

# Advances in research and application of photodynamic therapy in cholangiocarcinoma (Review)

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**Abstract.** Cholangiocarcinoma (CCA) is a disease characterized by insidious clinical manifestations and challenging to diagnose. Patients are usually diagnosed at an advanced stage and miss the opportunity for radical surgery. Therefore, effective palliative therapy is the main treatment approach for unresectable CCA. Current common palliative treatments include biliary drainage, chemotherapy, radiotherapy, targeted therapy and immunotherapy. However, these treatments only offer limited improvement in quality of life and survival. Photodynamic therapy (PDT) is a novel local treatment method that is considered a safe tumor ablation method for numerous cancers. It has shown good efficacy in various studies of CCA and is expected to become an important treatment for CCA. In the present study, the mechanisms of PDT in the treatment of CCA were systematically explored and the progress in the research of photosensitizers was discussed. The current study focused on the various PDT protocols and their therapeutic effects in CCA, with the objective of providing a new horizon for future research and clinical applications of PDT in the treatment of CCA.

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**Key words:** cholangiocarcinoma, photodynamic therapy, photosensitizer, combination therapy

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## 1. Introduction

Cholangiocarcinoma (CCA) is a highly malignant epithelial cell tumor that can occur in the bile duct tree and/or liver parenchyma. Based on its anatomical location, it can be classified as intrahepatic CCA, hilar CCA or extrahepatic CCA (1,2). In recent years, the incidence of CCA has increased globally (3). There are no obvious symptoms in the early stages of CCA and most patients show only clinically evident symptoms during the later stages of the disease, resulting in a 5-year survival rate of ~10% (4,5). Tumor resection is the primary treatment modality for CCA, but for the majority of patients, the surgical opportunity is often missed at the time of definitive diagnosis (6). Patients who cannot be treated surgically can only choose palliative care. Without effective palliative treatment options, patients may die from tumor metastasis, biliary obstruction-induced infection or liver cirrhosis. Currently, the efficacy of biliary drainage, chemotherapy and radiotherapy as treatment options for advanced or unresectable CCA is limited (7-9). Therefore, the study of new efficient treatment methods is of great significance for expanding the treatment options for patients with CCA. Photodynamic therapy (PDT) is a modern localized treatment method that selectively destroys pathological tissues through the interaction of laser-activated photosensitizers (PSs) with oxygen molecules, resulting in a photochemical reaction (10). PDT offers advantages such as selectivity, minimal trauma and fewer side effects compared to conventional treatments. Its favorable efficacy and potential for combination therapy with other treatment modalities have led to its wide use in cancer treatment, including for the treatment of CCA, since its first application in 1991 (11). The primary objective of PDT in advanced CCA is to alleviate biliary obstruction, promote bile duct drainage and slow tumor growth. It may also be used as a preoperative or postoperative adjuvant treatment for CCA.

PDT has superior efficacy and minimal adverse effects when used as a palliative therapy for advanced CCA. Therefore, PDT was included as a treatment modality for palliative therapy in the 2013 Asia-Pacific CCA consensus recommendations (12),

which indicated that PDT has significant clinical significance for CCA, particularly in advanced cases. The present review comprehensively summarizes the development of PSs in PDT, progress in understanding the mechanisms underlying the targeting of tumor cells and advances in PDT-based treatment for CCA from various perspectives.

## 2. PDT mechanisms and photodynamic reaction

PDT is based on the interaction between three components that induce photochemical reactions *in vivo*: PS, specific wavelength light and dissolved oxygen in the tissue. After local or systemic injection of PSs into patients, these agents selectively accumulate in actively proliferating cells, such as tumors. Subsequently, the PSs are activated by light at the appropriate wavelength. Upon absorption of photons, the PSs transition from the ground state ( $S_0$ ) to the singlet state ( $S_1$ ) or undergo intersystem crossing, leading to the formation of the triplet state. Then two competitive reactions, known as type I and II reactions, are initiated (13) (Fig. 1A).

Type I reactions involve the direct transfer of protons or electrons from triplet-state PSs to cellular substrates, resulting in the formation of cationic or anionic free radicals. These free radicals interact with oxygen molecules, generating hydrogen peroxide ( $H_2O_2$ ), superoxide anions ( $O_2^-$ ) and hydroxyl radicals ( $\cdot OH$ ), thereby producing reactive oxygen species (ROS) (14). By contrast, type II reactions involve the direct transfer of energy from triplet-state PS to oxygen molecules, creating cytotoxic singlet oxygen with strong oxidizing properties. The ROS generated can induce cell apoptosis or death, vascular damage and immune activation (15-17). Type I and type II reactions usually occur simultaneously and the ratio between these reactions depends on the type of PSs, the oxygen concentration and the affinity between the PSs and the substrate. In general, type II reactions dominate and singlet oxygen is considered to be the main cytotoxic factor in PDT and is involved in a variety of biological effects, including cell death.

Furthermore, the cytotoxic effect of PDT is dependent on the selective uptake of PSs by tumor cells. The concentration of PSs in tumor cells should be significantly greater than that in normal cells to ensure the killing of tumor cells while preserving normal cells. The main reasons behind the greater uptake of PSs in tumor cells than in normal cells are their physiological differences. These differences include increased tumor vascular permeability, abundant lipoprotein receptors, increased internal volume, decreased pH in the microenvironment and impaired lymphatic drainage (18,19). ROS affect tumor tissue development through a variety of reactions and have an important role in the disease course. However, further exploration of the multiple mechanisms of ROS in tumors, such as those related to increasing the enrichment of PS in tumor cells, thus increasing the killing power of PDT in tumors and reducing the damage to normal tissues, is needed; in the field of materials science or optics, more efficient ROS-generating PSs and more appropriate light can be developed.

## 3. Research and development of PS

As a core component of PDT, PSs have a crucial role in determining its effectiveness. An excellent PS should possess

the following characteristics: i) Strong absorption in the near-infrared and far-infrared region, but weaker absorption in the visible light regions; ii) high selectivity for tumor tissue over normal tissue; iii) high quantum yield, capable of efficiently generating ROS; and iv) high dark toxicity and biocompatibility, among others (20-23). PSs can be classified into different categories on the basis of their chemical structure and composition, such as porphyrins and phthalocyanines. The existing PSs have been developed from the first generation of traditional PSs to the third generation of functional PSs (Table I). First-generation PSs are derivatives of hematoporphyrin, such as Photofrin, which is the most widely used PS in PDT for CCA (24). However, first-generation PSs have limitations in deep tissue targeting due to their short excitation wavelength, relatively low selectivity and long residence time *in vivo*. Second-generation PSs include various compounds, such as porphyrin derivatives, metal phthalocyanines and chlorophyll degradation products. Compared to first-generation PSs, second-generation PSs generally have improved selectivity and clearance time, and reduced skin photosensitivity. These PSs are often single pure compounds. One example of a second-generation PS is meso-(tetrahydroxyphenyl)chlorin (mTHPC), also known as temoporfin, which is widely used in PDT for CCA. mTHPC exhibits high tumor selectivity and a high quantum yield of ROS generation (25-27).

Third-generation PSs are developed by combining second-generation PSs with biological or chemical components, with the aim of reducing their impact on normal cells, enhancing targeting efficiency, tumor cell selectivity and ROS production, and enabling active targeted transport. In a previous study by our group (28), near-infrared II fluorescent nanoparticles (NPs) were designed, which are novel organic NPs with efficient passive targeting and a high ROS generation rate. These NPs can accumulate in digestive system tumors to guide laser irradiation to kill deep tumor tissues, and their superiority was proved in mouse models, providing a potential strategy for PDT treatment of digestive system tumors (28). Stein *et al* (29) constructed stable mTHPC-albumin NPs using NP albumin-bound technology. They found that CCA cells (TFK-1) exhibited increased uptake of mTHPC and increased cytotoxicity, suggesting potential directions for the selection of PSs for PDT in CCA. m Grunebaum *et al* (30) prepared three new polymer-based meso-(tetrahydroxyphenyl) porphyrin-based NPs, of which the NPs prepared with Eudragit® E showed 200-fold drug accumulation in CCA cells. These formulations effectively overcome the problem of low drug solubility and demonstrate the high efficiency of PS transporters *in vitro*. Kurokawa *et al* (31) explored the effect of porphylipoprotein (PLP), a PS, on the efficacy of PDT in CCA cells. PLP is a lipid-like nanostructure based on a porphyrin analog. When the porphyrin moiety in PLP is quenched due to its structure, it dissociates into individual molecules, acting as a PS to induce cytotoxicity. Therefore, when PLP is not disturbed, normal cells do not undergo photodynamic reactions. They found that PLP accumulated significantly more in tumor tissue than in normal cells, and PDT using PLP demonstrated efficacy against CCA (31). Disas *et al* (32) fabricated interstitially targeted liposomes (ITLs) encapsulating zinc phthalocyanine (ZnPC) and aluminum phthalocyanine (AlPC) and performed *in vitro* and *in vivo* experiments on

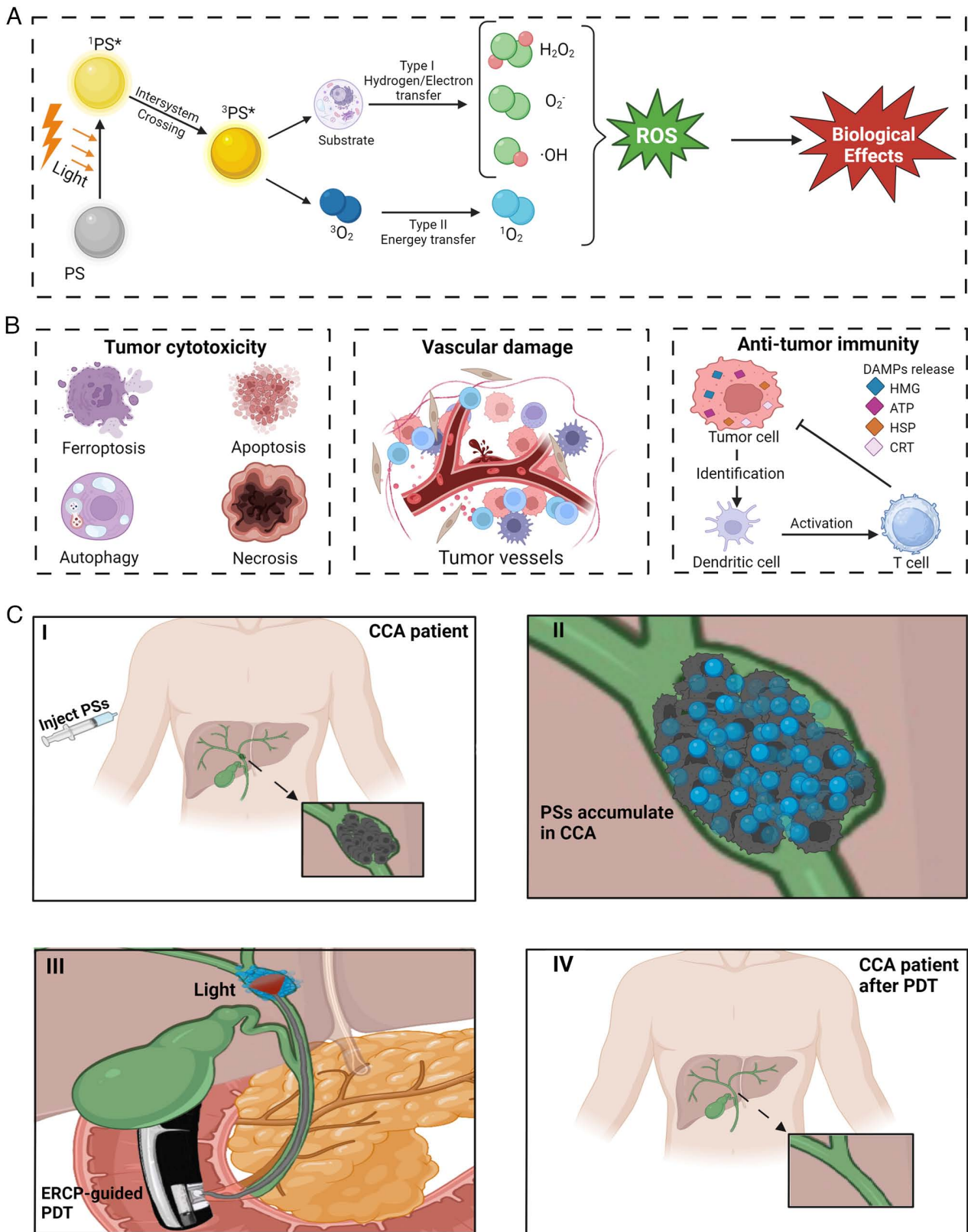


Figure 1. (A) Schematic illustration of the principle of PDT. (B) Anti-tumor mechanisms of PDT. (C) ERCP-guided PDT for CCA: I) Injection of PSs into patients with CCA; II) PSs are gathered in CCA tissue; III) ERCP-guided PDT for CCA; IV) the tumor tissue was ablated with PDT. ROS, reactive oxygen species; PSs, photosensitizers; CCA, cholangiocarcinoma; PDT, photodynamic therapy; ERCP, endoscopic retrograde cholangiopancreatography; DAMP, damage-associated molecular pattern; HSP, heat shock proteins; CRT, calreticulin; HMG, high-mobility group proteins.

ZnPC-ITLs, AIPC-ITLs and their derivatives (ZnPCS4 and AIPCS4). Among them, AIPCS4 is considered to be the least toxic and most effective PS of these agents, which is

conductive to its clinical translation (32). Near-infrared (NIR) light excitation can provide deeper penetration, but based on the low conversion efficiency and difficult molecular design of

Table I. Classification and examples of PSs.

PS	Generation	Components	Type of tumor	Model	Features	(Refs.)
Photofrin	1	Hematoporphyrin derivatives	Most cancers	Human	The first PS to receive regulatory approval. High accumulation in skin; poor tissue penetration	(24)
Temoporfin	2	Dihydroporphyrin derivatives	Advanced head and neck cancer; basal cell carcinomas; cholangiocarcinoma	Human	Improved selectivity and clearance time, and reduced skin photosensitivity; poor water-solubility, body clearance rate and photo-bleaching	(25)
mTHPC-NP	3	Albumin, mTHPC	Cholangiocarcinoma	TFK-1 cells	Improved biocompatibility and drug targeting	(29)
mTHPP-PLGA-NP	3	PLGA, mTHPP	Cholangiocarcinoma	TFK-1 cells, EGI-1 cells	Improved cytotoxicity	(30)
mTHPP-PLA-NP	3	PLA, mTHPP	Cholangiocarcinoma	TFK-1 cells, EGI-1 cells	-	(30)
mTHPP-Eudragit® E-NP	3	Eudragit® E, mTHPP	Cholangiocarcinoma	TFK-1 cells, EGI-1 cells	Increased intracellular accumulation capacity	(30)
Porphylipoprotein	3	Porphyrin-based NPs that mimic native lipoproteins	Gallbladder carcinoma	NOZ cells; BALB/c nude mice	Attenuate photosensitivity disorder; improved cytotoxicity and intracellular accumulation capacity	(31)
AIPCS4	3	Watersoluble tetrasulfonated derivatives of AIPC-ITLs	Cholangiocarcinoma; human triple-negative breast cancer	SK-ChA-1, HUVECs, NIH-3T3, RAW 264.7; zebrafish and chicken embryos, BALB/c nude mice	Low systemic toxicity in animal model; improved cytotoxicity and tumor-killing capacity	(32)

PSs, photosensitizers; PLA, poly(D,L-lactide); PLGA, poly(D,L-lactide-co-glycolide); ITLs, interstitially targeted liposomes; AIPC, aluminum phthalocyanine; mTHPP, 5,10,15,20-tetrakis(m-hydroxyphenyl) porphyrin; NP, nanoparticle.

traditional NIR photosensitization methods, Zheng *et al* (33) developed a simple lanthanide-triple state sensitization method that employs organic PSs coupled to lanthanide NPs to achieve high-performance near-infrared photosensitization. This method can efficiently generate ROS under ultralow near-infrared radiation and the depth of the treated tissue and therapeutic efficiency can be significantly improved compared with those of the PSs used in traditional PDT (33).

In comparison to first- and second-generation PSs, the combination of inorganic or organic components on the basis of PSs has become the main research direction for promoting tumor death or more precise targeting of lesion tissues. With the advancement of technology and the development of photo-dynamic diagnosis and PDT, accelerating the development of novel nano-PSs with diagnostic and therapeutic functions is a major task in the future. Furthermore, Lu *et al* (34) first applied PSs, which are considered a class of fourth-generation PSs, to metal-organic frameworks in PDT in 2014. This has advantages, such as high stability, high PS loading capacity and facilitation of ROS diffusion. In addition, its excellent image-guidance capability could facilitate PDT in deep tissues (35). Exploring the mechanisms of PDT may provide new ideas and directions for the development of novel PSs.

#### 4. Anti-tumor mechanisms of PDT

Currently, there are three well-known mechanisms of PDT-mediated tumor cytotoxicity: i) Direct tumor cell killing through photochemical reactions during PDT; ii) PDT-induced damage to the tumor vasculature; and iii) PDT-mediated enhancement of anti-tumor immunity. These three mechanisms interact with each other to induce the anti-tumor effects of PDT (Fig. 1B).

**Tumor cytotoxicity.** PDT damages tumors through multiple mechanisms. PDT generates a large amount of ROS, which causes oxidative damage to tumor cells and kills them through apoptosis or necrosis. When high light intensity is used, tumor cells undergo necrosis, which is characterized by swelling of the cytoplasm and organelles, rupture of cell membranes, release of cytotoxic substances and destruction of surrounding normal cells (36). When low light intensity is used, PDT typically induces apoptosis in tumor cells, characterized by cellular shrinkage, chromatinic condensation and the formation of apoptotic bodies. Programmed cell death does not affect surrounding normal cells, as there is no rupture of the cell membrane (37). In previous work, our group has developed a DNA nanosphere platform (DNS) by assembling multifunctional Y-scaffold DNA monomers, aptamers, small interfering RNAs and PSs. This DNS platform can not only enhance targeting and promote tumor cell apoptosis, but also monitor and guide PDT of tumor cells in real time through fluorescence imaging. A variety of treatment methods are integrated in a platform to achieve high-efficiency killing of tumors (38). In addition to combining cell apoptosis, composite PDT combining cell killing mechanisms such as autophagy and ferroptosis can also be constructed.

In addition, PDT can induce tumor cell death through autophagy. The specific mechanism of cell death triggered by PDT depends on various factors, such as the type and

dosage of PS and the location of the subcellular site (39,40). He *et al* (41) used the PS chlorin-A in PDT and reported that it enhanced the levels of the autophagy-related protein Beclin1 and the autophagy-mediated pathway PI3K/AKT/mTOR, thus increasing autophagy and apoptosis rates in human CCA cell lines. Autophagy also participates in the collective antioxidant system to prevent cells from excessive oxidative damage. Therefore, the role of autophagy in the mechanism of PDT needs to be further explored.

Ferroptosis, an emerging topic in tumor research, is a form of nonclassical programmed cell death caused by the accumulation of lipid peroxidation products. Casini *et al* (42) reported that PDT can induce ROS in CCA, resulting in a decrease in intracellular glutathione (GSH) and an increase in malondialdehyde (MDA). MDA and GSH have important roles in ferroptosis induction, suggesting that PDT can induce cell death through this mechanism. Using Cox regression analysis, Zhang *et al* (43) established a model of ferroptosis gene characteristics, and by analyzing and experimentally verifying CCA gene expression data after PDT, they identified four sensitive genes, providing new insight for screening target genes that improve PDT efficacy.

**Vascular damage.** Tumor angiogenesis depends on the growth factors present in tumor cells, and the survival of tumor cells also depends on the supply of nutrients provided by blood vessels. PDT can selectively damage blood vessels within tumors, leading to hypoxia-induced damage to the tumor tissue and ultimately resulting in apoptosis or necrosis of tumor cells. The effect of vascular damage caused by PDT has led to the development of vascular-targeted PDT (VTP). In contrast to PDT, VTP involves targeting and occluding tumor arteries and veins, causing stasis of tumor blood flow (44). Its characteristics include a short drug-light interval during which PSs remain localized within the blood vessels and significantly enhance the effect of vascular damage. Furthermore, studies have shown that VTP combined with verteporfin can induce dose- and time-dependent increases in tumor vascular permeability and decreased blood perfusion (45). Based on the vascular damage effect of PDT, Liu *et al* (46) developed a nanodrug called CeCA, which is composed of the vascular disrupting agent combretastatin A4 (CA4) and the PS chlorine e6. This PS not only generates a large amount of ROS under light irradiation but the CA4 component also mediates endothelial cell inhibition, leading to tumor bleeding and improving the therapeutic efficacy of PDT (46).

**Anti-tumor immunity.** PDT not only damages tumor tissue and blood vessels but also releases proinflammatory cytokines and tumor antigens. This transformation from nonimmunogenic to immunogenic is known as immunogenic cell death (ICD) and mediates the body's anti-tumor immune response. ICD releases damage-associated molecular patterns (DAMPs), which activate innate and adaptive immunity. ROS generated by PDT induce stress in the endoplasmic reticulum in tumor cells, contributing to ICD (47). DAMPs released by tumor cells are recognized by antigen-presenting cells such as dendritic cells, which activate immune cells and promote the activation of tumor-specific T cells. DAMPs involved in PDT-induced ICD include mainly adenosine triphosphate, heat shock

proteins, calreticulin and high-mobility group proteins (48). Researchers have developed various novel PS delivery systems based on the study of PDT-mediated ICD in tumor cells. For instance, Li *et al.* (49) synthesized a PS nano-system modified with an endoplasmic reticulum-targeting peptide, which induces strong endoplasmic reticulum stress on the surface of tumor cells under light irradiation, promoting ICD-related immunotherapy and effectively killing tumor cells. Our team developed an NP that enhances photodynamic effect-mediated ICD and real-time detection of ROS as a way to achieve more precise treatment (50). Recently, photodynamic-immune synergy therapy has attracted increased amounts of attention (51). In the future, the role of ICD in photodynamic effects and immunotherapy can be explored to develop new PSs to enhance the therapeutic efficacy for tumors.

## 5. PDT in CCA

Treatment of CCA relies primarily on radical resection of the lesion at R0. However, for patients with unresectable or non-R0 resected CCA, PDT, as a novel local treatment, is expected to become an effective therapeutic modality or adjuvant treatment. To date, a large number of studies have shown that, compared with traditional methods, PDT can prolong survival and improve patient quality of life in the treatment of various CCAs, which may be helpful for the treatment of CCA, particularly advanced CCA. Table II provides a summary of the clinical studies involved in the various current approaches to PDT for the treatment of CCA. It can be seen that, in both palliative treatment and as a preoperative adjuvant or neoadjuvant and in combination therapies, PDT can increase therapeutic efficacy and improve the prognosis of patients without increasing the risk of complications compared to the original therapies.

*PDT combined with stent implantation for unresectable CCA.* For unresectable CCA, palliative treatment is usually used to control local tumor progression. The primary objective of palliative treatment is to alleviate bile stasis by relieving biliary obstruction through drainage of the bile duct. Palliative treatment options include chemotherapy, radiotherapy, radiofrequency ablation and transarterial chemoembolization. However, satisfactory therapeutic effects are difficult to achieve with these methods (52). Currently, the common method of PDT for unresectable CCA is its combination with biliary stent implantation. The aim is to shrink the tumor and alleviate biliary obstruction by activating PSs in the tumor tissue through light irradiation.

A study divided 184 patients with hilar CCA into three groups: Surgical resection (n=60), PDT combined with stent implantation (n=68) and stent implantation alone (n=56) groups (53). The study investigated and analyzed prognostic indicators and revealed that the median survival time was 12.0 months in patients who underwent PDT combined with stent implantation and 6.4 months in those with stent implantation alone ( $P<0.01$ ). Furthermore, the median Karnofsky performance score before treatment increased by 2.3% in the PDT combined with stent implantation group, while it decreased by 8.3% in the stent implantation alone group ( $P<0.01$ ). This study demonstrated that, compared with stent

implantation alone, PDT combined with stent implantation in the palliative treatment of CCA is more effective at prolonging patient survival and improving patient quality of life. In the surgical resection group, the median survival time was 37 months for R0 resection and 12.2 months for R1 and R2 resection. Consequently, the researchers concluded that despite the promising results of PDT combined with stent implantation, complete tumor resection is necessary to achieve longer survival (53). A non-randomized trial conducted by Zoepf *et al.* (54) involving 32 patients with unresectable CCA also provided evidence that PDT can prolong the survival of patients. The median survival time for the stent combined with PDT and for those who received a stent alone was 630 and 210 days, respectively ( $P=0.0109$ ). Furthermore, combination treatment did not significantly differ from stent implantation alone in terms of cholangitis induction, and only one case of biliary bleeding was reported (54). A recent meta-analysis indicated that, compared with stent implantation alone, PDT combined with stent implantation significantly improved the overall survival (OS) of patients with hilar CCA ( $P=0.002$ ), without increasing adverse reactions (55).

Currently, endoscopic retrograde cholangiopancreatography (ERCP) and percutaneous transhepatic cholangioscopy (PTCS) are commonly used to guide the activation of PSs through laser irradiation (Fig. 1C). Compared with PTCS-guided PDT, ERCP-guided PDT offers the following advantages: i) Multiple segments can be treated at one time; ii) repeated punctures and long-term stent placement can be avoided for patients requiring multiple PDT sessions; and iii) the time required for sinus tract formation can be saved. However, because ERCP requires X-ray guidance, the ability of the operator to accurately locate and irradiate lesions is limited. Zhou *et al.* (56) addressed this issue by using SpyGlass to visualize bile ducts and guide PDT under direct vision. This technique allows more accurate localization, evaluation of treatment effectiveness and even distribution of light through central fiber placement, making it a potential future standard guidance method (56). However, its limitations include the high level of skill required and its narrow scope of application. Lee *et al.* (57) conducted a retrospective analysis comparing 24 patients who underwent PTCS-guided PDT and 13 patients who underwent ERCP-guided PDT. The study did not find any significant differences in prognosis or stent patency rate between the two guidance methods, but the median hospitalization time was longer in the PTCS-guided group (63 days) than in the ERCP-guided group (37 days) ( $P<0.001$ ) (57). Based on the literature, it may be indicated that for PDT combined with stent implantation in the treatment of unresectable CCA, ERCP guidance is more suitable due to its minimal trauma to anatomical structures and greater applicability. Other guidance methods are expected to evolve in the future, potentially expanding their scope of application or providing additional evidence of feasibility.

With respect to the implantation of stents in PDT for unresectable CCA, both plastic and covered metal stents can be chosen. PDT can prolong the patency time of metal stents and improve patient quality of life (58,59). Since the use of PSs combined with stent implantation is based on systemic injection, controlling the concentration in the tumor is challenging. Bae *et al.* (60) and Liang *et al.* (61) addressed this issue

Table II. Summary of trials investigating the effect of PDT on survival in patients with CCA.

Therapy	Study type	Treatment	Patients	Post-treatment status	Adverse events	(Refs.)
PDT combined with stent implantation	RS	PDT+stent	Total: n=68; Bismuth type: I (n=1)/II (n=0)/III (n=14)/IV (n=53)	MST: 12.0 months	Phototoxicity (n=8); Cholangitis (n=38)	(53)
		Only stent	Total: n=56; Bismuth type: I (n=5)/II (n=9)/III (n=12)/IV (n=30)	MST: 6.4 months	Cholangitis (n=32)	
	RCT	PDT+stent	Total: n=16; Bismuth type: I (n=0)/II (n=1)/III (n=0)/IV (n=15)	MST: 630 days	Cholangitis (n=3); Cholecystitis (n=1)	(54)
		Only stent	Total: n=16; Bismuth type: I (n=0)/II (n=0)/III (n=0)/IV (n=16)	MST: 210 days	Cholangitis (n=1)	
Meta-analysis	RS	PDT+stent	Total: n=235	1,2-year survival rate: 56, 16%	-	(55)
		Only stent	Total: n=211	1,2-year survival rate: 25, 7%	-	
	RS	PTCS-directed PDT	Total: n=24; Bismuth type: I (n=0)/II (n=3)/III (n=10)/IV (n=11)	Median hospital stay: 63 days; MST: 11.6 months	Cholangitis (n=2); Cholecystitis (n=1); Phototoxicity (n=1)	(57)
		ERCP-directed PDT	Total: n=13; Bismuth type: I (n=0)/II (n=3)/III (n=5)/IV (n=5)	Median hospital stay: 37 days; MST: 9.5 months	Cholangitis (n=1); Pancreatitis (n=1)	
RCT	RS	Plastic tube stent	Total: n=30; Bismuth type: I (n=0)/II (n=2)/III (n=8)/IV (n=20)	6-month patency rate: 20%; 50% patency period: 112 days	-	(58)
		Metal stent	Total: n=30; Bismuth type: I (n=0)/II (n=1)/III (n=8)/IV (n=21)	6-month patency rate: 81%; 50% patency period: 112 days	-	
	RS	PDT+metal stent	Total: n=18; Bismuth type: I (n=0)/II (n=3)/III (n=5)/IV (n=10)	MST: 356±213 days; median stent patency: 244±66 days	Cholangitis (n=1); Cholecystitis (n=1); Phototoxicity (n=1)	(59)
		Only metal stent	Total: n=15; Bismuth type: I (n=0)/II (n=4)/III (n=7)/IV (n=4)	MST: 230±73 days; median stent patency: 177±45 days	Cholangitis (n=2)	
Postoperative adjuvant therapy for PDT	RS	PTCD-PDT	Total: n=18; TNM stage: I-II (n=4)/III-IV (n=14)	MST: 23 months	Bleeding (n=2); Cholangitis (n=4); Pneumothorax (n=1); Blockage (n=7); Phototoxicity (n=2)	(65)
		Only PTCD	Total: n=21; TNM stage: I-II (n=6)/III-IV (n=15)	MST: 10 months	Bleeding (n=1); Cholangitis (n=3); Pneumothorax (n=1); Blockage (n=6)	
Neoadjuvant PDT for CCA	Phase II clinical trial	PDT before surgery	Total: n=7; Bismuth type: I (n=0)/II (n=1)/III (n=3)/IV (n=3)	All patients achieved R0 resection; 1-year recurrence-free survival rate: 83%; 5-year survival rate: 71%	Phototoxicity (n=1)	(66)

Table II. Continued.

Therapy	Study type	Treatment	Patients	Post-treatment status	Adverse events	(Refs.)
PDT combined with other treatments for CCA	Phase II clinical trial	PDT before surgery	Total: n=7; Bismuth type: I (n=0)/II (n=1)/III (n=3)/IV (n=3)	All patients achieved R0 resection; the overall 1, 3 and 5-year patient survival after surgery was 86, 57 and 43% MST: 20 months	-	(67)
	RS	PDT+chemotherapy	Total: n=36; Bismuth I-II (n=1)/III-IV (n=29)/distal (n=4)/metastasis (n=5)		Cholangitis (n=20); Haemocytopenia (n=7); Bleeding (n=4); Abscess (n=5); Phototoxicity (n=4); Infections (n=4)	(79)
		Only PDT	Total: n=34; Bismuth I-II (n=2)/III-IV (n=30)/distal (n=4)/metastasis (n=17)	MST: 15 months	Cholangitis (n=15); Bleeding (n=1); Phototoxicity (n=3); Infections (n=2)	
		Only chemotherapy	Total: n=26; Bismuth I-II (n=1)/III-IV (n=21)/distal (n=4)/metastasis (n=18)	MST: 10 months	Cholangitis (n=8); Haemocytopenia (n=8); Bleeding (n=1); Abscess (n=2); Infections (n=5)	
Phase II clinical trial		PDT+S-1	Total: n=21; Bismuth type: I (n=0)/II (n=1)/III (n=4)/IV (n=16)	1-year survival rate: 76.2%; MST: 17 months	Abscesses (n=1); Photosensitivity (n=2); Cholangitis (n=2)	(80)
		Only PDT	Total: n=22; Bismuth type: I (n=0)/II (n=5)/III (n=5)/IV (n=12)	1-year survival rate: 32%; MST: 8 months	Abscesses (n=2); Photosensitivity (n=2); Cholangitis (n=2)	

PDT, photodynamic therapy; CCA, cholangiocarcinoma; RS, retrospective study; RCT, randomized controlled trial; PTCS, percutaneous transhepatic cholangioscopy; PTCD, percutaneous transhepatic cholangial drainage; ERCP, endoscopic retrograde cholangio-pancreatography; MST, median survival time; S-1, an oral fluoropyrimidine prodrug.

by embedding PSs into the stent, allowing controlled release of the PSs in the tumor tissue and reducing their uptake by normal tissues. Furthermore, this approach allows repeated PDT to provide sustained relief from malignant obstruction and local tumor control, thus avoiding the discomfort of repeated catheterization. Therefore, when PDT is combined with stent implantation for the treatment of CCA, it may be possible to use PDT combined with metallic stent-mediated ERCP, which is perhaps one of the most definitive methods for improving the prognosis of patients, but a multicenter large-sample comparative study is still needed. At the same time, placing the PS into the stent to achieve a relatively controlled PDT may not only be easier for the physician in making the treatment easier, but also the continuous PDT may ease the burden of the patient.

*Postoperative adjuvant PDT for CCA.* For patients with CCA who have undergone surgical resection but have positive margins of tumor or experience local recurrence, adjuvant therapy can further eradicate tumor tissue to reduce the risk of tumor recurrence or metastasis to other sites and improve long-term survival. Previous adjuvant therapies for postoperative CCA include chemotherapy and radiotherapy (62,63). However, these methods have limited efficacy in suppressing tumor progression, carry the risk of adverse events and lack high-level evidence.

PDT can be used as adjuvant therapy for postoperative CCA through PTCS or ERCP. Both approaches are feasible, with preference given to PDT through the formation of a sinus tract guided by a stent. The use of PDT as adjuvant therapy for CCA postoperatively originated from a study by Nanashima *et al* (64), which evaluated adjuvant PDT in 8 patients with positive margins of the bile ducts or postoperative recurrence. The results showed significant destruction of tumor tissue, and 4 patients had no recurrence during follow-up (6-20 months). They concluded that PDT in the resection of postoperative positive margins/recurrence can reduce the rate of recurrence and improve OS in patients with postoperative CCA (64). Chen *et al* (65) conducted a retrospective analysis of 39 patients with postoperative recurrence, including 18 patients who underwent PDT guided by PTCS and 21 patients who underwent percutaneous transhepatic cholangial drainage (PTCD) alone. The median survival time was longer in the PTCS combined with PDT group (23 months) compared to the PTCD group (10 months) ( $P=0.00001$ ), and the difference in adverse event rates between the two groups was not statistically significant. This study demonstrated that adjuvant PDT after surgery can prolong survival in patients with postoperative recurrence of CCA without increasing the occurrence of complications (65).

The data and application of adjuvant PDT for postoperative CCA are limited, but existing studies have provided some evidence of its benefits in terms of long-term survival and patient quality of life. Furthermore, this approach does not increase the risk of complications. However, further large-scale prospective studies are still needed.

*Neoadjuvant PDT for CCA.* For unresectable or borderline resectable CCA, a feasible approach is neoadjuvant therapy, which involves controlling or reducing tumor infiltration and then performing radical resection. PDT selectively ablates

CCA tissue, reducing the volume of the primary tumor, clearing cancer cells and dysplastic epithelium from the entire wall of the bile duct, increasing the rate of resection R0, preventing resection R1/R2 and reducing local tumor recurrence.

Wiedmann *et al* (66) reported on the use of neoadjuvant PDT in 7 patients with CCA, for which the median treatment time was 6 weeks. The results showed that all patients achieved R0 resection, with no active tumor cells found in surgical specimens of 4 mm. The 1-year recurrence-free survival rate was 83% and the 5-year survival rate after surgery was 71%. Neoadjuvant PDT was considered effective at selectively ablating the inner 4 mm of bile duct tissue (66). In a phase II clinical study, Wagner *et al* (67) reported 7 patients with initially unresectable CCA who underwent radical resection 6 weeks after neoadjuvant PDT. All patients achieved R0 resection and 6 patients died after an average of 3.2 years after surgery. The 5-year survival rate was 43%, comparable to patients with R0 resection without treatment (67). In addition to using PDT to ablate tumor tissue in patients eligible for radical resection, liver transplantation is also considered a treatment option for advanced CCA. For patients awaiting liver transplantation, PDT can help control local tumors (68). One study reported that after neoadjuvant PDT, although the inner 4 mm of tumor tissue was completely necrotized, surviving tumor cells were observed within 5-10 mm (69). Therefore, future designs may focus on developing PSs and PDT delivery systems capable of deeper tumor penetration.

Furthermore, a large number of studies have shown that PDT can be used for precancerous lesions of the skin, esophagus and oral cavity, and has achieved considerable efficacy (70-75). However, there are few studies on the treatment of CCA precancerous lesions with PDT, but in a case report of intraductal papillary neoplasm of the bile duct, cholangioscopy-guided PDT was used to effectively remove local lesions, relieve obstructive symptoms and reduce bilirubin levels. Therefore, PDT may also be a promising way to treat precancerous lesions of CCA.

*PDT combined with other treatments for CCA.* For patients with advanced CCA, the main chemotherapy regimen is gemcitabine in combination with other platinum drugs. Several studies have indicated that gemcitabine combined with cisplatin is effective at alleviating and controlling the progression of advanced CCA (9,76,77).

PDT significantly inhibits the proliferation of gemcitabine-resistant CCA cells and the KLF10/EGFR signaling pathway may be involved in the process by which PDT inhibits gemcitabine-resistant CCA cells (78), providing a theoretical basis for the combined treatment of PDT and chemotherapy for CCA.

In a retrospective analysis of 96 patients with unresectable CCA, the patients were treated with PDT combined with chemotherapy (n=36), PDT alone (n=34) or chemotherapy alone (n=26) (79). The results showed that the combination of PDT and chemotherapy significantly improved OS compared to chemotherapy alone ( $P=0.022$ ). The median survival time in the combination treatment group was 20 months, which was significantly longer than the 10-month survival time in the chemotherapy alone group, suggesting that PDT in combination with chemotherapy prolongs survival and has fewer

adverse effects (79). In a phase II clinical trial comparing PDT in combination with S-1 therapy, an oral fluoropyrimidine prodrug, vs. PDT alone for unresectable CCA, the results showed higher 1-year survival rates, OS and progression-free survival with PDT combined with S-1 therapy, with no significant differences in adverse events or quality of life (80). A recent meta-analysis showed that PDT combined with chemotherapy can significantly prolong OS and reduce the risk of death compared to chemotherapy or PDT alone, while the incidences of adverse effects such as cholangitis and photosensitivity are similar (81). Hong *et al.* (82) performed a univariate analysis of patients with advanced CCA and the results suggested that serum bilirubin levels below 3.0 mg/dl before PDT, a TNM stage of <III, prompt PDT after diagnosis, repeat PDT and PDT combined with chemotherapy were all favorable prognostic factors. According to multivariate analysis, PDT combined with chemotherapy and multiple rounds of PDT were significant predictors of survival (82).

In recent years, targeted therapy and immunotherapy have shown effectiveness in the treatment of CCA. Precise targeting and control of drug release are crucial for immunotherapy in tumor treatment. As a precise treatment modality, PDT can enhance the efficacy of immunotherapy by combining immunotherapeutic drugs with PDT for active targeted delivery. Liu *et al.* (83) synthesized the photoactivatable prodrug cBMS-1 by incorporating [7-(diethylamino)coumarin-4-yl]methyl with the programmed cell death 1 (PD-1)/programmed cell death ligand 1 (PD-L1) inhibitor BMS-1, allowing the controlled release of PD-1/PD-L1 inhibitors to improve treatment efficacy and reduce immune-related side effects. Furthermore, PDT can activate the immune system, modify the tumor microenvironment, upregulate the expression and stability of PD-1 or PD-L1, enhance T-cell infiltration and improve tumor-killing effects (84,85). Gu *et al.* (86) designed NPs loaded with an anti-PD-L1 peptide that were able to specifically recognize matrix metalloproteinases and enable precise release of the anti-PD-L1 peptide. The NPs also have oxygen-independent free radical generation capability, further enhancing treatment efficacy by promoting ICD (86). With ongoing research on immunotherapy and PDT, future studies hold promise for CCA treatment. Therefore, in unresectable CCA, the combination of PDT and other treatments is a promising therapeutic strategy.

There are numerous studies on the use of PDT in superficial lesions such as skin, blood vessels and certain tumors. In deep tumors, insufficient penetrating light, among other things, prevents conventional PDT from being effective. Interstitial PDT (I-PDT) is a means of activating PS by inserting one or more laser fibers into a tumor or margins. It has been approved or used in clinical studies for the treatment of deep-seated tumors, such as head and neck tumors, prostate cancer, brain cancer and pancreatic cancer, and has achieved reliable efficacy (87-90). It makes up for the insufficient laser penetration of large or deep tumors in PDT.

*Postoperative complications of PDT for CCA.* PDT has shown promising efficacy in the treatment of CCA, but it also involves several complications. The occurrence of these complications is mainly associated with the location, morphology, depth of infiltration of the tumor tissue, PS dose, light dose and timing of light exposure. Common adverse reactions

include photosensitivity reactions, pigment deposition and cholangitis, while rare complications include biliary bleeding and stenosis (91).

Photosensitivity reaction is the main postoperative complication of PDT. It is a predictable but unavoidable side effect caused by the absorption of PSs not only by malignant bile duct epithelial cells but also by other tissues, such as the skin. It is characterized mainly by itching, pain, erythema and, in severe cases, ulceration and vesicles after exposure to direct sunlight to the skin. Therefore, patients are advised to avoid direct sunlight for 4-6 weeks after PDT treatment and, if exposed to sunlight outdoors, protective clothing is used as a barrier (92,93).

Although PDT can improve quality of life and improve biliary drainage in patients with CCA, it may also lead to cholangitis. The incidence of post-PDT cholangitis is similar to that of simple stent placement (94). Routine placement of biliary stents after PDT is necessary to ensure smooth bile drainage and minimize the occurrence of cholangitis. The possibility of cholangitis should be considered in patients who undergo PDT treatment and subsequently experience fever, jaundice and worsening abdominal pain (95,96).

A small number of patients may experience biliary bleeding, biliary perforation and acute pancreatitis, mostly related to ERCP and PTCD (97,98). As a rare complication, massive biliary bleeding is caused mainly by PDT-induced destruction of tumor tissue, leading to the formation of internal fistulas between the bile duct and the hepatic artery (99). Therefore, individualized treatment adjustments should be made for patients with tumors located near the hepatic artery or portal vein and those with tumor infiltration <4 mm.

In conclusion, for successful and safe implementation of PDT, an experienced PDT team is needed, and individualized treatment and appropriate patient education should be provided to minimize the occurrence of complications.

## 6. Conclusion

PDT is an effective palliative treatment for unresectable or advanced CCA. It significantly improves quality of life and prolongs OS in patients with advanced-stage CCA, without increasing the incidence of adverse reactions. Compared to other palliative treatments, PDT offers the advantage of local therapy with minimal damage to normal cells, low toxicity of PSs allowing repeated use, and longer survival in patients who undergo multiple sessions of PDT. Furthermore, PDT has achieved considerable efficacy in the postoperative adjuvant or neoadjuvant treatment of CCA.

However, there are several limitations of PDT for CCA that should be addressed in future studies. As mentioned earlier, the photodynamic effect has achieved reliable results in the treatment of tumors. However, in addition to treatment, we should also pay attention to the impacts of photodynamic effects on other factors in the body, such as metabolism, the microbiota, inflammation and tissue hypoxia. The prevention and treatment of complications such as photosensitivity and the precise control of the tumor-killing depth of PDT are also of concern. Due to the relatively late application of PDT in the treatment of CCA, although retrospective and uncontrolled trials have demonstrated its superiority, larger clinical

randomized controlled trials are needed to validate these findings and guide the clinical application of PDT.

R0 surgical resection is still the most effective treatment for patients with CCA. Therefore, it is still necessary to intervene in the progress of CCA in terms of early diagnosis and treatment. In PDT for CCA, there is a major lack of clinical evidence-based medical data, as well as clear clinical diagnostic and therapeutic technical specifications, such as the choice of PS, the appropriate PS dosage and the appropriate timing and dosage of light exposure. Because of individual differences, future specifications should grade patients with CCA to use more accurate PDT for patients at specific stages. In CCA, PDT is mostly used as a palliative treatment to relieve biliary obstruction and improve the prognosis and quality of life of patients. The high cost of PS limits the popularity of PDT. After treatment, patients are not exposed to sunlight for 6 weeks to avoid photosensitization, which in turn affects their quality of life and may prolong hospitalization.

PDT research is strongly accompanied by the development of PSs, and in clinical practice, recognized and reliable standard procedures, including PS dosage, irradiation time and device use, should be proposed as a way to control the quality of PDT and to avoid related complications. Furthermore, in PDT, image-based real-time detection and real-time dosimetry may be used to assess the photodynamic effect on tumors, monitor the treatment effect and duration, and predict treatment efficacy, so that individualized treatment can be improved.

Research on the development of PSs with deeper penetration and faster efficacy, as well as the study of the dose-response relationship, will contribute to a more efficient clinical use of PDT. At the present stage, PSs used in the medical field are mainly applied to trigger the photodynamic effect to ablate diseased tissues. According to different scenarios, different types of PSs can be developed, e.g., PSs with precise targeting function to lesions through nano drug delivery carriers, and PSs that are resistant to the anoxic tumor microenvironment and adapted to the treatment of deep-seated solid tumors. In addition, there are also new PSs derived from the combination of PDT with immunotherapy and chemotherapy, which will be a prospect for further research and development and use of PSs. PDT has traditionally been used for the treatment of superficial lesions, but the development of I-PDT and NP PSs will broaden the indications of PDT and enable it to act on deeper tumors or diseases. We look forward to the full application of PDT in patients with CCA and more tumor patients in the future.

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### Availability of data and materials

Not applicable.

### Authors' contributions

YFL and YHL performed the data analysis, literature search and manuscript writing; YHS and SLL contributed to the conception of the study. All authors have read and approved the final manuscript. Data authentication is not applicable.

### Ethics approval and consent to participate

Not applicable.

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Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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