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Review

A Snapshot of Photoresponsive Liposomes in Cancer Chemotherapy and Immunotherapy: Opportunities and Challenges

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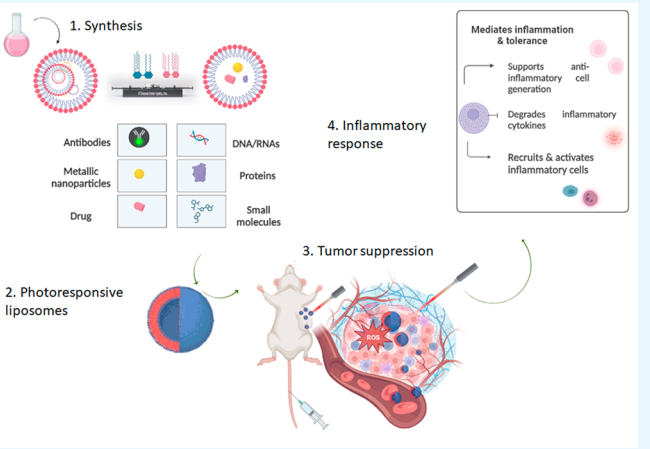
ABSTRACT: To provide precise medical regimens, photonics technologies have been involved in the field of nanomedicine. Phototriggered liposomes have been cast as promising nanosystems that achieve controlled release of payloads in several pathological conditions such as cancer, autoimmune, and infectious diseases. In contrast to the conventional liposomes, this photoresponsive element greatly improves therapeutic efficacy and reduces the adverse effects of gene/drug therapy during treatment. Recently, cancer immunotherpay has been one of the hot topics in the field of oncology due to the great success and therapeutic benefits that were well-recognized by the patients. However, several side effects have been encountered due to the unmonitored augmentation of the immune system. This Review highlights the most recent advancements in the development of photoresponsive liposome nanosystems in the field of oncology, with a specific emphasis on challenges and opportunities in the field of cancer immunotherapy.



1. INTRODUCTION

The development and use of novel nanomaterials for theranostic (diagnosis and therapeutic) purposes is known as nanomedicine.[1−3] These nanoscale carriers promote the targeted controlled delivery of various therapeutic molecules via increasing endocytosis, improving tumor cellular uptake, and enhancing the permeability and retention effect (EPR). Furthermore, these nanocarriers improve the pharmacokinetic properties and half-lives of the different payloads while minimizing their toxic off-target impact on healthy tissues.[2,4−7]

Liposomes are among the nanoscale carriers that represent well-known and reliable drug and gene delivery systems that are frequently employed in treatment of cancer, and the well-known liposomal doxorubicin (Doxil, Janssen Biotech, Inc.) represents the best example of a selective tumor targeting agent with decreased toxicity. It was the first FDA-approved liposomal medication for the treatment of several malignant tumors such as Kaposi's sarcoma, ovarian cancer, and multiple myeloma.

Liposomes that are triggered by external physical stimuli such as photoirradiation, magnetic fields, or X-ray irradiation represent novel nanocarrier systems that have an advantage over existing liposome drug delivery systems in that they allow on-demand payload release in response to external stimuli.[1,8]

Using light as an external stimulus for different photoresponsive nanocarriers has several benefits compared to other external stimulants, such as noninvasiveness, sharp spatial resolution, simplicity, and affordability.[9] Light of an optimized wavelength is utilized as a stimulus for the photothermal therapy (PTT), photodynamic therapy (PDT), and controlled release of loaded drugs/nucleic acids from photosensitive nanosystems containing light-dissociable compounds, such as coumarin derivatives, *o*-nitrobenzyl, or others.[10] Upon exposure to light at the optimized wavelength, the nanostructures release their payloads via either the disassembly of the nanosystem by the photothermal effect, photocleavage, or the structural deformations of the nanosystem caused by photoisomerization.[9] Three light wavelength ranges are used as triggers: (i) ultraviolet (UV, 200−400 nm), visible light (400−650), and near-infrared (NIR, 650−900 nm).[11] Even though UV stimulation generates more effective photochemical

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reactions and potent energy that are effective in both photodynamic and photothermal therapies (PDT and PTT), NIR stimulation is commonly used for the reasons presented in Table 1.

Table 1. Comparison between the Significant Features of Ultraviolet and near-Infrared Light Irradiations in Photodynamic and Photothermal Therapies

	UV stimulation	NIR stimulation
wavelength range (nm)	200−400	650−900
energy	more energetic	less energetic
photochemical reactions	more effective	less effective
penetration	poor body tissue penetration (<1 mm)	deep body tissue penetration (1−4 mm)
effectiveness against deep tumors	not effective against deep tumors effective against superficial tumors	effective against deep tumors
applicability in PTT and PDT	can be transformed into heat (for PTT) or reactive oxygen species (for PDT)	can be transformed into heat (for PTT) or reactive oxygen species (for PDT)
safety	hazardous to healthy tissues (could be absorbed by lipids and hemoglobin)	safer to healthy tissues

Lately, recent studies have exploited the advantages of both UV, such as low wavelengths, achieving high energy and effective photochemical reactions, and NIR, such as safety and deep tissue penetration irradiations.[12] In this regard, few strategies have been employed to convert NIR light to UV or visible light after passing the body tissues, such as using the two-photon absorption strategy and lanthanide-based nanostructures.[13] Visudyne (liposome for injection, Novartis AG) was the first to earn FDA approval in 2000 as a photo-engineered liposome for the treatment of myopia and age-related macular degeneration. Verteporfin (VP) is a synthetic porphyrin that is activated to produce highly hazardous reactive oxygen species when exposed to light at 690 nm, causing local damage to the neovascular endothelium.[14]

Immuno-oncology is the new era of cancer treatment.[15−17] It focuses mainly on exploiting this intrinsic tendency of the mammalian immune system to combat cancer.[18−20] The process of distinguishing cancer cells from healthy cells is known as immunosurveillance and is mainly carried out by the tumor-infiltrating lymphocytes such as adaptive cytotoxic T lymphocytes (CTLs) and innate natural killer cells (NK cells),[21,22] as shown in Figure 1. However, despite the apparent immunogenicity of cancer, the body often fails to eradicate it or prevent metastasis on its own. This is partly due to the evident evolution of malignant cells, which evade the immune system by various mechanisms, including the exploitation of immune checkpoints and T cell exhaustion.[23]

It has been evidenced that a liposome-based combination of phototherapy and immunotherapy can increase the therapeutic index of these modalities by improving the stability and biocompatibility of the cargo as well as minimizing the adverse effects.[24,25] In the same context, various studies have suggested that using nanocarriers in conjunction with immunotherapy and photothermal therapy could result in more significant antitumor immunological effects.[26,27]

Although scientists have spent the last ten years focusing on light-triggered liposomes in drug/gene delivery, the shallow depth of light irradiation still limits the spectrum of therapeutic indications because the light is applied externally. Furthermore, if free medications are freely delivered, they are likely to be activated in nontarget sites. As a result, the authors in this

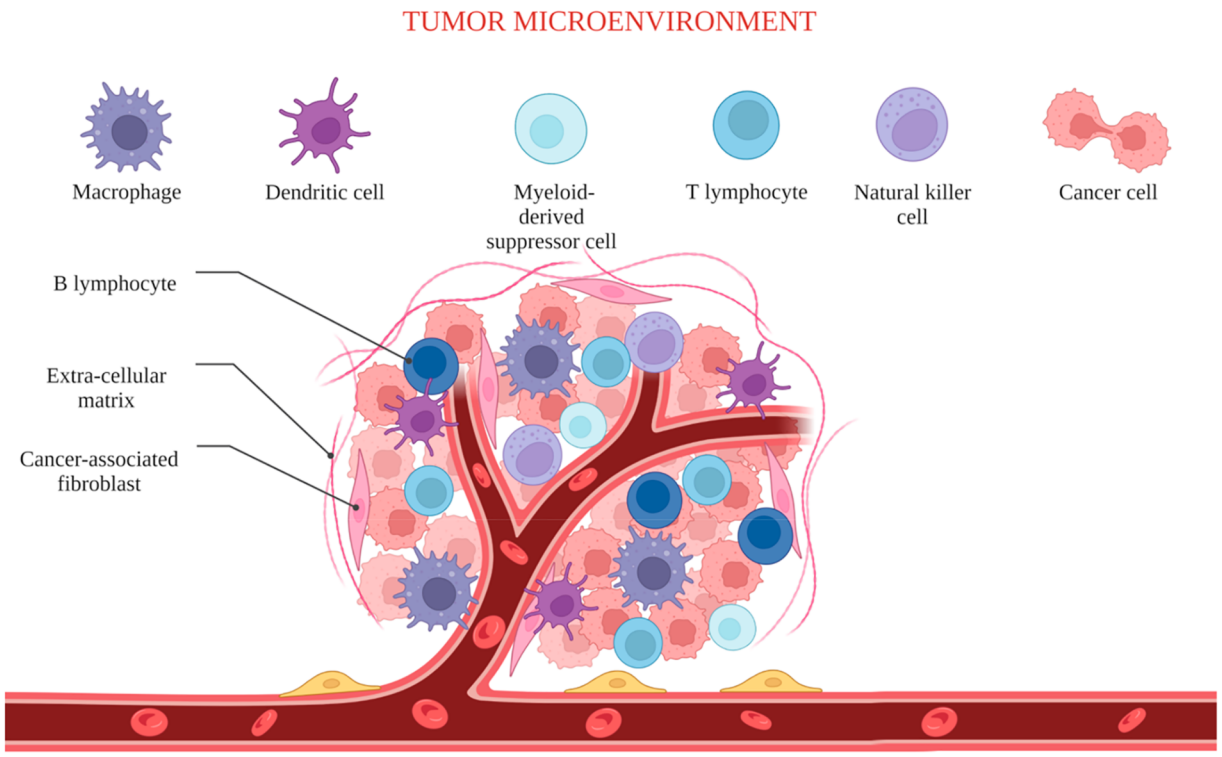

Figure 1. A schematic diagram illustrating the tumor microenvironment. Created by Biorender.com.

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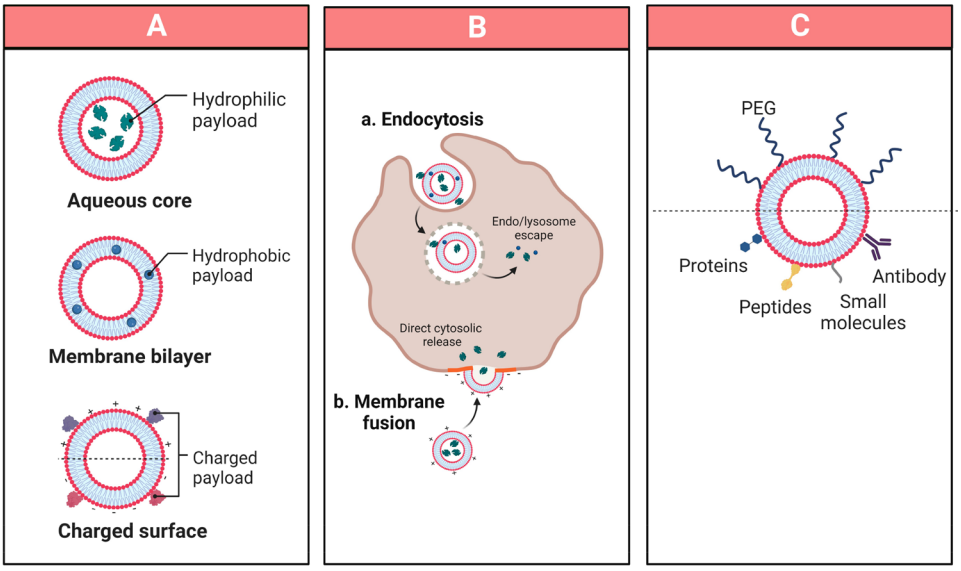

Figure 2. Schematic diagram illustrating (A) drug loading, (B) modes of crossing the tumor cell membrane, and (C) surface functionalization of liposomes. Created by Biorender.com.

Review address the most recent achievements in light-triggered liposomes with respect to their structure and encapsulation power for chemotherapeutic, immunotherapeutic, and oncological molecular targets, with a special emphasis on the potential alternative routes to these present obstacles for future research and clinical use.

2. LIPOSOMES AS A DRUG DELIVERY SYSTEM

2.1. Structure and Properties. Liposomes are developed by self-assembling biocompatible and biodegradable phospholipids and cholesterol in an aqueous medium to form concentric nanovesicles comprised of lipophilic tails (fatty acids chains) and polar heads (phosphate ester groups).[28]

The unique structure of liposomes, an aqueous center entrapped within lipid bilayers, facilitates the chaperoning of various hydrophilic and hydrophobic therapeutic molecules such as phytochemicals, chemotherapeutic or immunotherapeutic agents, and RNA molecules for cancer therapy (Figure 2A).[4,29,30] Charged therapeutic moieties could bind to the liposomal membranes through electrostatic interactions (Figure 2A). Additionally, liposomes can cross the tumor cell membrane through endocytosis or membrane fusion (Figure 2B), expediting the preferential cellular uptake and passive accumulation of loaded drugs and/or photosensitizers (PS) inside cancer cells.[31,32] In addition, using liposomes as nanocarriers in cancer therapy is auspicious because their surfaces could be tailored with different targeting moieties, such as folic acid, hyaluronic acid, antibodies, and peptides, ensuring the active targeting of liposomes to their target site of action (Figure 1C).[33,34] Additionally, using liposomes as nanocarriers for drug/RNA/PS delivery would improve the pharmacokinetics of the loaded therapeutic molecules and prolong their half-lives (>72 h) via surface coating with polyethylene glycol (PEG, Figure 2C).

The composition of the liposomes is crucial in controlling the integrity of the liposomes, as it gives the liposomes their unique features such as particle size, stiffness, fluidity, stability, and electrical charge.[35,36] The phospholipids (PLs) involved in designing liposomes might be natural, such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylserine (PS), or synthetic, such as 1,2-dipalmitoyl-*sn*-glycero-3-phosphorylethanolamine (DPPE), 1,2-distearoyl-*sn*-glycero-3-phosphorylethanolamine (DSPE), 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC). In addition, cholesterol steroid (Chol) is used in combination with the PLs to control the fluidity of the liposomal membrane. Surfactants (Span 60 and Tween 60) are also used in the formulation of liposomes to lower the surface tension between various immiscible phases, improving the drug/PS encapsulation and release profiles. Finally, polymers, such as PEG (polyethylene glycol), are used to decorate the liposomal surface to shield the liposomes from circulating proteins, increasing systemic circulation time and lowering immunogenicity while increasing therapeutic efficacy.[35] The major components used in the formulation of liposomes and their chemical structures are summarized in Table 2.

3. PHOTOTRIGGERED LIPOSOMES (PTLS)

3.1. Design, Optimization, And Physicochemical Properties. Since the FDA approved the first photoresponsive liposomes, Visudyne, in 2000, several studies reported the engineering and optimization of light-sensitive liposomes loaded with various therapeutic moieties to achieve controlled and convenient cancer therapy.[37,38] Numerous physical and chemical properties should be considered when fabricating photoresponsive liposomes, as they can influence the drug encapsulation capacity, liposomal stability in body fluids, payload release percentage in response to light stimulation, and, eventually, the therapeutic activity.[39] These factors are lipid membrane fluidity,[40] lipid phase transition (T_m),[41] and lipid polymerization.[42]

Lipid membrane permeability is an essential factor that should be optimized to achieve the maximum payload release capacity upon exposure to a light stimulus. The components used in formulating PTLs are key players that control liposome's physicochemical and biological features. For instance, using DSPC as one component of the PTLs led to an increase in the stability and entrapment efficiency of the liposomes as compared to using either dipalmitoylphosphati-

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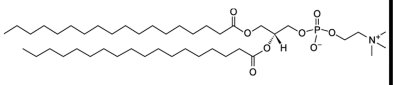
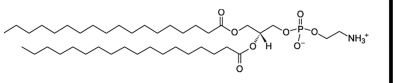
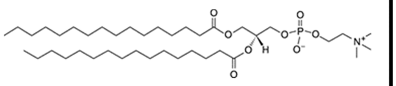
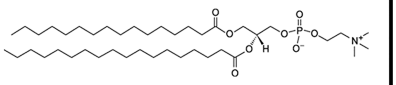
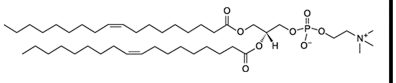
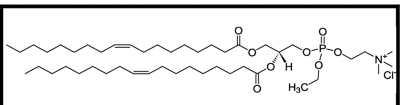
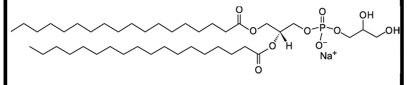
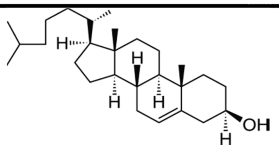
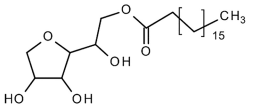
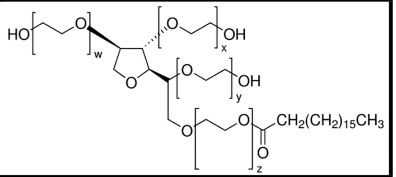
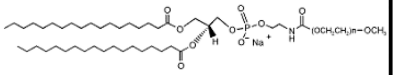
Table 2. Major Components and Their Chemical Structures Used in the Design of Liposomes

Liposome component	Type	Charge	Chemical Structure
DSPC (1,2-Distearoyl-*sn*-glycero-3-Phosphocholine	Phospholipid	Zwitterion	
DSPE (1,2-Distearoyl-*sn*-glycero-3-phosphorylethanolamine	Phospholipid	Zwitterion	
DPPC (1,2-Dipalmitoyl-*sn*-glycero-3 -phosphocholine	Phospholipid	Zwitterion	
HSPC (L-α-Phosphatidylcholine, hydrogenated (Soy)	Phospholipid	Zwitterion	
DOPC (1,2-Dioleoyl-*sn*-glycero-3-phosphocholine	Phospholipid	Zwitterion	
EPC (1,2-Dipalmitoyl-*sn*-glycero-3-ethylphosphocholine (chloride salt)	Phospholipid	Zwitterion	
DSPG (1,2-Distearoyl-sn-glycero-3-phosphoglycerol, sodium salt	Phospholipid	Anionic	
Chol (Cholesterol)	Steroid	Neutral	
Sorbitan monostearate (Span 60)	Surfactant	Non-ionic	
Polyethylene glycol sorbitan monostearate (Tween 60)	Surfactant	Non-ionic	
DPPE mPEG5000 (1,2-Distearoyl-sn-glycero-3-phosphoethanolamine -N-[methoxy (polyethylene glycol)-2000] (sodium salt))	Decorating polymer	Anionic	

dylcholine (DPPC) or EPC. The decoration of the PTLs with the DPPE mPEG5000 polymer generates a steric hindrance around the liposomal membrane, minimizing the reticuloendothelial system uptake. Additionally, the inclusion of DPPG in the design of PTLs was reported to enhance the capability of the liposomes to penetrate the tumor cells due to its fusogenic

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features.[37,43] Finally, it was reported that increasing the concentration of Chol led to increasing the stiffness of the liposomal membranes while decreasing their fluidity. Additionally, increasing the molar ratio of Chol was found to reduce the membrane's permeability.[41]

The lipid phase transition (T_m) is another critical factor affecting the cargo release from the lipid bilayer after light irradiation. It is affected by the composition of the phospholipids. Light illumination causes a photothermal effect, increasing the temperature above the T_m.

Consequently, a phase transition of the lipid bilayer occurs from the orderly solid phase to the disassembled one, improving the selective diffusion of the loaded therapeutic moiety out of the liposomal membranes at the tumor site (Figure 3).[41] For instance, a study reported the design of

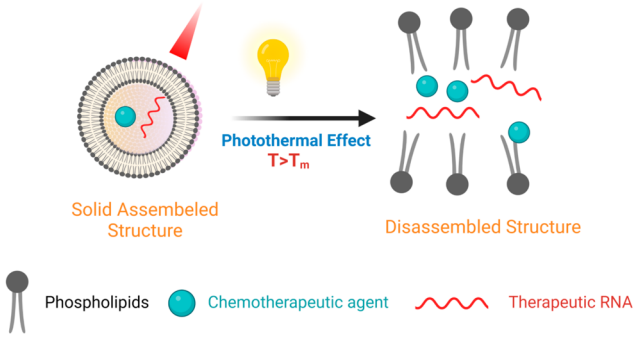

Figure 3. Photothermal effect on the payload release from the photoresponsive liposomes. Created by Biorender.com.

photoresponsive liposomes containing DPPC (T_m = 41 C) and gold nanoparticles. Upon irradiation with NIR radiation of 760 nm, the incorporated gold nanoparticles emit thermal energy (that exceeds the T_m of DPPC) and thus lead to the release of the loaded drugs at the site of action.[44]

Finally, lipid polymerization is another important property that affects the efficient release of loaded therapeutic agents upon light triggering. Photopolymerizable phospholipids, such as DPPC, are essential for disassembling the liposomal membranes, upon light irradiation, through the photo-cross-linking phenomenon. This leads to the selective release of the loaded drugs in the tumor tissues.[36]

3.2. Payload Release Modalities from the Photo-triggered Liposomes (PTLs). The design of PTLs involves incorporating light-responsive entities (such as azobenzene) or PS (such as porphyrins derivatives) within optimized ratios of phospholipids using several approaches, such as thin-film hydration, ultrasonic dispersion, injection, freeze-drying, and microfluidic methods.[45] The typical structure of liposomes is exploited where the light-responsive molecules (PS) are loaded based on their water-solubility; the hydrophilic ones are encapsulated in the aqueous core, while the hydrophobic ones are incorporated in the lipid bilayer.[46] Upon light irradiation, the PTLs are disassembled via either physical or chemical activation of the incorporated light-responsive molecules, leading to the efflux of the payloads at the site of action. Table 3 summarizes the release modalities of the loaded therapeutic agents through either photophysical or photochemical activation.

The liposomes coloaded by PS and anticancer agents are accumulated in the tumor site via passive targeting through the enhanced permeability and retention (EPR) effect. In addition, PTLs could be actively targeted to the intended site of action by functionalizing their surfaces with target moieties that bind to specific overexpressed receptors in the tumor cells (folate receptors, for instance). Both passive and active targeting could decrease the off-target effects and help in the preferential accumulation of PS and payloads in the tumor tissues. Then, upon the light irradiation of the desired site of action, activating the PS leads to the PTT (or PDT) effects.[47]

For instance, a study reported the design of GE11 peptide conjugated liposomes coloaded with curcumin, a natural

Table 3. Release Modalities of the Payloads from Photoresponsive Liposomes

payload release modality	example	mechanism	ref
A. photophysical activation			
1. photothermal conversion	indocyanine green (ICG, FDA-approved)	introducing molecular dyes (ICG) into the liposomes leads to the conversion of NIR into heat upon light irradiation at a definite wavelength, destabilizing the liposomal structure and, eventually, releasing the payloads at the tumor site	49
2. incorporation of plasmonic nano-structures	plasmonic nanoparticles such as gold nanoparticles (Au NPs)	including plasmonic nanoparticles (Au NPs) in the liposomal system upon light illumination (at a specific wavelength), the plasmonic nanoparticles induce heat (surpassing the lipid phase transition (T_m) through the surface plasmon resonance (SPR) effect, dismantling the liposomal lipid bilayers and releasing the loaded therapeutic moieties	50
3. incorporation of inorganic nano-structures	upconverting nanoparticles (UCNPs) such as graphene oxide (GO)	using UCNPs during the engineering of PTL can absorb NIR radiation and convert it to UV−vis energy for instance, GO (a two-dimensional (2D) nanostructure) can absorb NIR radiation and transform it into strong thermal energy via delocalizing the electron the released thermal energy exceeds the T_m of the phospholipids and leads to the disassembly of the liposomes upon phototriggering and light-controlled effluxing of their cargos	51
B. photochemical activation			
1. photocleavage	photocleavable molecules such as coumarin derivatives and the *o*-nitrobenzyl group	the photocleavable entities included in the light-responsive liposomes can irreversibly disrupt the integrity of the liposomes through photocleavage, rearrangement, and electron transfer reactions upon light illumination	52
2. photoisomerization	photoisomerizable entities such as azobenzene	the photoisomerizable compound incorporated in the liposomal lipid bilayer leads to photoinduced conformational modifications when irradiated by NIR radiation these conformational changes then result in the disassembly of the liposomal bilayer membrane and consequent payload release	53
3. photochemical internalization	photosensitizers (PS) such as porphyrins derivatives (verteporfin)	when exposed to light stimulation, the PS incorporated in the liposomes generate reactive oxygen species, which oxidize the liposomal phospholipids this leads to the rupture of the lipid bilayer and the subsequent release of the loaded therapeutic molecules.	54

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Table 4. Phototriggered Liposomes Encapsulating Chemotherapeutic and Immunotherapeutic Agents

liposome system	liposome components	photosensitizer (PS)	*in vitro*/*in vivo* model	therapy model	outcome	ref
NIL-IM-Lip	1-MT, IR780, NGR motif, Mal-MMP2 sensitive pep-IL-15, DPPC, HSPC, and DSPE PEG2000	IR780	B16F10, CT-26, MC38, and HUVEC cell lines	PTT	enhances the effectiveness of T and NK cells, induces immunogenic cell death, and suppresses regulatory T cells	70
poly I:C- and ICG-containing TRLs (piTRLs)	DPPC, MPPC, DSPE-PEG2000, and poly I:C	ICG	B16F10 CT-26 cell lines	PTT	activates dendritic cells (DCs) and cytotoxic T cells and accelerates cancer cell death	68
ICG thermosensitive liposome	DPPC, ICG, and cisplatin	ICG	HeLa 4T1 cell lines	PTT	enhances efficacy either for PTT alone or with chemotherapy	64
FA-L@MD@CAT liposome	SPC, DSPE-mPEG2k or FA-DSPE-mPEG2k, cholesterol, MBDP, and Dox	MBDP	4T1MCF7 cell lines	PDT	reduces hypoxic conditions in the tumor microenvironment and boosts the efficacy of PDT	66
Hyp-HPbCD-loaded liposomes and hypericin TEL liposomes	DSPC, DPPC, TEL Hypericin, and HPbCD	hypericin	SK-OV-3 cell lines	PDT	more precise delivery of the photosensitizer to the tumor site	60
AQ4N-64Cu-hCe6 liposome	DPPC, DSPEmPEG5k, AQ4N, and hCe6	hCe6 (hexadecylamine-conjugated chlorin e6)	Balb/c mice bearing 4T1 tumors	PDT	improves the elimination of cancer in a mice model via sequential PDT and hypoxia-activated chemotherapy AQ4N	67
zwitterionic liposomes	poly(12-methacryloyloxy)dodecyl phosphorylcholine DSPC	methylene blue (MB)	4T1 cell lines	PDT	increases cytotoxicity against breast cancer cells	65
chitosan-coated liposomes	DMPC, Cholesterol Chitosan	ICG	melanoma cell lines	PDT	increases permeability and phototoxicity against melanoma cell lines	61
lipopolyplexes (LPPs)	DOPC DPPC Cholesterol	curcumin	SK-OV-3 B16F10 cell lines	PDT	increases and improves gene delivery to SK-OV-3	62
stealth liposomes (decorated with PEG)	HSPC DPPE mPEG5000	curcumin	MUG-Mel2 SCC-25 cell lines	PDT/PTT	increased cytotoxicity against cancer cells and decreased cytotoxicity for normal keratinocytes.	63
GNOL (gold nanoshell-coated liposomes)	oleanolic acid cholesterol chitosan	gold	143B cell lines	PTT	increases target specificity and improved therapeutic efficacy of the encapsulated drug	71

chemotherapeutic, and indocyanine green (ICG), a photosensitizer. The presence of GE11 peptide facilitates the selective targeting of liposomes via binding to the epidermal growth factor receptor (EGFR) on the lung tumor surface. Then, upon NIR irradiation, the liposomes released their payloads, which synergistically generated reactive oxygen species (ROS) and induced apoptotic pathways, improving the anticancer effects while reducing off-target effects.[48]

4. PHOTOTRIGGERED LIPOSOMES IN CANCER CHEMOTHERAPY AND IMMUNOTHERAPY

4.1. Phototriggered Liposomes Encapsulating Chemotherapeutic and/or Immunotherapeutic Agents. As previously mentioned, cancer nanomedicine is a hot topic that has been highly tackled by researchers from different disciplines.[55] This displays an arsenal of nanotherapeutics with proven better efficiency in the sustained delivery of several anticancer agents.[56] Phototriggered liposomes have been on top of the list of nanocarriers tailored for cancer patients, prompting the development of personalized nanomedicine among cancer patients.[57,58] PDT and PTT are less invasive local therapy methods that eliminate cancerous cells by producing highly toxic reactive oxygen species (ROS) or hyperthermia upon light illumination.[59] For instance, Hyp-HPbCD-loaded liposomes containing hypericin (Hyp) as a photosensitizer showed a phototoxic effect against ovarian adenocarcinoma only after irradiation.[60] Additionally, the encapsulation of indocyanine Green (ICG) in chitosan-coated liposomes gave stable liposomes, enhanced their uptake by B16-F10 melanoma cancer cells, and increased and improved phototoxicity.[61] According to another study, a novel multimodal delivery system using curcumin-loaded lipopolyplexes (LPPs) in combination with PDT was created, and it enhanced the delivery of RNA molecules to the SKOV-3 cancer cell.[62] In a different study, curcumin was loaded into liposomes made of HSPC and DPPE-mPEG5000, which improved the stiffness of the liposomal bilayer membrane, prevented curcumin from leaking outside the lipid membranes, and led to increased cytotoxicity toward cancer cells.[63] Another study reported the fabrication of photoresponsive liposomes that contained both the anticancer medication cisplatin and ICG.[64] Through a photothermal action mediated by ICG under NIR illumination, cisplatin was selectively released to tumor tissues, demonstrating an improved anticancer efficacy either with PTT alone or in conjunction with chemotherapy.[64] Furthermore, Wu et al.'s study described the creation of zwitterionic liposomes containing methylene blue (MB) PS, which may produce reactive oxygen species (ROS) in response to light exposure and induce the death of cancer cells.[65] This work encapsulated MB into zwitterionic liposomes to protect loaded medicines from degradation in the bloodstream, prolong systemic circulation, and improve MB cellular uptake into cancer cells. DSPC was used to self-assemble a zwitterionic polymer−lipid, poly(12-(methacryloyloxy)dodecyl phosphorylcholine), in a 1:4 molar ratio, which showed enhanced cytotoxicity on breast cancer cells through the release of ROS[65] (Table 4).

The hypoxia caused by the tumor microenvironment is one of the issues that has been identified as an impeding factor for PDT's effectiveness. Consequently, numerous methods have been devised to address these issues, such as coloading nanoparticles to provide adequate oxygen self-generation, which improved the effectiveness of PDT by reducing tumor microenvironment (TME)-induced hypoxia. Another idea is creating a unique liposomal platform encapsulating the catalase (CAT) enzyme for combinatory photo/chemotherapies to tackle tumor hypoxia.[66] For instance, doxorubicin (Dox), catalase, and NIR photosensitizer-loaded liposomes were

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invented with an improved lysosome targeting capability. Catalase reduced the amount of in the hypoxic TME and boosted the PDT's efficacy by successfully causing the breakdown of H_2O_2 to produce *in situ* O_2.[66] Another intriguing work created novel light-triggered liposomes by coloading PS, chlorin e6, and the prodrug AQ4N, demonstrating a therapeutic potential in treating cancer under hypoxic conditions.[67] Under hypoxic conditions, the targeted delivered AQ4N was preferentially activated inside cancer tissues to release the more cytotoxic active form. This liposomal delivery system produced both the PDT effect and hypoxia-activated chemotherapy when exposed to LED light at 660 nm, which significantly improved the overall treatment results for the 4T1 tumor-bearing mouse model, as shown in Table 4.[67] Moreover, to test the viability and potential of combining PTT and immunotherapy, PTT-responsive liposomes coloaded with the immunostimulatory drug polyinosinic:polycytidylic acid (poly I:C) and indocyanine green were formulated.[68] The liposomal structure was disassembled upon irradiation with 808 nm laser light, inducing a photothermal therapeutic impact against B16 melanoma tumor and CT-26 colorectal carcinoma models and accelerating cancer cell death. The released poly I:C from the liposomes clearly activates dendritic cells (DCs) in nearby lymph nodes.

Additionally, a new triple-sensitive liposomal system called NIL-IM-Lip (pH/MMP2/temperature triple-sensitive) was reported to remodel the repressed tumor lymph node immune microenvironment (TLIME) by simultaneously mobilizing the adaptive and the innate immune arms at the TME, namely, cytotoxic T cells and natural killer (NK) cells, respectively.[69] Following the pH-sensitive release of the NGR motif (that binds to CD13) and the MMP2-responsive release of IL-15, the temperature-sensitive NIL-IM-Lip is directed to the lymph nodes. In addition, it contains IR780 (photosensitizer) and 1-MT (IDO inhibitor) that simultaneously induce immunogenic cell death and suppress regulatory T cells during photothermal stimulation.[70]

4.2. Phototriggered Liposomes Modulating Tumor Microenvironment and Cancer Cellular Behaviors through Encapsulating Genetic Material. The application of gene therapy to combat cancer oncological signaling pathways has been prompted by a growing body of knowledge in regard to its modulatory role for tumor cells and their surrounding TME. Gene therapy includes exogenous nucleic acids, such as genes, gene segments, oligonucleotides, microRNAs (miRNAs), or small interfering RNAs (siRNAs). Such genetic materials are delivered through *ex vivo* or *in vivo* modalities through several carriers, such as liposomes, nanoparticles, viral vectors, and many others.[72] In this section, the authors will shed light on the use of phototriggered liposomes in delivering genetic materials into cancer cells.

For instance, a study reported the usage of noncationic photoresponsive liposomes—Al(III) phthalocyanine chloride disulfonic acid (AlPcS2a)—and siRNAs ,where it was reported that the noncationic LPS results in minimizing off-target effects and improving the selective uptake of the siRNAs by cancer cells such as HepG2 and SK-HEP-1.[73] Several studies have recently reported the successful delivery of the CRISPER/Cas 9 system using liposomes. For example, the fabrication of liposomes loaded with photosensitizer verteporfin (VP) and sgRNA against the TNFAIP3 gene (that encodes the A20 protein, which is a tumor promoter in numerous cancer types, such as breast and liver cancers, and glioblastoma) was reported. The usage of these phototriggered liposomes resulted in a decrease in the TNFAIP3 mRNA level and increased knockdown efficiency.[74] Another multifunctional vehicle was reported for the delivery of Cas9-sgRNA. It was based on a lipid/AuNPs complex coated by TAT peptide for nucleus targeting and sgRNA targeting Plk-1 (a master mitotic regulator that is frequently overexpressed in tumor cells). This multifunctional system was successful enough to produce a PlK-1 knockout model. The designed liposomes led to effective tumor (melanoma) target gene (Plk-1) knockouts and tumor suppression owing to the synergic effect and the interaction between the photothermal-mediated intracellular release of the CP and the CP-induced cell death.[75] Table 5 summarizes the photoresponsive liposomes encapsulating genetic materials used in cancer therapy.

4.3. Phototriggered Liposomes As Carriers for Novel Immunotherapeutic Agents and TME-Remodeling Agents. Immune surveillance is the process by which different immune system elements safeguard the host against the establishment of primary tumors, promote tumor escape, or both, either by sculpting tumor immunogenicity or attenuating antitumor immune responses and forming what is called the TME.[15,78]

Cancer immune editing or immune escape is the process by which cancer cells evade the immune system using many tactics, such as defective antigen presentation, inducing apoptosis for cytotoxic T cells and NK cells, immune suppressive mediators, immune checkpoints, and other mechanisms, and the best way to restore the antitumor effect of the immune system is by immunotherapy.[16,17]

Various immunological elements are found upon cellular examination of a typical TME, indicating an immune battle against the tumor.[21,78−80] The appearance of multiple cytokines, macrophages, neutrophils, and tumor-infiltrating lymphocytes (TILs), among others, signify an attempt of the host immune system to eradicate the tumor.[22,81−84]

As previously mentioned, the recent introduction of immunotherapeutic agents in the field of oncology has been considered a revolution that restored faith in many desperate cancer victims.[19,21,85] Cancer immunotherapy has significantly improved patients' chances of survival and quality of life as compared to earlier standards of care (such as chemotherapy, radiation, and surgery), and there are many strategies for cancer immunotherapy like vaccination therapy, adoptive T cell therapy, and immune checkpoint inhibition on the molecular and cellular scale.[84] In this section, the authors will highlight the recent literature discussing the role of phototriggered liposomes in immunotherapy.

4.3.1. Liposome-Mediated Delivery of Immune Checkpoint Inhibitors. Immune checkpoints, by definition, are signals received by immune cells to either stimulate or repress their action. In a functional scenario, these direct the immune system to properly aggress during the initial stages of response before prompting it to regress during later stages to prevent autoimmune damage/prolonged inflammation. One example of an immune checkpoint dynamic is the interaction between the costimulatory T-cell receptor CD28 and the inhibitory cytotoxic T lymphocyte-associated antigen 4 (CTLA4), a receptor expressed in relatively low numbers only after T-cell activation. While CD28 prompts T-cell amplification upon binding to its B7 ligands (CD80 and CD86), CTLA4 counteracts this due to its higher affinity to the identical

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Table 5. Photoresponsive Liposomes Encapsulating Genetic Materials Used for Cancer Therapy

liposomes	liposomes component	photosensitizer	cargo	*in vitro*/*in vivo* model	outcomes	ref
noncationic LPs	DOPC	Al(III) phthalocyanine chloride disulfonic acid (AlPcS2a)	siRNA	HepG2 SK-HEP-1 cell lines	minimizing off-target effects and providing selectivity to hepatoma cancer cells	73
CRISPER/Cas 9 liposomes	DOTAP, DOPE, cholesterol	verteporfin (VP)	CRISPER/CAS 9 sgRNA against TNFAIP3	HEK293 cell lines	increased knockdown efficiency	74
TAT peptide-modified Lipid Encapsulated Gold Nanoparticles	AuNPs (DOTAP, DOPE, cholesterol, PEG2000-DSPE	gold	CRISPER/CAS 9sgRNA-targeting Plk-1	Melanoma cell lines and *in vivo* tumor model	Plk-1 knockout and tumor suppression *in vitro* and *in vivo*	75
light-triggerable liposome (lipVP)	DOPC DOTAP	verteporfin (VP)	antisense oligonucleotides against PAC1R	PC12 cell lines	increased PAC1R repression efficiency	76
X-ray triggerable liposomes	DOPC DOTAP	verteporfin (VP)	antisense oligonucleotides Against PAC1R	PC12 HCT 116 cell lines	increased PAC1R repression efficiency	77
liposome−polycation−DNA (LPD) nanocomplexes	DOTAP, PEGylated neutral lipid and cholesterol	Verteporfin (VP)	polyethylenimine (PEI)/plasmid DNA (pDNA) encoding EGFP	HCT116 cell lines	improves transfection efficiency	46

ligands and resultant competition with CD28, as shown in Figure 4.[86]

As the other key player in terms of immune checkpoints, PD-1 is a chief target of immune checkpoint blockade. A notable aspect of PD-1 is that its ligand, PD-L1, is commonly overexpressed on malignant cell surfaces to serve as another method for suppressive immune evasion method. The interaction between PD-1 and PD-L1 is characteristic of T-cell exhaustion and thus the abrogation of this interaction has been proposed to effectively "reverse" this exhaustion, as shown in Figure 4. Henceforth, treatments by either anti-PD-1 mAb or anti-PD-L1 mAb have been tested and showed promising results.[87,88]

Liposomes have been recently introduced to immune checkpoint blockade therapy, especially targeting PD-1/PD-L1 inhibitors, increasing their efficiency, and reducing their previously mentioned possible side effects. A double-layer system liposome has been employed by Lang et al.[89] to encapsulate the PD-1 inhibitor HY19991 and thioridazine. The liposomes successfully increased the accumulation of the HY19991 and thioridazine drugs inside the tumor tissue.[89]

From another perspective, Merino et al. employed liposomes coupled with PD-L1 mAbs to modulate the immune system from the other side of the cancer−immune synapse.[90] Such approach results showed that liposomes containing 5% PEG showed the highest hindering of the PD-L1 suppressor effects. Furthermore, Hei et al. developed another type of liposomes coupled with anti-PD-L1 on their surface, yet they also contain the catalase enzyme inside them; those liposomes showed better therapeutic effects and, most importantly, low general toxicity.[91] Gu et al. also reported the reduced systemic toxicity and tissue damage of PD-L1 antibodies when formulated with liposomes in mice melanoma models.[92]

It is worth noting that a combination of phototriggered liposomes and immune checkpoint inhibitors has been reported to be effective in improving the immunogenic profile of cancer cells. Lan et al. designed a new phototriggered liposome called Lp(DHA)@CP (composed of a polyunsaturated fatty acid-doped liposomal hydrogel) loaded with the PD-L1 antibody (αPD-L1) and photosensitizer chlorin e6 (Ce6). In response to the NIR light irradiation, abundant ROS were produced by the integrated Ce6, which then caused the liposomal hydrogel to disintegrate for the on-demand sustained release of PD-L1. Additionally, the produced ROS in TME was able to make low-immunogenic "cold" tumors "hot" by boosting the T cell infiltration in tumors, which in turn increased their potential responses to ICB treatments.[93]

Another liposomal system coloaded with a photothermal agent (IR780), folic acid (FA) (that increases tumor targeting and also increases the enhanced permeability and retention (EPR) effect of the liposomal system), oxaliplatin (OXA), and PD-L1 inhibitors (BMS-1) was fabricated. The created liposomal system demonstrated advantageous tumor tissue biodistribution and tumor cell endocytosis, boosting the photothermal and anticancer effects, as well as improved dendritic cell maturation and infiltration of antigen-specific T lymphocytes into the residual tumors and blocking the immune checkpoint as summarized in Table 6.[94]

4.3.2. Liposome-Mediated delivery of Immunogene Therapy. Fusogenic liposomes represent an optimum RNA delivery vehicle, since they protect the RNA moiety from endocytic sequestration and lysosomal destruction.[95] Anionic fusogenic liposomes were created by Stremersch et al. and

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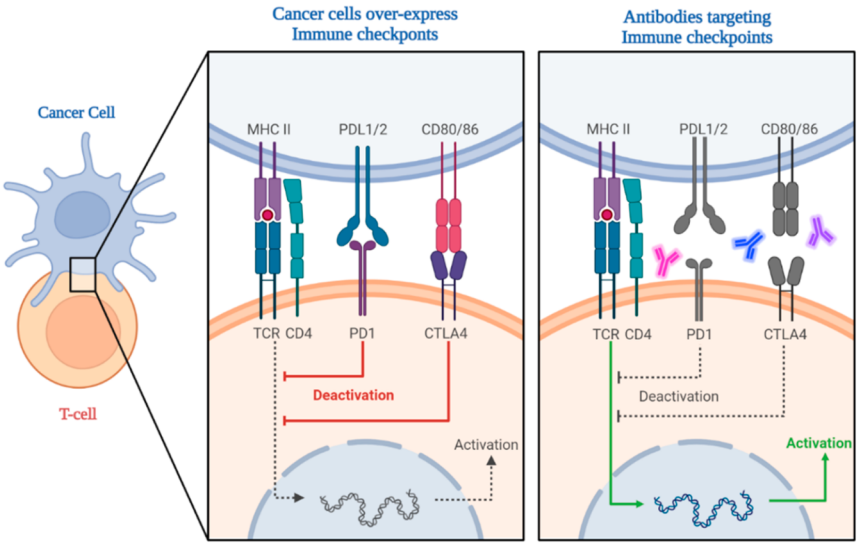

Figure 4. Mechanism of action of antibodies targeting overexpressed immune checkpoint proteins on cancer cells. Created by Biorender.com.

Table 6. Photoresponsive Liposomes Encapsulating Immunotherapeutic and TME-Modulating Agents

liposomes	immunotherapeutic agent	photosensitizers	immunotherapy strategy	effector cells	cancer cell	outcomes	ref
Lp(DHA) @CP	αPD-L1 (Ab)	Ce6	PD-L1 inhibition	$CD4^+$ T cells $CD8^+$ T cells macrophages	4T1 cells	activated tumor-infiltrating lymphocytes	93
FOIB@Lip	FA, BMS-1 oxaliplatin	IR780	PD-L1 inhibition	$CD8^+$ T cells dendritic cells	CT26 cells	improves dendritic cell maturation and infiltration of antigen-specific T cells into the tumor site	94
IND@RAL	indoleamine-2,3-dioxygenase (IDO) inhibitor	porphyrin	TIME-remodeling agent	$CD8^+$ T cells $CD4^+$ T cells NK cells	4T1 cells	activates innate and adaptive immune arms promotes antigen presentation increases T cell infiltration	103
FA-L@ MD@ CAT	encapsulated catalase (CA) and doxorubicin (Dox)	MBDP	TIME-remodeling agent	tumor-associated macrophage	4T1 cells	boosts the efficacy of the PDT by decreasing hypoxia decreases M2 tumor-associated macrophages (TAM)	66
PGIL	galectin-3 inhibitor (low molecular citrus pectin (LCP))	chlorin e6 (Ce6)	TIME-remodeling agent	NK cells	A375 cells	Inhibition of migration and invasion capacities of tumor cells Immune activation of NK cells	104

were assessed for their ability to transport siRNA to the monocyte/DC (JAWSII) cell line and B16F10 cancer cells.[96] Another interesting delivery method for DC targeting is RNA lipoplexes. When delivered systemically to lymphoid organs, they can shield RNA from extracellular ribonucleases and permit the selective expression of their RNA cargo in local APCs. By enhancing *in situ* CD8 T cell immunity, Salomon et al. revealed RNA lipoplexes encoding CD4 T cell-recognizable neoantigens and created powerful adaptive T cell responses.[97] Hybrid lipopolyplexes containing *N*1-methyl pseudouridine nucleoside-modified mRNA has been reported by Thielemans et al. to diminish inflammatory responses without impairing T-cell immunity. Compared to mRNA and lipoplexes in this investigation, immunization with lipopolyplexes demonstrated robust T cell immunity and higher efficacy in suppressing tumor growth.[98]

4.3.3. Phototriggered Liposomes: TME Remolding Agents. In addition to the conventional photosensitive liposomal effects in the ablation of tumors, PTT and PDT have been recently employed to alleviate the immunosuppressive TME intrinsically and, at the same time, act as a carrier for several immunotherapeutic agents.[99]

On the mechanistic level, the produced heat during either PTT or PDT radiation can induce the eradication of cancer cells by activating the involved immune cells at the TME. This is mainly accompanied by the release of tumor-associated antigens, ATP, and high mobility group box 1 protein into the TME and the translocation of calreticulin to the cell surface.[100] Such release will result in the uptake of the antigens by antigen presentation cells and the activation of effector T cells and thus initiation signals for antitumor immunosurveillance episodes.[101] In addition, PTT-mediated intrinsic immunogenic effects can further enhance antitumor immune responses of immunotherapeutic agents, leading to combinational action for effectively eradicating cancer cells and alleviating the immune-suppressive TME.[102]

Liu and colleagues formulated a photosensitive liposomal system coloaded with porphyrin as a photosensitizer and indoleamine-2,3-dioxygenase (IDO) (which is an enzyme that changes tryptophan (Trp) to kynurenine (Kyn), which could impair the survival and activity of $CD8^+$ T cells) inhibitor as an immune-metabolic adjuvant. This designed liposomes were reported to initiate the cytotoxic T cell-mediated eradication of cancer cells through the induction of the immunogenic cell death (ICD) cascade and the simultaneous blockage of the

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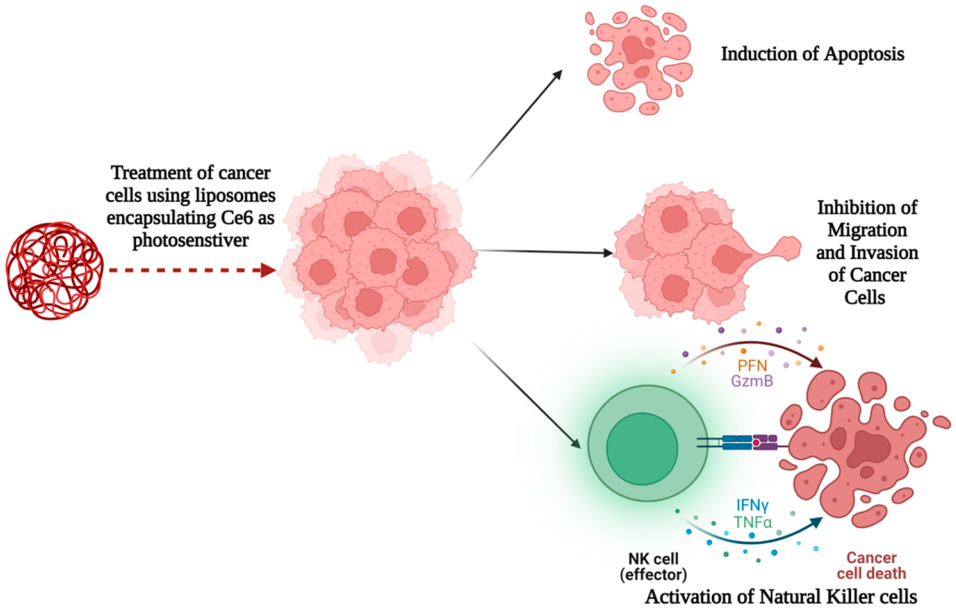

Figure 5. Mechanistic role of chlorine e6 liposomes in halting cancer cells through oncological and immunological pathways. Created by Biorender.com.

IDO pathway.[103] This highlights the synergistic spectacles that could happen upon combining PDT and immunotherapeutic agents and the potent reversal of the immunosuppressive TME, fighting against metastatic cancers.[103] Shi et al. developed a liposome-based immune activation method that dramatically enhanced tumor growth inhibition when paired with photodynamic therapy. Specifically, they developed a novel liposomal-encapsulated catalase (CAT), lyso-targeted NIR photosensitizer (MBDP), and doxorubicin (Dox) to achieve combinatory chemo-PDT to promote tumor oxygenation and antagonize the immunosuppressive effects of the TME.[66]

Another study by Wang et al. reported that a chlorine e6 (Ce6) liposome formulation encapsulating galectin-3 inhibitor low molecular citrus pectin (LCP) was used against melanoma by combining PDT with immunotherapy.[104] It was speculated that Ce6 induced PDT therapeutic effects against melanoma via apoptosis, inhibition of migration and invasion capacities of tumor cells, and eventually immune activation effects, as shown in Figure 5. Kim et al. reported the potent immunomodulatory effects of light-triggered liposomes in a xenograft mouse model bearing human liver bile duct carcinoma cell line.[105] The Kim group formulated the liposomes using a PS-conjugated lipid encapsulating gemcitabine as a conventional chemotherapeutic agent. A confirmation of immunomodulatory cells at the TME recruited as a result of light-triggered liposomes has been done using immunohistochemical analysis and also helped in the maturation of dendritic cells, production of proinflammatory cytokines and, finally, activation of T cells.[105]

Photoresponsive liposomes encapsulating immunotherapeutic and TME-modulating agents are summarized in Table 6.

5. CHALLENGES AND OPPORTUNITIES OF TRANSLATIONAL PHOTOTHERAPY

Although various photoresponsive liposomes are considered the mainstay of phototherapy, poor light penetration limits the applicability to cutaneous conditions.[59] However, different solutions to this problem have been suggested, such as the creation of medical gadgets that make it easier to apply light endoscopically. In the case of Photofrin, an optic guide fiber diffuser was designed for employing endoscopically administered light to irradiate the lungs.[106]

Another option is to construct photosensitizer in nanoformulation, limiting systemic circulation and managing the photosensitizer's action. A phase 1/2 clinical trial (NCT02367547) is currently being conducted to investigate using PDT against cancer utilizing a nanoscale lipid vesicle gel composition.[106]

A phase 3 clinical trial is currently being conducted in primary liver cancer patients to assess the efficacy of combining microwave hyperthermia, radiofrequency, thermal ablation, or high-intensity focused ultrasound with ThermoDox/OPTIMA, a liposomal drug that releases DOX sealed in lysolipid thermally sensitive liposomes at temperatures above 40 °C.[37,107,108] This raises the question of whether the external stimulus that generates heat could be replaced with a light source, increasing the expandability of this nanoplatform.

6. CONCLUSIONS AND FUTURE PROSPECTS

Despite the fact that phototriggered liposomes have been at the top of the list of nanocarriers that have prompted the development of precision cancer nanomedicine, there are still many challenges that limit the application of photoresponsive liposomes, particularly in solid tumors where the external shallow application of light may not be able to penetrate. This opens up the possibilities to new solutions, such as the development of nanoformulation photosensitizers that allow target-specific penetration or by embedding medical devices that allow for the use of endoscopically administered light.

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