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Liposomal nanostructures for drug delivery in gastrointestinal cancers

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GI: Gastrointestinal

MPS: Mononuclear phagocyte system

PEG: Polyethylene glycol

EPR: Enhanced permeation and retention

ABSTRACT

Gastrointestinal (GI) cancers like liver, pancreatic, colorectal, and gastric cancer remain some of the most difficult and aggressive cancers. Nanoparticles like liposomes had been approved in the clinic for cancer therapy dating as far back as in 1995. Over the years, liposomal formulations have come a long way, facing several roadblocks, and failures, and advanced by optimizing formulations and incorporating novel design approaches to navigate therapeutic delivery challenges. The first liposomal formulation for a gastrointestinal cancer drug was approved recently in 2015, setting the stage for further clinical developments of liposome-based delivery systems for therapies against GI malignancies. This article reviews the design considerations and strategies that can be employed to deliver drugs to GI tumors, the wide range of therapeutics that had been explored, in preclinical as well as clinical studies, and the current therapies that are being investigated in the clinic, against GI malignancies.

Introduction

Gastrointestinal (GI) cancers are one of the major contributors to cancer-related mortalities worldwide. Colorectal and pancreatic cancers are ranked at 3rd and 4th in the list of top ten cancers by death rates among both males and females, and cancers of liver and intrahepatic bile duct rank at 5th and 8th in men and women in the list of estimated deaths, respectively, in the United States (Siegel et al., 2018). Chemotherapy is one of the primary treatment modalities used to treat cancer, with radiation therapy and surgery. However, drug delivery in diseases localized in the GI tract is challenged by the requirements of low systemic distribution and maximizing therapeutic concentrations along the tract. Further, the GI tract can present an extreme environment for therapeutic delivery with factors like pH, immune response, intestinal permeability, and mucosal barriers posing hurdles (Ensign et al., 2012). Additionally, solid tumors in themselves can impose intricate barriers in the altered tumor microenvironment and aberrant vasculatures (Sriraman et al., 2014). Together, these challenges in drug delivery require therapeutic delivery in high doses which contributes to systemic toxicity and drug resistance.

Nanoscale platforms like liposomes present significant opportunities in rational and targeted drug deliveries in gastrointestinal cancers, due to its superior efficiency to encapsulate drugs of variable physicochemical character and excellent biocompatibility, and capability to survive in hostile GI environment after suitable modifications. Further, nanoparticle encapsulated therapeutics have the potential to minimize chemotherapy mediated toxicity and improve biodistribution and prevent premature metabolism of the active therapeutic agent (Allen and Cullis, 2013; Min et al., 2015; Sousa et al., 2018).

Liposomal structures are spherical vesicles with a lipid layer encapsulating an aqueous core and were described as early as 1965 (Bangham et al., 1965). Since then, liposomes were explored widely as a delivery platform for gene therapies, biologics, and chemotherapeutic drugs, leading to the earliest clinical approvals of liposomal drug formulations against cancer with Doxil® (1995)

and DaunoXome® (1996), three decades after the discovery of liposomes (Sercombe et al., 2015; Bulbake et al., 2017). Onivyde®, a liposomal irinotecan formulation (**Fig. 1**) was recently approved for metastatic pancreatic cancer patients resistant to gemicitabine (Kipps et al., 2017). Currently, liposomal irinotecan is being explored clinically against multiple GI cancer types including colorectal, gastroesophageal, and biliary cancers (**Table 1**). Herein, we concisely review the liposomal technologies used to deliver therapies against GI cancers and the formulation strategies applied to circumvent the barriers of drug delivery to GI and solid tumors.

Design considerations in anticancer therapy

GI cancers offer challenging barriers for drug delivery, by combining the hurdles associated with solid tumors, the GI tract physiological environment, and the tight epithelial tissue barriers if systemic delivery is required (Tscheik et al., 2013). Liposomal compositions need to be tuned to the intended therapeutic challenge and route of administration. However, some general guidelines can be used for designing the nanoplatforms. For *in vivo* delivery of nanoparticles, several factors must be taken into consideration, like colloidal stability, interaction with proteins in the serum ,shelf life, blood circulation time, mononuclear phagocyte system (MPS) clearance, tissue extravasation, and cytokine induction (Cheng and Lee, 2016). Advancements in pharmacokinetic properties of liposomal formulations, and enhancement of cargo encapsulation using active loading principles were two key events in liposomal design that eventually led to the clinical approval of Doxil®, PEGylated liposomal doxorubicin, in 1995 (James et al., 1994; Harrison et al., 1995; Northfelt et al., 1997). Steric stabilization of liposomes by incorporation of PEGylated phospholipids significantly increased blood circulation time (Blume and Cevc, 1990; Klibanov et al., 1990), which further enabled passive accumulation in organs with leaky vasculature, by virtue of the “enhanced permeation and retention” (EPR) effect (Matsumura and Maeda, 1986; Torchilin, 2011; Maeda et al., 2016). Further, active loading principles, exploiting pH gradient between the interior versus exterior of the liposome, allowed higher drug encapsulation, and minimized non-

encapsulated drug loss (Mayer et al., 1986; Bally et al., 1988). Till date, active loading of liposomes continues to remain a clinically relevant approach for weakly basic drugs and can be exchanged through the liposomal membrane when subjected to a pH gradient. The recently approved Onivyde® (2015), liposomal formulation of irinotecan, for patients with metastatic pancreatic adenocarcinoma and resistant to gemcitabine, exploits multivalent anionic polymeric/non-polymeric trapping agents like sucrose octasulfate (**Fig. 1**) providing an electrochemical gradient to facilitate drug retention in the interior of liposomes (Drummond et al., 2006; Passero et al., 2016; Drummond et al., 2018). In this section, we will review the different strategies that can be exploited to design formulations for drug delivery in GI cancers.

Oral delivery of formulations

Oral delivery is usually a preferred route for gastroenterological conditions, however, with metastatic cancers, systemic delivery of therapeutic is ideal to enhance drug distribution. Moreover, oral delivery using platforms like liposomal nanoparticles may suffer from instability and degradation of the carrier in the GI tract, mediated by gastric acids, lipases secreted from pancreas, and bile salts (Hu et al., 2013a; He et al., 2018), and scalability. Higher drug doses are usually required for oral formulations, requiring scaling up of liposomes, leading to inter-batch variability (He et al., 2018). If permeation across intestinal epithelia is desired, it can be a further challenge. Constant renewal of the dynamic gastrointestinal mucus barrier can restrict intestinal absorption of nanoparticle systems like liposomes (Ensign et al., 2012). Mucoadhesive and mucopenetrating polymers can be used to modify liposomes to enhance intestinal delivery of therapeutics (Liu et al., 2018). Alteration in the composition of gut microbiome may also affect drug delivery by impacting intestinal and colonic transit time, mucus production, and immune cell infiltration (Mittal et al., 2018). There had been efforts to exploit cells lining the epithelium, like M cells to facilitate transport of liposomal nanoparticles (Shukla et al., 2016). For localized cancers, therapeutic release in a site-specific manner is ideal, when surgical resection is not an option. For

malignancies of lower GI tract like cancers of colon and rectum (Gulbake et al., 2016), factors like temporal control, pH, enzymatic, and pressure can be used to actuate drug release in a specific section of the GI tract as the formulation passes through the gut. So far, there are limited successes in enhancing oral formulation targeting to diseased vs. normal gut tissue (Hua, 2014), however, active targeting principles can further be exploited to further improve uptake in cancerous tissues by decorating nano-delivery systems with ligands that can bind to receptors on cancer cell surface (He et al., 2018).

Enhanced permeation and retention (EPR) effect

Nanoparticle-based platforms can accumulate preferentially in tumors taking advantage of the EPR effect (Matsumura and Maeda, 1986). Previous studies demonstrated increased accumulation of nanoparticle systems within the tumor, compared to normal tissues corresponding to the same organ, in several of the GI malignancies like pancreatic adenocarcinoma, colorectal cancer, and stomach cancer (Natfji et al., 2017). Nanoparticles due to their sizes can preferentially accumulate in tumors, liver, spleen, due to leaky endothelial barriers of these tissues (Li and Huang, 2008). Further, it is possible to tune the size of the nanoparticles to enhance blood circulation time (Liu et al., 1992), and to minimize clearance due to internalization by the cells of the mononuclear phagocyte system (MPS). Surface modification of the liposomal carriers with polyethylene glycol (PEG), commonly referred to as PEGylated liposomes, may reduce opsonization after interaction with the components in the blood, and subsequent uptake by the MPS (Deshpande et al., 2013). Improving blood circulation time and reduction in opsonization can further increase EPR-mediated uptake as it allows the formulation to circulate through the intended site of drug delivery (Torchilin, 2007). Nanoparticle size play a critical role in guiding uptake in the solid tumor, and the magnitude of the difference in accumulation between smaller and larger liposomes increase with larger tumors as they tend to be more vascularized (Fanciullino et al., 2014). However, it must be noted, that clinically, there is

a high degree of inter- and intrapatient heterogeneity when it comes to EPR effect in human tumors (Maeda, 2015; Clark et al., 2016). Further, in some human malignancies, vascular permeability is not as high as in preclinical models of cancer, and these factors need to be accounted for while selecting patients for treatment using nanomedicine as drug delivery systems (Jain and Stylianopoulos, 2010; Bae and Park, 2011; Lammers et al., 2012). Using labeled PEGylated liposomes to assess the distribution of formulation in the patient's lesions by non-invasive imaging is an insightful approach to predict the utility of passive drug targeting while recruiting patients for therapy in the clinic (Harrington et al., 2001).

PEGylated liposomes: Issues with Anti-PEG immunity

While PEGylation of liposomes is a commonly used stealth strategy, recent reports demonstrate that the immune system can induce an antibody response against PEG, resulting in accelerated clearance of PEGylated liposomes and other protein therapeutics from the blood (Yang and Lai, 2015; Zhang et al., 2016b). Healthy individuals who are never treated with PEGylated therapeutics can have pre-existing titer of anti-PEG antibodies. A recent report showed the presence of either anti-PEG immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies in about 44% of patients (Chen et al., 2016). Further, pre-existing antibodies against PEG can be a barrier to treat patients with PEG-modified therapeutics. A recent Phase IIb trial investigating a PEGylated aptamer drug was suspended after three patients were observed to develop a severe allergic reaction against the drug (Povsic et al., 2016). When analyzed retrospectively, these three patients were observed to have elevation in the levels of anti-PEG IgG antibodies, suggesting the association of pre-existing anti-PEG antibody titer with the potential to induce immune-related adverse events. Currently, alternative stealth polymers like poly(2-oxazoline) are being investigated to shield nanocarriers while bypassing PEG-mediated immune reactions (Bludau et al., 2017).

Active targeting approaches

Nanoparticles can be modified with small molecules, peptides, monoclonal antibodies or antibody fragments to target tumor cells (Fernandes et al., 2015; Jain and Jain, 2018). A wide variety of ligands had been explored in the past to facilitate tumor-specific uptake of liposomes. Of these surface engineering approaches, some organs are more accessible to target than others. Liposomes and lipid nanoparticles preferentially accumulate in the liver, which can further be tuned with ligand-receptor interactions to shift biodistribution to specific cell populations in the liver like hepatocytes (Longmuir et al., 2009; Goodwin et al., 2016; Huang, 2017). Galactose, N-Acetylgalactosamine (GalNAc), heparan sulfate, and transferrin are some of the common ligands that had been used in the past to target nanoparticles to the hepatocytes (Li et al., 2009; Hu et al., 2013b). A whole class of functionalized liposomal platforms, the so-called immunoliposomes, have emerged, exploiting antibody or antibody fragments for targeted delivery (Eloy et al., 2017). Monoclonal antibody and antibody fragments targeting CD44 and VEGF receptor 2 (VEGFR2) were explored in preclinical models of hepatocellular carcinoma (Roth et al., 2007; Wang et al., 2012). VEGFR2 targeted PEGylated formulation of liposomal doxorubicin demonstrated superior efficacy over non-targeted liposomal formulations in murine models of colon cancer (Wicki et al., 2012). Transferrin-conjugated PEG had been used for targeting in preclinical models of gastric cancer as well, demonstrating efficacy over non-targeted liposomes (Iinuma et al., 2002). Cationic liposomal platforms targeted with single chain antibody fragments against the transferrin receptor (Camp et al., 2013) had also been explored, to deliver wild-type p53, a tumor suppressor gene, whose mutation can drive oncogenesis in multiple cancers including cancers of the pancreas (Freed-Pastor and Prives, 2012). Peptides are also exploited to enhance distribution to tumor cells. Integrin targeting was achieved with tri-peptide motifs, enabling specific binding to colon (Schiffelers et al., 2003) and gastric tumors (Akita et al., 2006; Chen et al., 2008), and penta-peptide motifs were used to drive accumulation of liposomes in angiogenic sites within murine

colon tumors (Shimizu et al., 2005). Further, multiple studies exploited immunoliposomes to mitigate toxicity of chemotherapeutic drugs and improve efficacy in GI malignancies. Antigen-binding sites of antibodies referred to as F(ab′)2 fragments were explored for targeting liposomes encapsulating doxorubicin in patients with metastatic or recurring cancers of the stomach (Hosokawa et al., 2004; Matsumura et al., 2004). Liposomes targeting human epidermal growth factor receptor 2 (HER2) had also been used recently to treat HER2 overexpressing gastric cancers (Espelin et al., 2016). Another ErbB receptor family member, epidermal growth factor receptor (EGFR) targeted oxaliplatin liposomes were also explored in murine colorectal cancer models (Zalba et al., 2015).

Although ligand-decorated nanoparticles were demonstrated to facilitate internalization in targeted cells in numerous preclinical applications, they do not improve biodistribution to tumor tissues, as nanoparticles are distributed predominantly by EPR guided passive targeting. Hence, nanoparticles having similar blood circulation were observed to have a similar distribution, regardless of active targeting (Goren et al., 1996; Kirpotin et al., 2006; Riviere et al., 2011; Allen and Cullis, 2013). Recent studies are therefore focused on understanding binding site barriers (BSB) to improve the cellular disposition of nanomedicine inside solid tumors (Miao et al., 2016).

The BSB can present itself in many forms. The extracellular matrix, and the stromal cells near blood vessels, can restrict the diffusion of nanoparticles, and further actuate internalization in stromal cells driven by targeting receptor expression. Although these barriers are obstacles to drug delivery, the so-called off-target delivery to fibroblasts can potentially be exploited in anticancer therapy by targeting fibroblasts for therapeutic delivery, inducing secretion of cytotoxic mediators in the tumor-stroma environment (Miao et al., 2017b). Further, the insights gained from BSB models invite detailed analyses of tumor cell populations to determine receptor expression in distinct cell populations, if specific delivery to cancerous cells in the tumor is critical for therapeutic efficacy.

Stimuli-sensitive designs

Physiological triggers like pH, light, enzymes, and redox can be exploited for stimuli-responsive drug delivery, by driving structural changes within the liposomal delivery platform, allowing the release of therapeutic cargo in the intended biological environment (Heidarli et al., 2017; Lee and Thompson, 2017). pH-responsive polymers had been exploited in the past to design liposomes capable of releasing the encapsulated cargo in mildly acidic conditions, like the environment around cancer cells. Copolymer modified rapamycin liposomes had been investigated as a pH-sensitive delivery platform for colon cancer cells *in vitro* (Ghanbarzadeh et al., 2014). Multifunctional nanoparticles targeting tumor cells, and using pH sensitivity to release cargo in cancer cells had been designed as well (Garg and Kokkoli, 2011). Overexpression of matrix metalloprotease-9 (MMP-9) in the tumor extracellular matrix had been exploited to release encapsulated cargo in the tumor microenvironment driven by MMP-9 mediated lipopeptide cleavage (Kulkarni et al., 2014). Liposomes can be co-loaded with superparamagnetic magnetite and chemotherapeutic, and the drug release can be triggered by hyperthermia induced by electromagnetic field (Clares et al., 2013). Thermosensitive liposomes capable of releasing drug cargo triggered by mild hyperthermia was also explored in pancreatic cancer cell lines (Affram et al., 2015). Some of these stimuli-responsive liposomes had also been tested in the clinic against GI cancers. Recently, the results from a Phase III trial investigating radiofrequency ablation (RFA) in combination with heat sensitive liposomes encapsulating doxorubicin, in patients with hepatocellular carcinoma, was reported (Tak et al., 2018). The study did not demonstrate a significant difference in progression-free survival between patients treated with RFA or RFA in combination with thermosensitive doxorubicin liposomes. However, benefit in patients with solitary lesions was observed. Overall, there are many challenges associated with translating complex nanocarriers to clinic and concerns about cost vs. benefit of targeted nano delivery

systems (Cheng et al., 2012), and these factors need to be taken into consideration for successful clinical translation of multifunctional nanoparticle systems like stimuli-responsive liposomes.

Combination therapy by liposome-assisted co-delivery

Liposomal drug delivery technologies can be exploited to load combination therapeutics in controlled proportions, further tuning the release of drugs from the delivery vehicle, allowing translations of *in vitro* combination chemotherapeutic synergies to *in vivo* therapies (Allen and Cullis, 2013; Zununi Vahed et al., 2017). However, co-encapsulation of drugs with different physicochemical properties, like solubility and stability, is challenging. Precise control over loading ratio of drug combinations, that is required for synergistic anti-cancer therapy, can be difficult to achieve. Nevertheless, distinct classes of drugs like irinotecan with floxuridine, cytarabine with daunorubicin, cisplatin with daunorubicin, and doxorubicin with salinomycin had been co-loaded into liposomes and investigated in murine models of pancreatic and colorectal cancers (Mayer et al., 2006; Sriraman et al., 2015; Gong et al., 2016; Li et al., 2016). Liposomes can also be used to combine different types of payloads, beyond chemotherapeutics. Recent studies exploited multi-pronged delivery vehicle design approaches, like pH-sensitive cationic liposomes to load a tyrosine kinase inhibitor drug with short interfering RNAs (siRNA) (Yao et al., 2015), and galactose-targeted liposomes to encapsulate doxorubicin and siRNAs, against murine models of liver cancer (Oh et al., 2016). Other nucleic acid therapeutics like micro RNA (miRNA) can be similarly co-loaded in liposomes with chemotherapeutics like doxorubicin (Fan et al., 2017). Further, it is possible to design complex systems of hybrid liposomes encapsulating drug conjugated to metal nanoparticles, and free drug, allowing an initial rapid release of cargo, and subsequent maintenance of drug level at the target tissue site for prolonged intervals (Zhang et al., 2016a). Another recent study exploits thermosensitive liposomes to encapsulate a drug targeting pancreatic stellate cells, and human serum albumin nanoparticles of paclitaxel against *in vivo* models of pancreatic ductal adenocarcinoma (Wei et al., 2017). Liposomes are also

routinely used in theranostic applications, like co-loading gadolinium and kinase inhibitors, for MRI guided treatment of hepatocellular carcinoma (Xiao et al., 2016). Biologics like monoclonal antibodies can also be co-loaded with photosensitizers, to enhance tumor killing mediated by photodynamic therapy, and had been investigated in pancreatic cancer models (Tangutoori et al., 2016). There is at least one combination chemotherapeutic liposomal formulation that had been tested in the clinic against GI malignancies called CPX-1, an equimolar combination of irinotecan HCl with floxuridine in a Phase II trial (NCT00361842) on colorectal cancer. However, the status of the drug combination in clinical development is unknown.

Payloads & Applications in GI cancer therapy

As we had discussed in the previous segments, liposomes are suited to encapsulate a wide range of therapeutics, varying in their physicochemical characteristics and mechanisms of action. Liposomes had been widely investigated in different GI cancers preclinically (Zhang et al., 2013a), and clinically (**Table 1**) (Cascinu et al., 2011; Wang-Gillam et al., 2016; Tak et al., 2018). In this section, we briefly highlight the different therapeutics that had been encapsulated using liposomal drug delivery systems for GI malignancies.

Small Molecules

Several liposomal formulations of small molecule-based therapeutics had been investigated in GI cancers over the years. Targeted and non-targeted liposomal doxorubicin formulations were explored in numerous studies involving preclinical models of colorectal cancer alone (Chang et al., 2010; Falciani et al., 2011; Lin et al., 2012; Wicki et al., 2012). Other chemotherapeutics and small molecule drugs like anti-angiogenic, anti-fibrotic, and anti-inflammatory agents, photosensitizers, and kinase-targeted therapeutics were also routinely investigated (Kan et al., 2011; Mullauer et al., 2011; He et al., 2013; Ranjan et al., 2013; Di Corato et al., 2015; Sriraman et al., 2015). Chemotherapeutic drugs can be modified before encapsulating in liposomal

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formulations, to mitigate cytotoxicity in free tissues, and taking advantage of tumor microenvironment specific factors. PEGylated formulation of mitomycin C prodrug was explored in multiple GI tumor models including colon, gastric, and colon cancer, exploiting drug release mediated by reductive factors in tumor tissues (Gabizon et al., 2006; Gabizon et al., 2012). Alternately, active metabolites of drugs were also formulated to overcome drug resistance mediated by mutational changes within the tumor cells. Gemcitabine triphosphate, a pharmacologically active nucleotide analog, and a metabolite of gemcitabine (Zhang et al., 2013b), was formulated in lipid calcium phosphate (LCP) nanoparticles, a nanoformulation with an asymmetric lipid bilayer (Li et al., 2012), and was reported to be efficacious in pancreatic cancer. Tumor-specific factors can further be harnessed to tailor the delivery of small molecules using liposomal formulations, in the clinic. Merrimack Pharmaceuticals, who developed Onivyde® earlier is currently investigating (NCT03076372) an ephrin receptor A2 (EphA2) antibody directed liposomal docetaxel in solid tumors including gastric and pancreatic cancer. Immunohistochemistry of clinical tumor samples were used to develop a framework for screening patients for inclusion in the clinical trial based on EphA2 expression in the patient's tumor (Kamoun et al., 2016). Overall, these approaches suggest that liposomes have many advantages in mitigating the cytotoxicity of chemotherapeutics and other small molecules and augmenting therapeutic benefit by improving drug delivery and release in the tumor. Taking lessons from the early clinical trials, a new generation of liposomal formulations are moving into clinical trials, bringing hope for several GI malignancies with limited therapeutic options.

Gene Delivery

Exogenous nucleic acids like DNA, messenger RNA (mRNA), siRNA, short hairpin RNA, miRNA, antisense oligonucleotides (ASO), and aptamers had been widely investigated preclinically and clinically for therapy in cancer and other genetic diseases (Ozpolat et al., 2014; Yin et al., 2014; Ramamoorth and Narvekar, 2015; Xiang et al., 2017). Several oligonucleotide therapeutics

received regulatory approval over the years (Stein and Castanotto, 2017), including the recent RNA interference (RNAi) drug Patisiran™, an siRNA formulated in lipid nanoparticles, after a successful Phase III clinical trial in patients suffering from hereditary transthyretin amyloidosis (Adams et al., 2018). There are a few studies that had explored liposomes for delivery of viral vectors in GI cancers (Liu et al., 2011; Wang et al., 2011). The primary objective in those studies was to explore if liposomes can be exploited to protect adenovirus from neutralizing antibodies. There had been, however, extensive efforts investigating liposomes and other lipid-based products for non-viral gene therapy (Guo and Huang, 2012). The reports of early phase clinical trials for nucleic acids using liposomes in cancer date as far back as 2004. An ASO complementary to c-raf-1 proto-oncogene were investigated in twenty two patients with advanced solid tumors, and colorectal cancer was the most common cancer type in the patients (Rudin et al., 2004). While hypersensitivity reactions associated with liposomal formulations hindered the therapeutic administration, this paved the way for preclinical optimization of formulations in the subsequent years.

Nucleic acids, in general, are susceptible to degradation by nucleases. Complexation with cationic/ionizable lipids can prevent degradation of nucleic acids. Further, nucleic acids are required to be delivered into a specific subcellular compartment to achieve its therapeutic function. Liposomes possess the capability to protect nucleic acids along its physiological journey and mediate cargo release in a specific compartment inside the cells, facilitating cytosolic trafficking and nuclear transport if required (Guo and Huang, 2011; Hu et al., 2013b; Saffari et al., 2016). To mitigate the toxicity of the cationic liposomes without compromising efficacy, extensive efforts were made to design ionizable lipid-based liposomes. The lipid head groups remain unprotonated during circulation, however, undergo protonation in the acidic pH of the early or late endosome, facilitating interaction with anionic endosomal membrane lipids, and promoting cargo release into the cytosol (Kanasty et al., 2013).

A broad range of nucleic acid cargos had been delivered using liposomal formulations in GI cancers (Ozpolat et al., 2014; Harrison et al., 2018). Liposomal formulations of plasmid DNA had been explored in multiple studies with intraperitoneal colon cancers (Kline et al., 2009; Lan et al., 2010; Aoyama et al., 2017). To further increase the stability of the plasmid cargo and achieve improved control over drug release, several polymers like polyethyleneimine, poly-L-lysine, and other cationic polymers and polycations had been used to complex with anionic DNA and encapsulate in liposomal nanoparticles (Goodwin and Huang, 2014). Recently, plasmid DNA based therapeutics were explored for non-viral gene therapy and immunotherapy in several GI malignancies including cancers of colon, pancreas, and liver (Goodwin et al., 2016; Miao et al., 2017a; Shen et al., 2018; Song et al., 2018; Zhou et al., 2018). Plasmid therapy using liposomal formulations had also been explored in patients with advanced solid tumors in a Phase I trial, including cancers of colon and rectum (Senzer et al., 2013). The study demonstrated p53 gene expression in tumors and toxicities observed were low-grade, leading to a Phase II clinical trial in patients with metastatic cancer of the pancreas, which is ongoing recruiting as of October 2018 (NCT02340117).

Liposomal nanoformulations were also investigated extensively for RNAi therapies in GI malignancies and other solid tumors (Zhang et al., 2013a; Xin et al., 2017). Atu027, an siRNA against Protein Kinase 3 was encapsulated in liposomes and had been explored in a phase Ib/IIa trial against metastatic pancreatic cancer as a combination therapy with gemcitabine (Aleku et al., 2008; Schultheis et al., 2016). The therapy demonstrated dose-dependent benefit, and therapy was well-tolerated, although grade 3 toxicities were recorded in most patients. Other emerging nucleic acid-based therapeutics like miRNA (Shah et al., 2016) had also been explored in the clinic in GI malignancies like liver cancer using liposomal formulations (NCT01829971). Although the expression of target genes was repressed based on patient tumor analyses, the trial had to be suspended as five patients suffered from immune-related adverse events (Peltier et

al., 2016; Beg et al., 2017). It is unclear if the adverse events were mediated by liposomal formulation, alterations in gene expression driven by the miRNA therapeutic, or inflammation actuated by double-stranded RNA (Dempsey and Bowie, 2015). However, future translational efforts of nucleic acid-based therapeutics needs to consider these factors to warrant strong anti-tumor activity while mitigating toxicity associated with the therapeutic or its carrier.

Harnessing liposomal drug delivery to treat liver metastases

The liver is a common site of metastasis for a wide variety of primary GI tumors, including colorectal and pancreatic adenocarcinoma (de Ridder et al., 2016). Metastatic dissemination significantly impacts mortality in cancer, accounting for about 90% of cancer-related deaths (Chaffer and Weinberg, 2011). In colorectal cancer patients, five-year overall survival differs significantly between patients with (16.9%) and without liver metastases (70.4%) (Engstrand et al., 2018). Liposomes are well-suited for hepatic delivery because of enhanced distribution in the liver which is further tunable with active targeting. As we discussed previously, several ligands had been used in the past to direct therapeutics to hepatocytes.

Recently, LCP nanoparticles with asymmetric lipid bilayer were shown to be efficacious in delivering plasmids expressing protein traps in murine liver metastasis models of colorectal and breast cancer (Goodwin et al., 2016; Goodwin et al., 2017). LCP nanoparticles delivering phosphorylated adjuvants and peptides also demonstrated efficacy in arresting colorectal cancer metastasis (Goodwin and Huang, 2017). As discussed earlier, a liposomal formulation of miRNA had also been explored in patients with primary cancers of liver or liver metastases (Beg et al., 2017). Stimuli triggers were also used preclinically for treatment of liver metastases. Iron oxide and oxaliplatin were loaded in PEGylated liposomes, and drug release was triggered by an alternating magnetic field (Gogineni et al., 2018). In the recently reported Phase III trial results from thermosensitive liposomal doxorubicin in combination with RFA in patients with unresectable hepatocellular carcinoma, treatment of patients with multiple lesions was found to be challenging

as repositioning the probe for ablation resulted in the loss of local tissue concentration of doxorubicin (Tak et al., 2018). Overall, there remain significant challenges associated with drug delivery targeting metastatic tumor sites like liver warranting increased attention in clinical drug development targeted to metastases over the primary tumor (Ganapathy et al., 2015).

Liposomal vaccines

Liposomes are well-suited as vaccine delivery systems in cancer, and beyond. Cationic lips and liposomal formulations can augment the immune response to an antigen by stimulation of innate immune response (Alving et al., 2016). As carrier systems, liposomes can facilitate the antigen uptake, trafficking, processing, and presentation by enhancing lymphatic tissue drainage and tuning the release of antigen and adjuvant cargo from the delivery system (Watson et al., 2012; Schwendener, 2014). Several types of macromolecules like DNA, mRNA, peptides, and proteins as antigens can be loaded in liposomes as cancer vaccines (Banchereau and Palucka, 2018). Two distinct classes of antigens can be explored as candidates for cancer vaccines, nonmutated tumor antigens that are overexpressed in cancer tissues with otherwise restricted expression pattern, and neoantigens, created by alterations in DNA resulting in the formation of new protein sequences absent from a normal host genome (Schumacher and Schreiber, 2015). Liposomes allow coencapsulation of different classes of adjuvants with antigens in a cancer vaccine, allowing delivery and therapeutic efficacy in highly aggressive and metastatic GI tumor models (Goodwin and Huang, 2017). In the past, liposomal formulations of adjuvants like monophosphoryl lipid A were encapsulated with protein tumor antigens and the vaccine formulations were explored in human patients with colorectal cancer (Neidhart et al., 2004). Further, active targeting principles had been employed to target liposomes to immune cell populations like dendritic cells *in vivo* (Xu et al., 2014; Goodwin et al., 2017), to bypass the challenges and hurdles of *ex vivo* antigen priming using donor dendritic cells (Cintolo et al., 2012). As personalized cancer vaccines are slowly coming of age with peptides and RNA as antigens (Ott et al., 2017; Sahin et al., 2017), we are

optimistic about the prospect of liposomal formulations in the development of cancer vaccines in GI malignancies.

Summary and Perspectives

Liposomes have come a long way from conceptualization to drug carriers in the clinic, as delivery systems in a wide range of marketed pharmaceutical products. The journey was not a smooth ride, and there were a lot of failures and bumps along the clinical development pathway. Lessons from early clinical trials facilitated optimizations of formulations to mitigate toxicities and adverse events. Liposomal formulations are still relevant in cancer drug development, and we witnessed the first clinically approved liposomal drug formulation for a GI malignancy recently in 2015, using a similar remote loading principle that was exploited to develop Doxil® about twenty years ago. Liposomes and other lipid-based nanoparticles have also established their positions as carriers for nucleic acids. As drug delivery to extra-hepatic targets is improved, we can hope to see more gene therapy interventions against GI cancers in the clinic. There are a few therapeutics with liposomal formulations, that are actively explored against GI malignancies in human patients. One of these studies (Camp et al., 2013; Senzer et al., 2013) is targeting to restore normal p53 tumor repressor gene function, a protein that is mutated in most patients with pancreatic ductal adenocarcinoma, and currently being explored in a Phase II clinical trial (NCT02340117). Further, nanomedicine drug development is slowly coming of age, as more considerations are applied to recruiting patients. Approaches like analyses of the patient tumor with proportions of cells expressing the protein used for active targeting of liposomes are used to determine if a specific patient is suitable for treatment with the targeted liposomal drug formulation. This patient selection strategy can also be expanded to take advantage of passive targeting in the right subset of patients. Considering the heterogeneity of EPR, ‘one size fits all’ approach may not be suitable, and non-invasive imaging should be exploited to determine if the patient’s tumor is leaky to be passively targeted by liposomal formulations. In the upcoming days, we can expect to see more

rationally designed therapeutics bypassing the challenges of safety, efficacy, and regulatory hurdles, and making into the clinic to tackle some of the challenging GI malignancies, achieving specific therapeutic objectives.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Das, and Huang.

Disclosure Statement

Huang is a co-founder of OncoTrap, Inc.

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Footnote

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Legends for Figures

Table 1 Selected liposomal products in clinical development in gastrointestinal cancers

Figure 1 Onivyde®, a clinically approved liposomal formulation in metastatic pancreatic cancer. Multivalent anionic trapping agents are used to retain irinotecan cation inside the liposome

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Conditions	Intervention	Phase	Sponsor	Clinical Trials Identifier
Advanced Colorectal Cancer	CPX-1 (Irinotecan HCl:Floxuridine)	II	Jazz Pharmaceuticals	NCT00361842
Gemcitabine resistant Metastatic Pancreatic Cancer	Liposomal irinotecan (Onivyde®)	III (Approved 2015)	Merrimack Pharmaceuticals	NCT01494506
Gastric, Gastroesophageal, and Esophageal Adenocarcinoma	Oxaliplatin in transferrin conjugated-liposome	I/II	Mebiopharm Co., Ltd.	NCT00964080
Non-resectable hepatocellular carcinoma	ThermoDox (Thermally sensitive liposomal doxorubicin)	III	Celsion	NCT02112656
Locally advanced/metastatic pancreatic cancer	EndoTAG-1 (Paclitaxel in cationic liposomes)	III	SynCore Biotechnology Co., Ltd.	NCT03126435
Pancreatic Cancer	(LE-DT) Docetaxel	II	INSYS Therapeutics Inc	NCT01186731
Metastatic/unresectable GI cancers	Liposomal irinotecan	I/II	Emory University	NCT03368963
Liver Cancer	Liposomal Doxorubicin with TTFields (alternating electric fields)	I	M.D. Anderson Cancer Center	NCT03203525
Liver Cancer	MTL-CEBPA (CEBPA small activating RNA (saRNA) in liposomes	I	Mina Alpha Ltd.	NCT02716012
Metastatic colorectal cancer	SN-38 liposome	II	Alliance for Clinical Trials in Oncology	NCT00311610

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Solid tumors including gastric/gastroesophageal/pancreatic ductal adenocarcinoma	(MM-310) Docetaxel in liposomes targeted with antibodies to EphA2 receptor	I	Merrimack Pharmaceuticals	NCT03076372
Locally advanced/metastatic esophageal carcinoma	PNU-93914 (Liposomal paclitaxel)	II	Memorial Sloan Kettering Cancer Center	NCT00016900
Metastatic pancreatic cancer	SGT-53 (Human p53 plasmid DNA in cationic liposome)	II	SynerGene Therapeutics, Inc.	NCT02340117
Primary liver cancer	MRX34 (Liposomal miRNA-34a mimic)	I	Mirna Therapeutics, Inc.	NCT01829971
Metastatic pancreatic cancer	Atu027 (Liposomal Protein Kinase N3 siRNA)	I/II	Silence Therapeutics GmbH	NCT01808638

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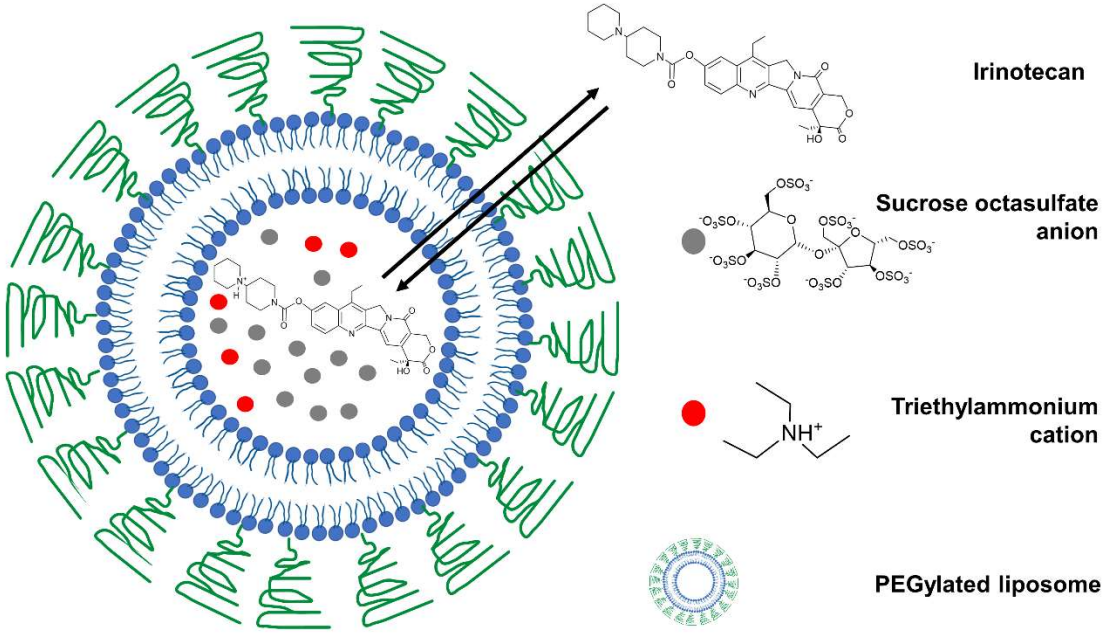

Figure 1 *Onivyde®, a clinically approved liposomal formulation in metastatic pancreatic cancer. Multivalent anionic trapping agents are used to retain irinotecan cation inside the liposome*