ROS generation. Artesunate was found to cause cell death in PC cells presenting resistance to apoptotic pathways [155]. Thus, further investigation into the potential for PC treatment of this commercially available drug, perhaps on liposomal combination therapy, would be beneficial.

6. Liposomal Formulations in Clinical Trials

There is large presence of irinotecan liposomal therapies in recent clinical trials. This is no surprise after FDA approval of Onyvide® in 2015. We see that there are numerous investigations into the safety and efficacy of Onyvide® with other drugs in combination, however there is very little emerging liposomal formulations in current clinical trials. Studies, active at time of reporting, of current treatments for pancreatic cancer with liposomal formulations in clinical trial are highlighted in **Table 2**. Predominately LP formulated chemotherapy drugs are seen in current clinical trials; gene therapy nor immunotherapy liposomal formulations are seen in any current clinical trials. However, a liposomal formulation for gene therapy using BikDD was proposed for Phase 1 trial in 2015 then later withdrawn due to stability issues, (clinicaltrials.gov, NCT00968604).

Table 2: Summary of some recent and active clinical trials involving liposomal treatment for pancreatic cancer.

Condition	Treatment	Study Phase	County	ID # (clinicaltrials.gov)
Locally Advanced or Metastatic Pancreatic Cancer	Irinotecan Liposome	3	Germany	NCT03468335
Metastatic Pancreatic Cancer	Irinotecan liposome Oxaliplatin 5-Fluorouracil, Leucovorin	3	Various Global	NCT04083235
Locally Advanced or Metastatic Pancreatic Cancer	Irinotecan liposome, 5-Fluorouracil, Leucovorin	3	China	NCT05074589
Advanced Pancreatic Cancer	Mitoxantrone hydrochloride liposome	2	China	NCT05100329
Advanced Pancreatic Cancer	paclitaxel liposome S-1.	4	China	NCT04217096
Non-resectable Primary Pancreatic Tumours	Liposomal Doxorubicin (ThermoDox®) (and Focused Ultrasound)	1	United Kingdom	NCT04852367

7. Challenges, future perspective, and clinical translation

Liposomal formulations encapsulating anti-cancer therapeutics are well established and have been widely recognized for their beneficial properties for a long time. The challenge of poor efficacious chemotherapeutics was overcome by incorporating them in LPs, evidenced in bountiful pre-clinical studies. Nonetheless, liposomal delivery of such chemotherapeutics still poses several challenges according to the gap between the results of pre-clinical studies and clinical translation of l LPs [156]. In liposomal delivery, efficient tumor penetration and cellular internalization depends on LPs' physicochemical properties (i.e. including their size, charge, lipid composition, and number of lamellae), which poses a challenge around formulation development.

Lipid content and surface charge modification are examples of strategies that can be used to improve the delivery effectiveness of the liposomal formulations, minimize toxicity and avoid clearance by the RES, resulting in enhance the circulation time and tumor-specific accumulation [9, 157]. For instance, it has been shown that that *in vivo* pre-administration of positively charged LPs followed by gold nanorods treatment minimized RES clearance, prolonged circulation time, and improved tumor uptake of gold nanorods [158]. However, vital consideration is that *in vivo* behaviour of LPs using animal models differs from clinical translation in humans, therefore further extensive studies are needed. Moreover, liposomal formulations can be functionalized with PEG as a stealth coating to avoid rapid excretion based on surface properties. For instance, PEGylated liposomes, have been demonstrated to prolong GEM plasma half-life and intratumoral drug concentration, to the extent that a 10-fold lower drug dose could be applied to achieve in vivo tumor inhibition, without signs of systemic toxicity [159]. However, recent reports suggest that PEGylation of liposomal formulations can compromise their efficient interaction with the desired target, due to steric hindrance imposed by PEG chains, hence, hampering endocytosis process and the long circulation time [156, 160-162]. Ishida *et al.* summarized findings of an extensive investigation on PEG-induced immune responses [143]. They are exemplifying the need for extensive research efforts before clinical translation. Several findings have revealed the failure of many liposomal formulations in clinical trials against PC that worked perfectly in pre-clinical studies [144]. Results demonstrated the alteration in therapeutic outcomes in PC and that *in vivo* animal or xenograft models are incompetent to predict the therapeutic efficacy of formulation in advanced stages or metastatic PC [163, 164].

The protein corona (proteins interacting with LPs forming aura around it) is another important factor to consider as it influences LP's fate *in vivo* and targeted delivery of drugs and nucleic acid [156]. Nucleic acid delivery also poses biological challenges including degradation by exogenous RNAs, high negative charge, hepatic clearance, and high molecular weight [165, 166].

The challenges posing cancer treatment, begins with the lack of early-stage diagnosis. Cancer symptoms are usually not revealed until they are locally advanced or metastasized, in which state even the surgical resection cannot be successful and palliative care is the best option. Despite ongoing advancements in cancer therapy, the survival rate of PC is still low, and there is an urgent need for more beneficial approaches and clinical trials. Current frontline therapies in PC treatment, i.e. yet, even with the advancement in delivering strategies of chemotherapeutics or nucleic acids, PC has shown resistance to these therapies and is projected to become the second deadliest cancer in the US by 2025 [167]. Poor response to the standard treatments, especially chemotherapy, contributes to the poor prognosis of PC. Unsuccessful chemotherapy can be attributed to multi-drug resistance; usually observed in GEM treatment, leading to decreased drug efficacy. Scientists have utilized combination therapy and anticancer agent-loaded nanoparticles including LPs, to overcome drug resistance but the results have not been hoped full and there were no significant changes in survival rate [10, 168]. On top of this, unfortunately, to date, no clinically specific targeting agents are available to target genetic mutations that occur in 90% of cases [169].

Together, with poor treatment efficacy, the genetically mutated signaling pathways including KRAS (present in more than 90% of cases), P53, and CDK2NA, plus immunosuppressive property of pancreatic tumor microenvironment are crucial causes of the high mortality rate. Application of various targeted therapies using chemotherapy and chemo drug-loaded NPs, including LPs, were disappointingly unable to improve the treatment outcomes [169].

Active targeting through surface functionalization of the LPs with antibodies, carbohydrates, peptides, aptamers, and other ligands overexpressed in the tumor microenvironment is a strategy often applied to induce specific and efficient LP uptake. Despite successful pre-clinical results, clinical trials on ligand-functionalized LPs for cancer therapy, including PC therapy, still presents some challenges [170, 171]. For example, DOX-loaded PEGylated LP functionalized with a single chain fraction of an anti-HER-2-monoclonal

antibody, also known as MM-302, initially presented promising results in phase I clinical trials as a potential nanotechnological strategy for HER-2+ breast cancer but it failed in other clinical trials to get the FDA's approval [172]. Among failed ligand-functionalized liposomal formulations for cancer treatment purposes is a liposomal formulation of a docetaxel prodrug that targets the ephrin receptor A2 receptor on cancer cells (MM-310) Although this antibody-targeted liposomal formulation improved the tolerability and anticancer efficacy of the active drug in multiple *in vivo* models, it failed during phase I clinical trials on different solid tumors, including PC [173, 174]. Employing DOX-loaded Anti-EGFR-immunoliposomes (C225-ILs-DOX) for targeting the EGFR in triple negative breast cancer is another example of unsuccessful clinical trials prematurely terminated [173].

Overall, the many challenges of both treatments for and PC itself continue to hinder the advancements in the field. There is no sole challenge to overcome, and creating a new therapy for PC is complex. However, there is vital research ongoing with promising potential for these treatments once the challenges are addressed. The main challenge going forward for the field should be bridging the gap between pre-clinical and clinical studies.

A new generation of delivery system has been created as lipid-polymer hybrid nanoparticles. These particles take advantage of the distinctive qualities of LPs and polymeric NPs that contributed to their clinical efficiency while overcoming drawbacks like structural disintegration, constrained circulation, and drug leakage. This technique is particularly intriguing as a multimodal drug delivery technology in cancer because of its two-in-one structure. For effective localization of anticancer therapy, transport of DNA or RNA materials, and usage as a diagnostic imaging agent, the outside surface can be embellished in numerous ways to take full advantage of the system [175].

The targeting problems with anticancer medications can be resolved by the special properties of mesoporous silica nanoparticles (MSNPs). Lipid-coated MSNPs, also known as protocells or silicasomes, have demonstrated to be promising therapeutic and theranostic drug delivery systems. Lipid-coated MSNPs are formed by the encapsulation of the MSNPs within a supported lipid bilayer. The encapsulating supported lipid bilayer can be PEGylated and functionalized with targeting and/or trafficking ligands to generate drug delivery systems presenting an effective tumor-targeted cargo delivery while preserving in vivo colloidal stability [176]. Therefore, lipid-coated MSNPs synergistically combine into one drug delivery system the

benefits of LPs (i.e. high biocompatibility, low prolonged circulation times), with the advantages of MSNPs (i.e. tuneable particle size and shape, and an high surface area of uniformly sized pores whose size and surface chemistry can be tuned to carry a wide range of cargos) [177]. Lipid coating of MSNPs can further improve the stability and biocompatibility of nanoparticle at the same time. Additionally, the lipid wrapping can increase drug delivery to the tumor location and decrease drug release across the body. Lipid coating may act as a barrier to prolong drug release and prevent early leakage. Better cellular absorption can also be obtained by utilizing such nanoparticles. The cellular toxicity of lipid-coated mesoporous silica nanoparticles is much higher than uncoated particles. By supplying and retaining an adequate concentration of therapeutics at the tumor site without causing systemic side effects, lipid coated MSNPs represent a successful method for treating cancer [178].

Examples of lipid-coated MSNPs (silicasomes or protocelles) in PC therapy [179] include co-delivery of GEM and PTX, in which GEM was loaded in MSNPs and PTX in the lipid bilayer [180]; irinotecan delivery, achieving higher stability and prolonged release profile when compared to LP counterparts [181]and, in the case of iRGD-modified lipid bilayer-coated irinotecan-loaded silicasomes, enhanced transcytosis and internalization of the nanosystem [182]; combination of irinotecan-loaded silicasomes with anti-programmed death-ligand 1 (PD-L1), an immune checkpoint blockade inhibitor, was also explored for chemo-immunotherapy of PC [183]; oxaliplatin delivery in combination with anti-PD-L1 [184]; oxaliplatin and indoleamine 2,3-dioxygenase (IDO) inhibitor co-delivery for reversing PC immunosuppressive microenvironment and induce immunogenic cell death [185]; and delivery of signaling hub kinase GSK3 inhibitor [186].

Another interesting approach is the combination of LPs and mesoporous silica nanoparticles in a two-wave approach to address PC-specific challenges such as the dense and desmoplastic stroma, which acts as a treatment barrier by restricting blood vasculature access. For instance, a first-wave carrier composed of MSNPs functionalized with a polycationic polymer was developed to deliver a small-molecule inhibitor of the TGF-β receptor kinase, which increases vessel permeability through pericyte ablation. After 1–2 h this first-wave carrier opened vascular fenestrations enabling a second-wave nanocarrier application, a LP, to efficiently deliver the chemotherapeutic drug GEM to the tumor location [187].

8. Conclusion

PC remains one of the leading causes of death. The natural tendency of PC cells to metastasize rapidly and their resistance to chemotherapy, resulting in growing cases and mortality rates. Its characteristically dense tumor microenvironment, inhibitory immune niche, and un-targeted genetic mutations, make it one of the most challenging diseases. Yet, low efficacy chemotherapeutics and nucleic acid molecules remain standard care. Despite the many treatment strategies/delivery systems in the development pipeline or passed the early stages of clinical trials, identifying target therapy and designing novel strategies for treatment for this disease remains a moving target. The delivery and stability of nucleic acids and chemotherapeutics are challenging, thus several delivery systems including LPs have been employed in PC treatment. LPs are important lipid-based NPs used in PC drug delivery, carrying anticancer agents in single and combinational forms [10]. For instance, the application of GemLip® which is a GEM encapsulating LP-based formulation (hydrogenated egg phosphatidyl cholin/cholesterol) demonstrated a 35-fold more therapeutic improvement and higher half-life of gemcitabine in PC models [188].

These systems need further improvement to enhance their performance. As different challenges are desired to be overcome by liposomal delivery of chemotherapeutics in PC, the use of novel targeting strategies, smart materials, and neglected genetic mutations should be supportive in improving survival [156]. Likewise, the therapeutic potential of anti-cancer drugs and nucleic acids either alone or in combination, has been proved extensively. Co-delivery of multiple medications and non-coding RNAs (siRNA) by LPs s is another strategy to overcome chemotherapy side effects and reach a more effective targeted therapy [168].

Nevertheless, combination therapy appears to be appealing owing to its benefits in the treatment of PC in pre-clinical and clinical trial stages. The development of multifunctional liposomal systems is making waves in PC treatment. Controlled delivery and release can be achieved by combining stimuli-responsive moieties and targeting agents. LPs offer vast therapeutic strategies with their easy functionalization. However, more clinical trials need to be performed in order to find a potential ligand-functionalized LPs formulation for regulatory authorities approval. Overall, both investing in designing a novel, innovative delivery system and careful assessment

of advanced methods could significantly impact the clinical translation of liposomal formulations and improve the quality of PC treatment. Such findings appear to be promising for future application of liposome-based anti-cancer agents in PC.

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Conflict of interest and disclosure

The authors report no financial or personal interests.

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Figure captions

Figure 1. Schematic illustration of liposome-based drug delivery systems in PC diagnosis and treatment. Liposomal delivery systems are capable of being applied for early detection of the PC through MRI and optical imaging technologies plus delivering various therapeutic agents including genes and anti-cancer drugs to the exact tumor site for an effective targeted therapy.

Figure 2. Schematic illustration of GCSDL (composed of HSPC, CHOL, DOPE-GSH, and embedded DOX) application in which the GGT enzyme catalyzes the γ-glutamyl transfer reactions of GSH moiety that results in cationic primary amines generation and the anionic GCSDL conversion into the cationic form **(A)**. Following intravenous injection (1) and circulation in the bloodstream (2), a few of GCSDL or Doxil diffuse into the tumor periphery through extravasation of the leaky blood vessels (3); GCSDL / TVEC contact and GGT catalyzation, leads to the conversion of the anionic GCSDL into cationic form (4); The caveolae-mediated endocytosis is activated due to the cationization and proceeds the vesicle-mediated trans-cytosis, resulting in the increased tumor accumulation and deep penetration into interior parenchyma (5) **(B)**. Tumor blood vessels' ultrastructures captured by TEM **(C, D)**. GCSDL transcytosis suggested by TEM **(E)**. Luminescence intensity of BxPC3-Luci tumors-bearing mice during the experiment **(F)**. Dissected tumors images and the tumor weight average at the end of the experiment **(G, H)**. Adapted with permission from reference [61], copyright Small (2020).

Figure 3. An illustration representing the preparation and *in vivo* application of TSL/has-PE nanocarriers in nude mice bearing BxPC-3 and HPaSteC, treated with intravenously injection of these formulations, at doses of PTX 5 mg/kg and EA 4 mg/kg, for about 2 weeks **(A, B)**. Tumor volume curves during the experiment (The suffixes "HT" and "NT" in the curves indicate various heat treatments of the tumors) **(C)**. The tumor xenograft images and tumor weight (B-1: Saline (HT); B-2: Taxol (HT); B-3hasSL/HSA-PE (Nhas B-4: HSA–PTXhasT); has: HSA–PTX + HSA–has (HT); B-6: TSL/HSA-PE (HT) **(D)**. Adapted with permission from reference [69], copyright Clinics and research in hepatology and gastroenterology (2019).

Figure 4. Schematic illustration showing the potential therapeutic mechanisms of MR-T-PD (composed of DOX, PCAL, and MR)-loaded thermosensitive liposomes in a PC mouse model bearing BxPC-3 and HPaSteC xenografts. The MT1-MMP on the surface of tumor endothelial cells (ECs) activates MR-T-PD to release cRGDfK which promotes MR-T-PD accumulation in the tumors. Additionally, under heat treatment, MR-T-PD releases PCAL and DOX into the interstitium. The released DOX induces apoptosis in the tumor cells whereas the PCAL prodrug is converted to CAL and CAL promotes the antitumor effects of DOX **(A)**. Tumor volume curve during the treatment **(B)**. Tumor weight curve **(C)** and representative images of tumor-bearing mice and tumor tissues at the end of the treatment (the black arrows indicate the tumors) **(D)**. Adapted with permission from reference [74], copyright Advanced Functional Materials (2021).

Figure 5. EPR-independent delivery of miRNA/siRNA in PC treatment. Herein, anti-miR-210 and siKRAS^{G12D} – loaded PCX nanoparticles were injected intraperitoneally in an orthotopic pancreatic tumor. Following injection, the PCX nanoparticles internalized deeply into the tumor and resulted in metastasis blockade, immunosuppression attenuation, and desmoplastic stroma modulation *via* cancer-stroma interaction inhibition and pancreatic stellate cells inactivation. Adapted with permission from reference [90], copyright ACS nano (2020).

Figure 6. A Schematic illustration indicating the tumor growth and metastasis hindering through innate and adaptive immunity evoke and immune memory effects employing Node-Targeted Cholesterolized TLR7 Agonist Liposomes **(A)**. CT26-bearing Balb/c mice were injected with 4 mg kg^{-1} 1V209-Cho-Lip on day −1 or 0, 9, 12, 15, 18 and the mice were sacrificed on day 23 **(B)**. Average weight of post-dissection tumors after treatment with PBS control and 1V209-Cho-Lip **(C)**. Representative of lung tumor signals by IVIS on 7, 10, and 14 days after receiving the CT26 cells i.v **(D)**. Image of post-dissection lungs, H&E and the numbers of lung nodules after harvesting lungs on day 23 **(E, F)**. Average popliteal lymph nodes weight at the end of treatment in the CT26 lymphatic metastasis model **(G)**. Adapted with permission from reference [117], copyright Nano Lett (2021).

Figure 7. This schematic illustration shows the application of mild hyperthermia plus FAP-α responsive size-adjustable nanoparticles (HSA-BMS@CAP-ILTSL) for combinational treatment of photothermal therapy and immunotherapy **(A)**. Subcutaneous tumor and artificial metastatic induction and treatment in Pan 02 female C57BL/6 mice models which were under NIR laser irradiation after 4 h treatments **(B)**. The curves of mice weight (n = 5) and the tumor growth **(C, D)**. Representative images of tumors **(E)**. Mice tumor weights (n = 5) and H&E staining images of tumors, lungs and livers (arrows and dashed lines indicate the metastatic areas) **(F, G)**. Serum levels of cytokine IL-6 and IFN- γ measured by Elisa kits after the treatment (n = 3) **(H)**. Adapted with permission from reference [65], copyright Acta Biomaterialia (2021).

Figure 8. A schematic illustration representing the preparation of MC-T-DOX, a doxorubicin (DOX) loaded smart liposome **(A)**. MC-T-DOX improves the tumor blood perfusion and drug delivery in PC. Low density MT1-MMP-activated cilengitide (MC) is modified onto DOX-loaded thermosensitive liposomes (TSLs), yielding MC-T-DOX. Following IV injection of MC-T-DOX into the hypo perfused pancreatic tumor in BxPC-3 mice models at a low dose of cilengitide, every 4 days for four cycles at an identical DOX dose of 3 mg kg^{-1}, MT1-MMP on tumor ECs could activate MC-T-DOX to release cilengitide, which then promotes ECs migration and angiogenesis, resulting in higher levels of MC-T-DOX accumulation and distribution in the tumor site which would be improved after subsequent heat-triggered DOX release, in the interstitium **(B)**. Representative photos of tumor and functional blood vessels plus blood density quantitative analysis and functional blood vessels percentage **(C, D)**. Tumor growth curves (the arrows indicate the time points for treatment [138]) **(E)**. Body weight changes during the experiment (n = 6) and quantitative analysis of cell apoptosis (n = 6) **(F, G)**. Adapted with permission from reference [14], copyright Advanced Science (2020).

Figure 9. Schematic illustration of the nanocarrier LRC-GEM-PFD in which, PFD is inserted into the hydrophobic chamber of β-CD, and the liposome encapsulates GEM. Cleavage of LRC-GEM-PFD by MMP-2 results in the regulation of the PSCs by PFD and recognition of the PTCs by GEM-loaded liposome **(A)**. Expression levels of Collagen I and TGF-β in tumor tissue after LRC, PFD, and LRC-PFD treatment. (**Up:** IHC stained slices images. **Down:** Statistic quantitative analysis of collagen I and TGF-β from IHC results) **(B)**. Tumor volume curves of PSCs/Panc-1 pancreatic tumor in mice models treated by various GEM formulations with a GEM dose of 20 mg/kg **(C)**. Rhd penetration into the PC tissues (Panc-1 and PSCs coimplanted) following IV injection of different PFD formulations (Red: Rhd) **(D)**. Adapted with permission from reference [12]**,** copyright ACS applied materials & interfaces (2016).

Table captions

Table 1: Various nano-systems for different therapeutic strategies.

Table 2: Summary of some recent and active clinical trials involving liposomal treatment for pancreatic cancer.

Figures

Figure 1

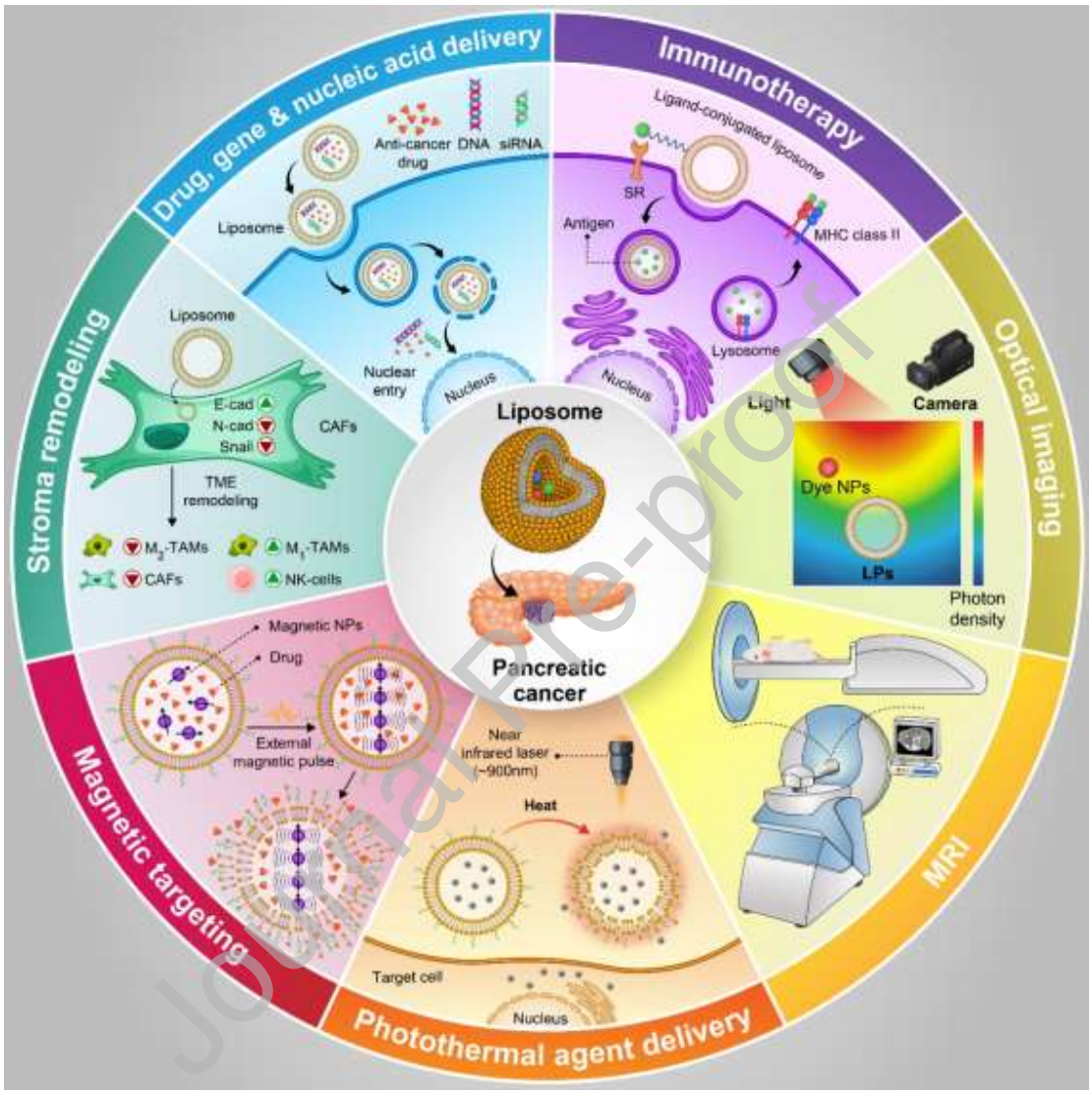
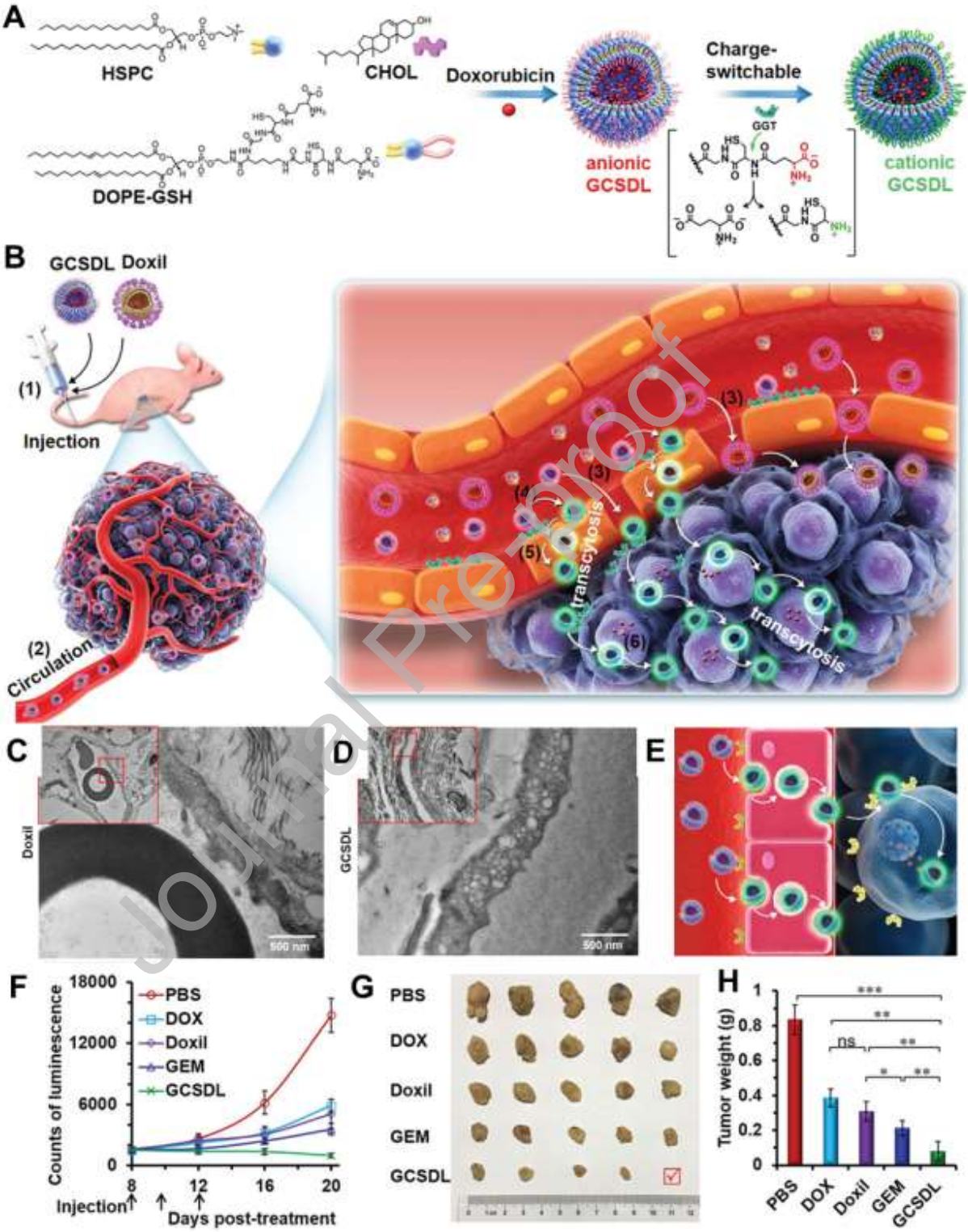


Figure2



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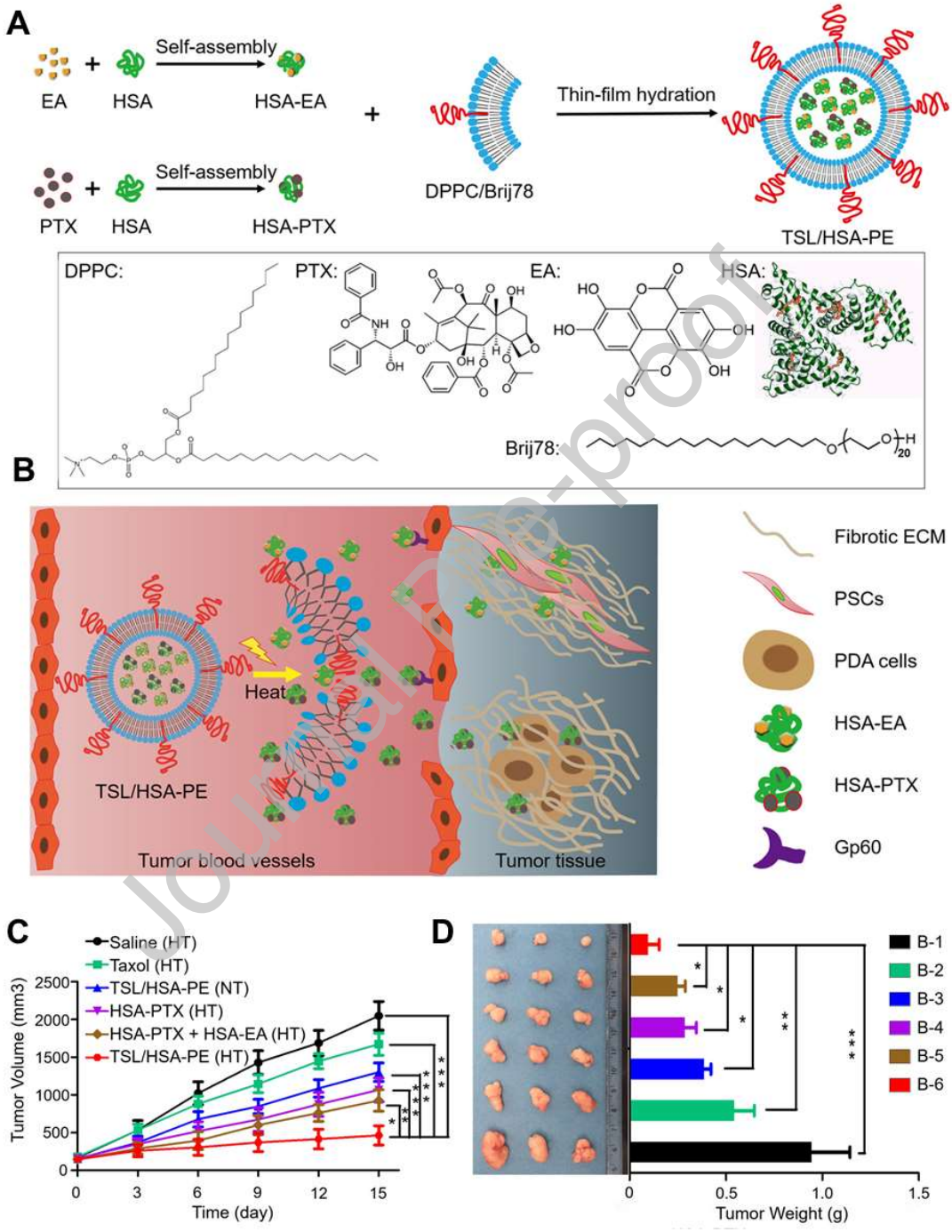


Figure 4

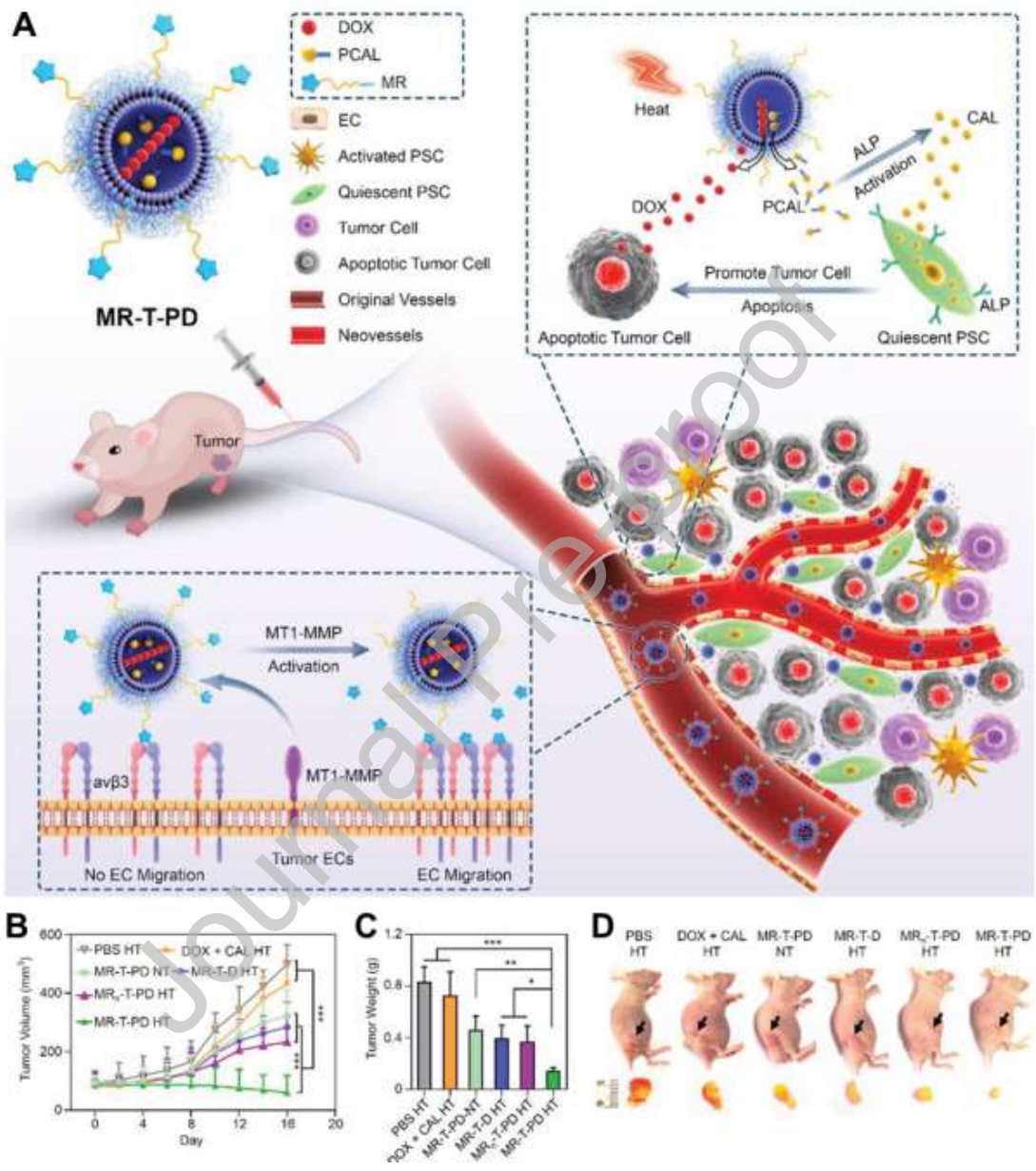


Figure 5

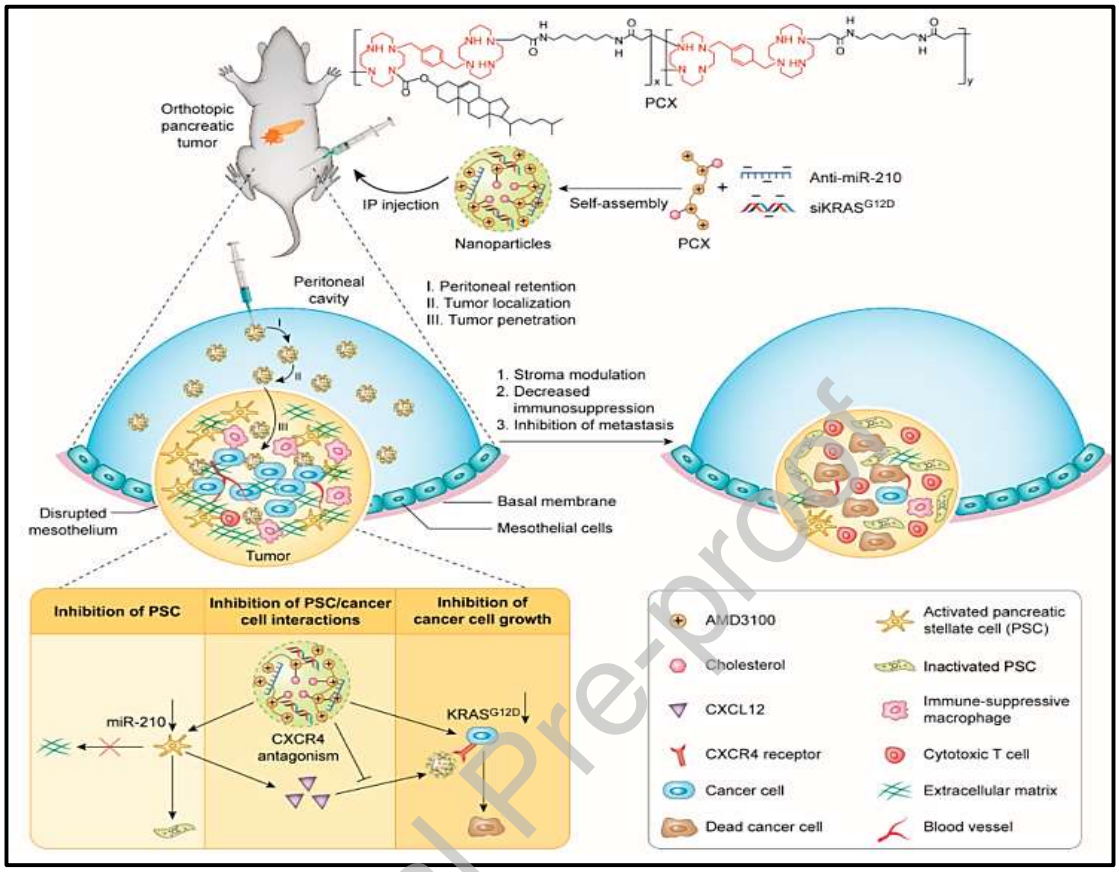


Figure 6

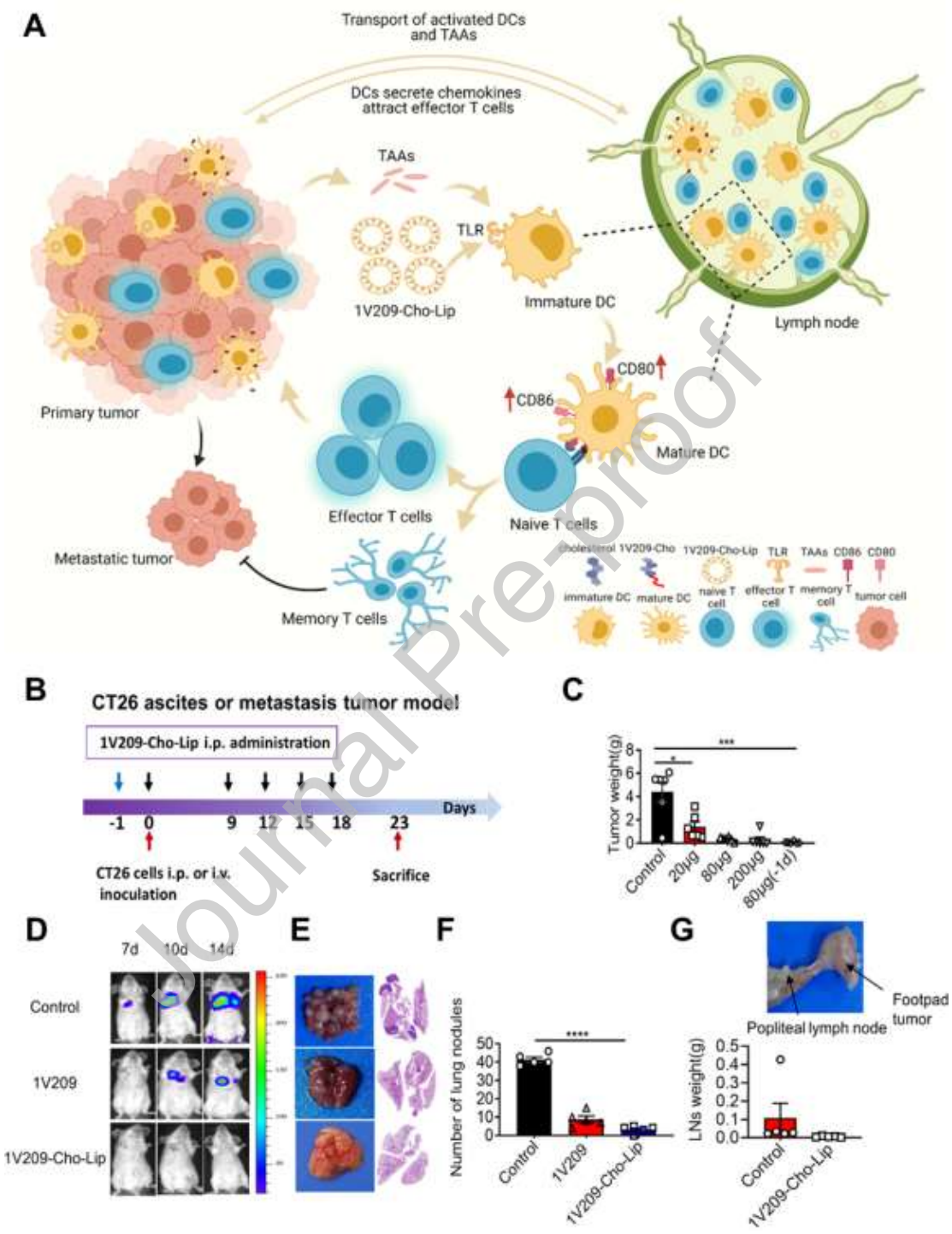
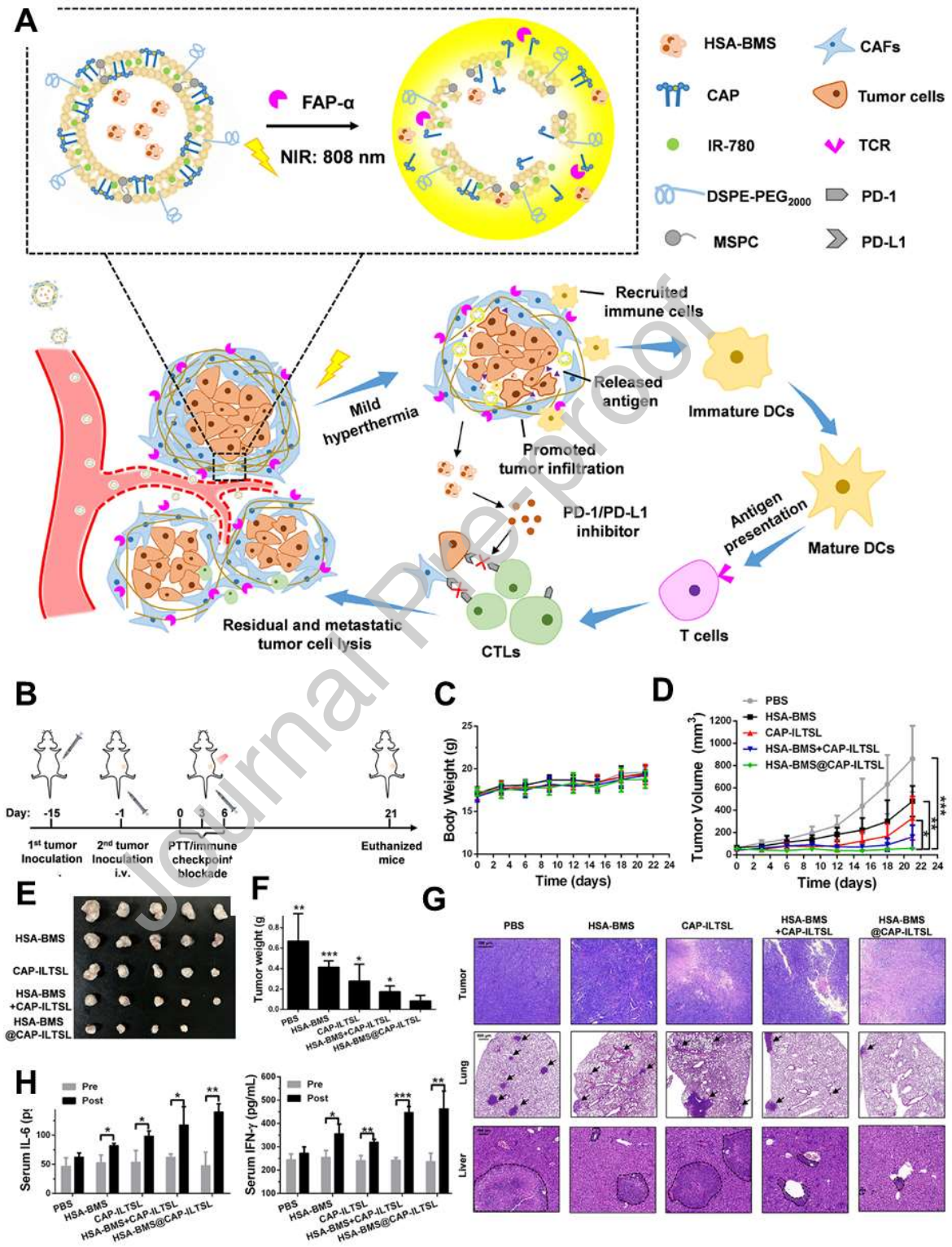
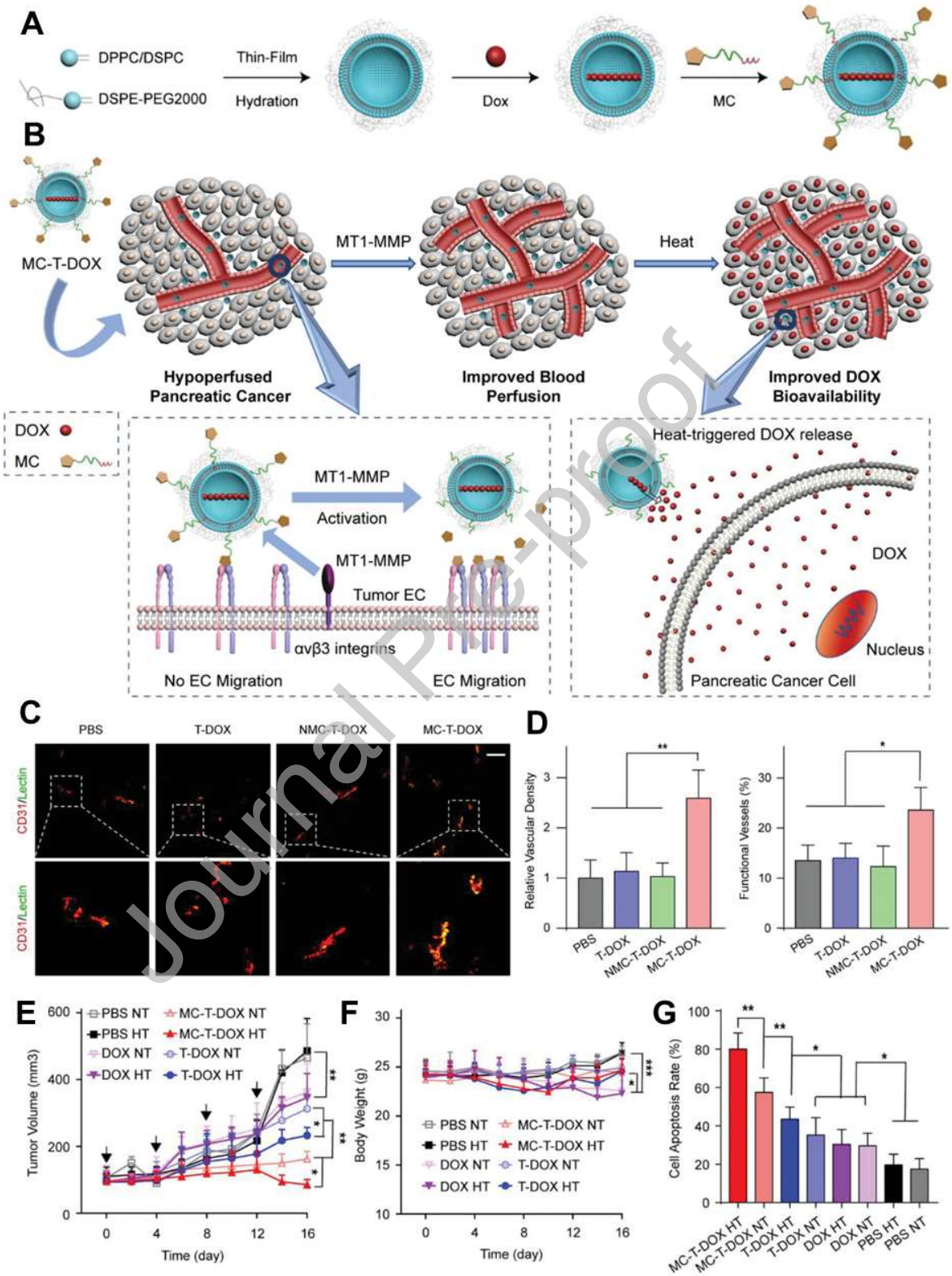


Figure 7

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Pancreatic Cancer
Improved Blood
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Body Weight (g)
Time (day)
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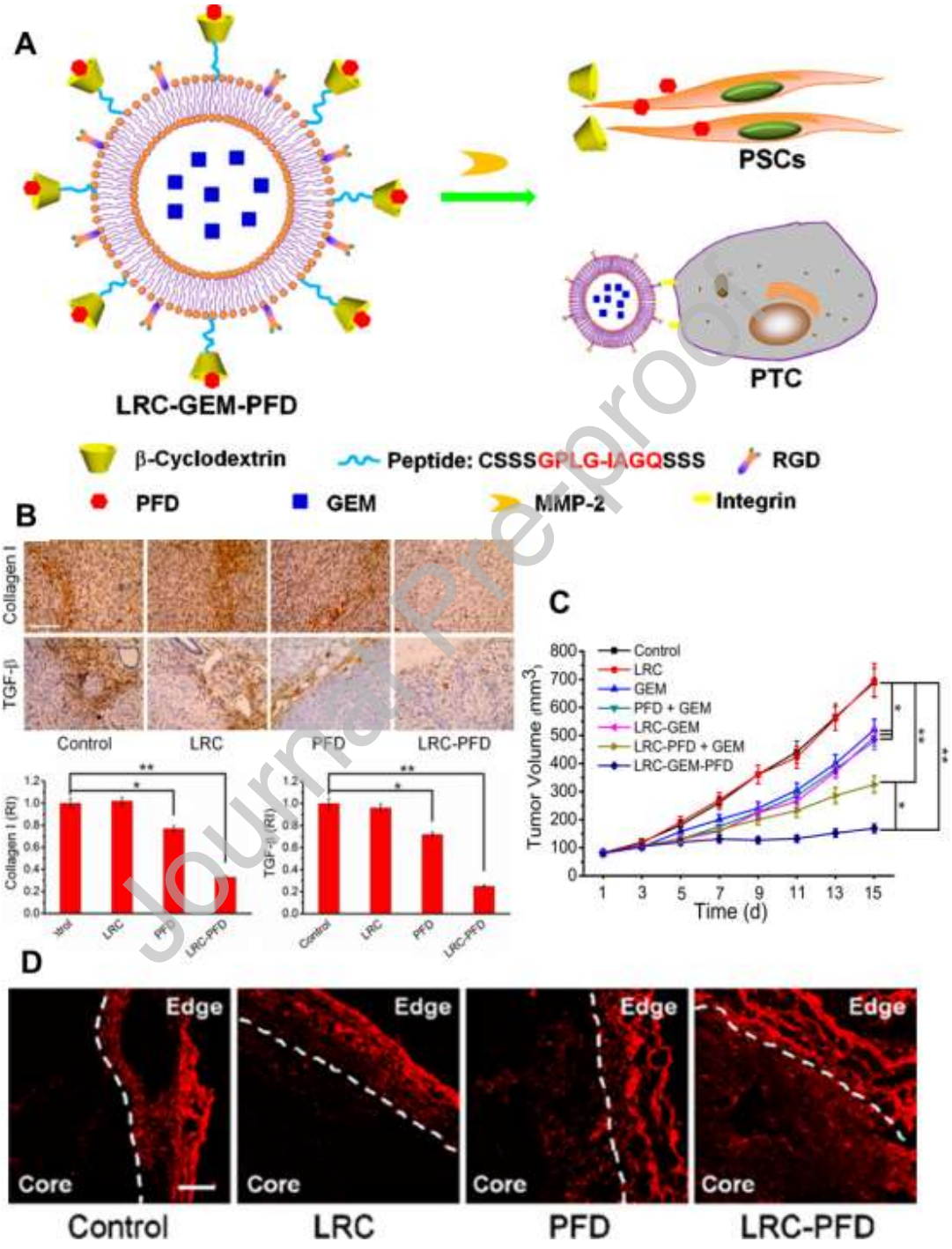
Figure 8

Figure 9



Tables

Table 1: Various nano-systems for different therapeutic strategies.

Composition	Size/loading/encapsulation	*In vivo* models	Findings	Therapeutic Strategy	Ref
Lipid encapsulated gemcitabine	Size 79 ± 2 nm Encapsulation >96%	Mice with Capan-1 or BxPC-3 tumors	Suppression of tumor growth	Chemotherapeutic loaded liposomal therapy	[60]
GSH surface modified liposome with encapsulated doxorubicin.	Size 65.2 ± 5.7 nm DOX encapsulation > 95% and a DOX loading content ~10%	Mice bearing subcutaneous Huh7 tumors and pancreatic ductal adenocarcinoma (PDA) BxPC3 cell line	Inhibited tumor growth	Liposome with targeting agent and cytotoxic agent	[61]
TR-PTX/HCQ-Lip	Size 135.47 ± 2.85 nm loading PTX 83.72 ± 1.96% HCQ 80.96 ± 2.38%	BxPC-3 orthotopic pancreatic cancer model	Suppression of tumor growth and inhibition of autophagy and stroma fibrosis	Liposome with agent to modify stroma pathways	[62]
HSA-BMS@CAP-ILTSL	Size 121.5 ± 2.8 nm loading efficacy of BMS-HSA in CAP-ILTSL was 10.75 ± 1.7%	Pan 02 subcutaneous mouse model	Suppression of tumor growth	Immunotherapy and photothermal	[63]

CpG-DNA-peptide-liposome complex		TM4SF5-expressing mouse PDAC cells (PANC02-hTM4SF5)	Suppression of tumor growth	Gene Therapy	[64]
TLR7 agonist, conjugated with cholesterol prepared into liposomes	Size 110 nm	CT26 colorectal cancer, 4T1 breast cancer, and Pan02 pancreatic ductal cancer models.	Suppression of tumor growth and metastasis	Lymphatic Targeting	[65]

Table 2

Table 2: Summary of some recent and active clinical trials involving liposomal treatment for

Condition	Treatment	Study Phase	County	ID # (clinicaltrials.gov)
Locally Advanced or Metastatic Pancreatic Cancer	Irinotecan Liposome	3	Germany	NCT03468335
Metastatic Pancreatic Cancer	Irinotecan liposome Oxaliplatin 5-Fluorouracil, Leucovorin	3	Various Global	NCT04083235
Locally Advanced or Metastatic Pancreatic Cancer	Irinotecan liposome, 5-Fluorouracil, Leucovorin	3	China	NCT05074589
Advanced Pancreatic Cancer	Mitoxantrone hydrochloride liposome	2	China	NCT05100329
Advanced Pancreatic Cancer	paclitaxel liposome S-1.	4	China	NCT04217096
Non-resectable Primary Pancreatic Tumours	Liposomal Doxorubicin (ThermoDox®) (and Focused Ultrasound)	1	United Kingdom	NCT04852367

pancreatic cancer.

Statement of significance:

Considering that conventional treatments for pancreatic cancer are highly associated with sub-optimal performance and systemic toxicity, the development of novel therapeutic strategies holds outmost relevance for pancreatic cancer management. Liposomes are being increasingly considered as promising nanocarriers for providing not only an early diagnosis but also effective, highly specific, and safer treatment, improving overall patient outcome. This manuscript is the first in the last 10 years that revises the advances in the application of liposome-based formulations in bioimaging, chemotherapy, phototherapy, immunotherapy, combination therapies, and emergent therapies for pancreatic cancer management. Prospective insights are provided regarding several advantages resulting from the use of liposome technology in precision strategies, fostering new ideas for next-generation diagnosis and targeted therapies of pancreatic cancer.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: