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Overcoming colorectal cancer and cancer stem cell resistance with photodynamic therapy: new frontiers in oncology

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Colorectal cancer (CRC) is a major global health issue characterized by abnormal cell growth leading to high morbidity and mortality rates. While conventional treatments like chemotherapy and surgery exist, they often fall short due to severe side effects and the emergence of treatment resistance, particularly from cancer stem cells (CSCs). These CSCs contribute to tumor initiation, progression, and recurrence, posing challenges for effective management. Photodynamic therapy (PDT) offers a promising alternative, leveraging light-activated photosensitizers (PSs) to selectively target and destroy tumor cells while minimizing damage to healthy tissue. Recent advancements in PDT, including the use of nanoparticles (NPs) and novel PSs, enhance its efficacy against CRC and CSCs. Preclinical studies have demonstrated PDT ability to induce apoptosis and inhibit tumor growth in various models, highlighting its potential as a therapeutic strategy. Additionally, combining PDT with immune checkpoint inhibitors may further improve treatment outcomes by activating robust immune responses. This review discusses the mechanisms of resistance in CRC, the role of CSCs, and the evolving landscape of PDT, emphasizing the need for continued research to optimize combination therapies for enhanced efficacy against colorectal cancer.

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1 Introduction

Cancer continues to be one of the primary contributors of mortality worldwide. It is characterized by uncontrolled cell proliferation that can invade and metastasize to various organs in the body.[1] Colorectal cancer ranks as the third most prevalent cancer worldwide. Understanding its geographical and temporal burden offers valuable insights into the prevalence of risk factors and advancements in strategies for cancer control. Early detection significantly improves survival rates.[2] The onset of CRC is often associated with a blend of genetic factors and environmental influences. Around 25% of CRC cases are hereditary, while the remaining instances are linked to environmental contributors. Genetic risks are elevated by inherited conditions such as familial adenomatous polyposis (FAP), hereditary non-polyposis colorectal cancer (HNPCC), and various other familial cancer syndromes. Environmental elements that may heighten the risk of developing CRC include a diet that is low in fiber but high in fat and red meat, excessive alcohol intake, and lifestyle choices such as physical inactivity, smoking and obesity. These lifestyle habits can create a conducive environment for cancer development by promoting inflammation, altering gut microbiota, and affecting overall metabolic health, underscoring the importance of both genetic and environmental factors in CRC risk.[3] According to the WHO the estimated number of new cancer cases worldwide (both sexes, all ages) is projected to increase from 20 million in 2022 to 35 million by 2045.[4] This projected increase underscores the urgent need for enhanced prevention, early detection, and effective treatment strategies to combat this growing public health challenge.[5] Although conventional treatments for colorectal cancer, such as chemotherapy and surgery, are widely available, they often come with significant limitations, including severe side effects, invasiveness, and reduced efficacy in advanced stages.[6] CSCs play a pivotal role in treatment failure and resistance across various cancers, including colorectal cancer. These cells have the unique capacity to self-renew, differentiate, and evade conventional therapies such as chemotherapy and radiotherapy, which primarily target the rapidly dividing tumor mass. Due to their resistance mechanisms, CSCs often survive treatment, leading to recurrence and metastasis. Addressing CSCs is therefore critical in overcoming these therapeutic challenges and opens the door for novel approaches like PDT to combat resistance.[7]

Despite advances in drug development and treatment strategies, resistance to conventional therapies and the associated toxicity to healthy tissues remain significant barriers in cancer treatment. PDT offers a promising alternative by using light, a photosensitizing agent, and oxygen to selectively destroy tumor

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cells. This therapy induces oxidative damage, disrupts tumor vasculature, and stimulates immune responses against cancer cells. PDT presents several advantages over traditional chemotherapy, including precise tumor targeting, the ability to bypass resistance in recurrent cancers, flexible light and drug dosing, and superior cosmetic outcomes with minimal or no scarring.[8]

Although PDT offers several advantages over conventional treatments, its efficacy is often limited by poor penetration of light and PSs in deep-seated or malignant tumors. Recent advances in nanocarrier-based delivery systems have significantly addressed this limitation, improving the accumulation and activation of PSs at tumor sites. This review highlights the emerging role of CSCs in CRC and examines how integrating nanotechnology with PDT can selectively target CSCs, overcome therapy resistance, and enhance treatment outcomes. By combining insights into CSC biology with innovative nanotherapeutic strategies, this work explores new frontiers in precision PDT for CRC.

2 Chemical basis and function of PSs in PDT

Photosensitizers are agents that are activated with a suitable wavelength of light to either elicit fluorescence, phosphorescence, or trigger cell death.[9] An ideal PS for PDT modalities should be able to discriminate tumour cells from normal cells.[10] An excellent PS should emit fluorescence at both low and high wavelengths of light, to generate cytotoxic species that can effectively obliterate cancer cells. Moreover, the PSs must exhibit high chemical purity and stability, negligible toxicity in the dark, rapid body clearance and ease of synthesis.[11] PSs are generally devided into three classes, namely first, second, and third generation in line with their photochemical and photophysical properties, as well as their cellular mechanism of action.[12] Additionally, structural characteristics have further grouped PSs into four classes (phthalocyanines, porphycenes, chlorins and porphyrins).[9]

2.1 First-generation PSs for PDT application

First-generation PSs encompass early photosensitizing molecules, which were first explored for PDT applications.[10] First-generation PSs such as haematoporphyrin derivative (HpD) and photofrin paved the way for the development of PDT as a therapeutic approach for cancer treatment. However, they had several disadvantages relating to off-target toxicity, hydrophobicity, prolonged skin hypersensitivity, and poor penetration into deep-seated tumours.[9] The purified haematoporphyrin derivative Photofrin was the first PS to be approved for use in PDT and PDD studies; it demonstrated operational eradication of various cancers. Despite photofrin having the ability to fluoresce at lower wavelengths for diagnosis, it exhibits extended retention in cutaneous tissues, causing severe photosensitivity, and is ineffective at generating ROS at 630 nm, resulting in poor penetration into deeper tissues and, ultimately, minimal cell death. However, the numerous drawbacks of first generation prompted ongoing research into PSs with exceptional properties that allow for synchronized PDD and PDT applications.[9]

2.2 Second-generation PSs and PDT applications

Second-generation PSs refer to a class of compounds developed to address shortcomings of first-generation PSs.[9] Second-generation PSs entail porphyrins, chlorins, phthalocyanines, benzoporphyrin, bacteriochlorin, curcumin, methylene blue analogues, and many others.[13] They have been widely employed in preclinical and clinical trials for PDD and PDT applications due to their superior optical, photophysical, and photochemical properties.[9] These PSs have characteristics such as improved selectivity, reduced skin sensitivity, undetectable dark toxicity, and enhanced absorption within the therapeutic window (600–800 nm), which allows for a deeper penetration of light for activation of PS retained in bigger or deeper-seated tumour.[11]

Benzoporphyrin, also referred to as verteportin. They are hydrophobic PSs that absorb light strongly at 690 nm wavelengths, allowing for high tissue depth penetration, ROS quantum yields, and rapid clearance from the body. However, verteporfin is not eligible for PDT applications since it doesn't have a peak at short wavelengths.[14]

Chlorins are tetrapyrrole molecules derived from porphyrins consisting of a pyrrole ring short of at least one double bond. This modification to the chlorin structure enhances its absorption to 650–690 nm, which allows for limited scattering, deep tissue penetration, and maximum extinction coefficients for better PDT outcomes.[9] Pheophorbide-a, a chlorophyll-derived photosensitizer, has emerged as a potent candidate for PDT due to its strong absorption in the red spectral region (665–680 nm), enabling deeper tissue penetration and effective ROS generation. Studies have shown that Pa induces mitochondrial dysfunction and cell death in cancer cells *via* oxidative stress, as demonstrated in breast cancer cell lines (MDA-MB-231 and MCF-7).[15–17] A study demonstrated that Pheophorbide-a (Pheo-a) effectively induced photodynamic cell death in doxorubicin-resistant MCF-7 breast cancer cells using a 2.5 μM concentration and 660 nm laser irradiation at 10 J cm^{-2}. The findings highlight chlorophyll-based PSs as a promising approach to overcoming chemotherapy resistance in cancer.[18] Another study showed that Pheophorbide-a (Pa) exhibits antitumor effects in PDT (Pa-PDT), though its role in anticancer immunity is yet to be studied. In HepG2 hepatoma cells, Pa-PDT inhibited growth with an IC_{50} of 0.35 μM at 24 hours, inducing apoptosis through phosphatidylserine externalization, cytochrome c release, and caspase activation.[15] These findings highlight the potential of PPBa for PDT, but its hydrophobicity and limited stability require the development of advanced delivery systems to improve clinical effectiveness. Although second-generation PSs show promise, their hydrophobic nature and toxicity to healthy tissues hinder their broad clinical adoption, emphasizing the need for smart drug delivery approaches.[19]

2.3 Third-generation PSs and PDT applications

Third-generation PSs are developed by conjugating second-generation PSs with targeting moieties such as amino acids or

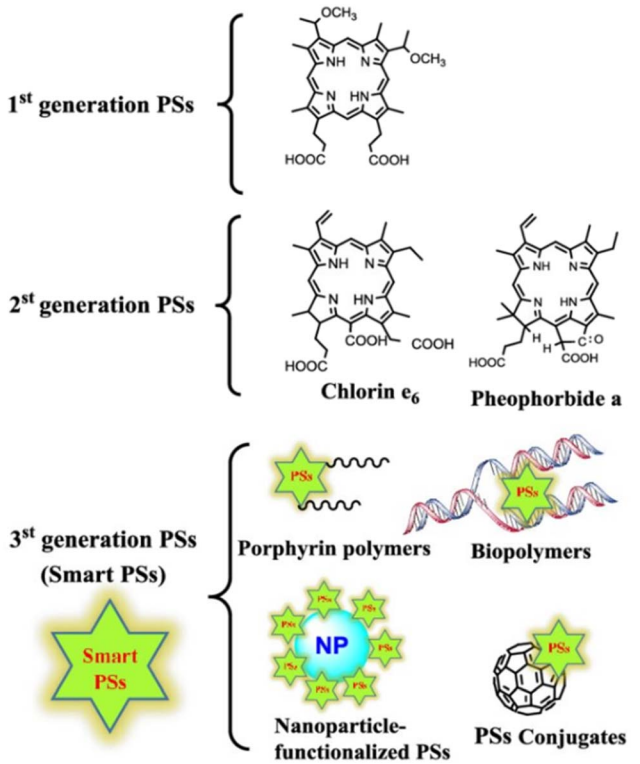

Fig. 1 Generational classification of PSs developed to date.[12]

peptides, or by encapsulating them within nanocarriers like liposomes, micelles, polymers, or NPs.[20,21]

Encapsulation and the use of nanocarriers enhance the biocompatibility and selectivity of PDT systems while boosting their phototherapeutic effects.[22] These strategies also increase the stability and hydrophilicity of nanodrugs, minimize side effects, enable consistent photosensitizer delivery, ensure targeted drug accumulation in tumor cells, and reduce dark toxicity.[23] Fig. 1 illustrating the progression from first-generation PSs with limited selectivity to second- and third-generation PSs featuring improved efficacy, tumor targeting, and reduced side effects.[12]

3 Structural modification for solubility enhancement in early drug discovery

Enhancing the solubility of poorly soluble lead molecules remains a significant challenge for medicinal chemists and formulation scientists. Chemical modification is currently considered an effective strategy to improve solubility while maintaining bioactivity.[24] Common approaches include introducing or removing functional groups to achieve an optimal acid–base balance (pH), adjusting the chemical structure to optimize the hydrophilic–hydrophobic balance (log *P*), incorporating hydrogen bond donors or acceptors, introducing heterocyclic ring systems, and employing bioisosteric replacements with hydrophilic groups. These modifications enhance the interaction of drug molecules with the aqueous environment, thereby improving solubility.[25] In some molecules, poor solubility arises not only from hydrophobicity but also from a high melting point. This elevated melting point often results from tightly packed molecular arrangements, increased molecular planarity, and symmetrical structures that exhibit higher residual entropy in the solid state.[26,27] Enhancing solubility by disrupting molecular symmetry and planarity is considered an effective strategy in modern lead optimization.[28]

The introduction of hydrogen bond donors and acceptors, such as –OH and –NH_2 groups, can improve aqueous solubility by facilitating hydrogen bonding with water molecules. Interestingly, it has also been reported that removing certain hydrogen bonds can increase solubility, possibly by lowering the melting point, making this a valuable approach for solubility enhancement.[29,30] Additionally, molecules with hydrogen bond-donating and -accepting groups often participate in hydrogen bonding within the solid state.[31] Another prodrug strategy is a well-established alternative for addressing challenges in drug discovery, including poor solubility, and has been widely reviewed.[32,33] A prodrug is an inactive compound that undergoes chemical or enzymatic conversion within the body to release the active parent drug in a controlled and predictable manner.[34] Prodrugs are generally classified into two main categories: carrier-linked prodrugs and bio precursors.[35] Carrier-linked prodrugs offer a practical approach for addressing major solubility challenges. In this strategy, the parent drug is covalently attached to a nontoxic carrier or promoiety to modify or eliminate undesirable physicochemical properties. These prodrugs are designed to undergo enzymatic or nonenzymatic cleavage, releasing the active drug.[36] Carrier-linked prodrugs can be classified as bipartite (where the carrier is directly attached to the parent drug) or tripartite (where a spacer links the carrier to the parent drug). Several types of carrier-linked prodrugs have been developed to enhance solubility, including amide, phosphate, imine, carbonate, and ether prodrugs.[37]

Incorporating hydrophilic and ionizable groups is one of the most common and classical approaches for improving solubility. Studies have shown that introducing these groups can enhance solubility by lowering the lipophilicity (log *P*) of lead compounds. This technique has proven effective even for highly hydrophobic molecules, including large structures like C_{60}.[38] Enhancing water solubility by disrupting molecular planarity and symmetry has emerged as a recent strategy in small-molecule drug discovery. Unlike classical methods such as adding hydrophilic groups or reducing hydrophobicity which can sometimes compromise bioactivity this approach offers an alternative route.[39] Poor solubility is often linked to tightly packed crystal structures, where high crystallization energy, driven by flat molecular configurations and intramolecular hydrogen bonding, contributes to reduced solubility.[40,41]

Bioisosteric replacement is a classical and widely used strategy to enhance solubility, potency, selectivity, and other key pharmaceutical properties of challenging molecules. Bioisosteres are generally categorized into two types: classical bioisosteres, which include monovalent, divalent, trivalent

Table 1 Computational tools for predicting solubility of compounds

Tool/method	Approximate calculation time	References
QSAR	1–2 hours	47
QSPR	1–2 hours	48
Molecular dynamics (MD)	12–24 hours (depends on simulation time in ns)	49 and 50
COSMO-RS	6–12 hours	51 and 52
SP (solubility parameter)	2–3 hours	53 and 54
Free energy calculations	6–12 hours	55
Flory-Huggins parameter	6–12 hours	56

atoms or groups, tetra-substituted structures, and ring equivalents; and non-classical bioisosteres, which comprise cyclic and non-cyclic functional groups.[42] Replacing the phenyl group of a lead molecule with heterocycles such as pyridine or thiophene can enhance solubility along with other pharmaceutical properties.[43]

Inaccurate solubility predictions are a major contributor to misinterpretation of results in many *in vitro* assays.[44] High lipophilicity and strong intermolecular interactions make drug solubilization energetically demanding. Consequently, there has been significant interest in developing *in silico* models to predict compound solubility based on molecular structure. Studies indicate that nearly 77% of compounds screened during drug discovery are insufficiently soluble for further evaluation, leading to considerable time and resource wastage.[45] Therefore, predicting solubility early in the drug discovery process using computational tools is a valuable strategy to improve the likelihood of clinical success for drug candidates (Table 1).[46]

4 Interactions of cancer stem cells with nanotherapeutic approaches

Nanotechnology offers tremendous potential as a therapeutic platform, enabling the delivery of a wide range of agents such as small molecules, genes, RNAs, peptides, and imaging probes. When applied in systemic cancer therapy, it can significantly improve drug pharmacokinetics and enhance the therapeutic index of many treatments.[57–59] Several key parameters such as average particle size, uniformity, surface charge, and drug-loading capacity play a crucial role in determining the delivery efficiency and therapeutic potential of NPs.[60,61]

By carefully engineering nanomaterials (NMs) with defined characteristics such as size, charge, hydrophilic–hydrophobic balance, and surface functional groups, it is possible to achieve selective recognition and binding to CSC surface markers, enabling precise CSC targeting and controlled biological activity *in vivo*. Understanding the structure–activity relationship of NMs on CSCs further supports the design of tailored nanotherapeutics that minimize off-target toxicity and adverse effects. Since CSC survival and proliferation are strongly influenced by specific microenvironmental conditions, NMs also hold significant potential for modulating the tumor microenvironment (TME).[62,63] Designing nanotherapeutics with tailored physicochemical properties enables effective modulation of the CSC microenvironment, thereby suppressing their growth and metastasis. Recently, the integration of artificial intelligence (AI) with nanomedicine has created new opportunities for precision-targeted therapies against tumors and CSCs. For instance, Wei *et al.* utilized a fully connected deep neural network model to analyze the relationship between NPs and their properties through machine learning, enabling both classification and quantitative prediction of the enzyme-like activities of nanozymes.[64] Jiang *et al.* applied edge-transitive nets to model the intricate architectures of multicomponent MOFs, offering algorithmic guidance for the development of new nanomaterials.[65] Google DeepMind's GNoME (Graph Networks for Materials Exploration) model and the MatterGen model introduced by Zeni *et al.* have together advanced the discovery of new nanomaterials. While GNoME focuses on building extensive libraries of novel crystalline materials, MatterGen enables the generation of diverse inorganic structures. Collectively, these approaches greatly enhance the stability and diversity of newly developed nanomaterials.[66,67] The recently developed MatterChat model advances material synthesis by accurately predicting key physical properties.[68] In the field of NP targeting, Wang *et al.* employed AI models to accelerate the screening and design of ionizable lipids and targeted lipid NPs.[69] Similarly, May *et al.* applied supervised machine learning to analyze vascular and TAM density data, establishing a biomarker scoring system to distinguish nanomedicine accumulation within tumors.[70] AI has also shown remarkable promise in predicting structure–activity relationships. Deng *et al.* introduced DeepNano-seq, a tool that predicts nanoantibody–antigen interactions using protein sequence data, demonstrating strong cross-species generalization and robust virtual screening capabilities.[71] Similarly, Chen *et al.* developed the TopoFormer model, which transforms three-dimensional protein–ligand complex structures into one-dimensional topological sequences, enabling the capture of key interactions across spatial scales.[72] This provides a theoretical framework for designing nanomaterials that selectively target CSC surface markers. Collectively, the integration of AI into nanomedicine ranging from nanomaterial structure prediction and synthesis design to targeting efficiency and structure–activity relationship modelling offers not only extensive candidate libraries and advanced design tools but also establishes a strong foundation for developing personalized therapeutic strategies against CSCs.[73]

In summary, advancements in nanotherapeutic strategies have provided renewed hope for clinical cancer treatment by enabling the precise targeting and elimination of CSCs. Progress in cancer cell and molecular biology has fueled interdisciplinary research, driving the development of novel therapies. By progressively addressing current challenges associated with CSCs and nanomaterials, it is expected that safer and more effective nanotherapeutics will be developed, ultimately allowing for highly targeted and precise cancer treatment.

5 Cancer stem cells in colorectal cancer

Cancer stem cells are a distinct population of cells found within tumors that have the unique capabilities of self-renewal and differentiation. These cells play a crucial role in the initiation and progression of tumors, as well as in metastasis. Their ability to generate diverse cell types within the tumor contributes to its heterogeneity and resilience against therapies. This characteristic allows CSCs to survive conventional treatments, leading to recurrence and making them a significant focus in cancer research for developing more effective therapeutic strategies. In CRC, CSCs are implicated in disease recurrence, resistance to treatment, and the formation of metastases.[74] Several cell surface markers, such as CD133, CD44, ALDH1, EpCAM, and LGR5, are commonly used to identify colorectal CSCs. Among these, CD133 and CD44 are the most extensively studied and linked to CSC properties in CRC.[75] CD133 is a transmembrane glycoprotein often overexpressed in CRC stem cells. It is associated with a poor prognosis and is a key player in tumorigenesis. Studies have shown that CD133-positive cells exhibit enhanced tumor-initiating capabilities and are more resistant to chemotherapy and radiation. CD133 interacts with several intracellular signalling pathways, including the Wnt/β-catenin, Hedgehog, and Notch pathways, which are critical for maintaining stemness and promoting tumor growth.[76,77] Another marker CD44, a cell surface glycoprotein overexpressed in CSCs, plays a critical role in colorectal cancer by promoting self-renewal, epithelial mesenchymal transition (EMT), and resistance to chemotherapy and radiotherapy. Specific CD44 isoforms are key in tumor development, progression, metastasis, and therapy resistance. Through its interaction with hyaluronan (HA), CD44 helps maintain CSC stemness, making it a promising target for cancer therapy.[78] ALDH1 is recognized as a CSC marker in CRC, with its elevated expression strongly associated with poor cellular differentiation, enhanced metastatic capacity, and advanced disease stages. Moreover, ALDH1 significantly contributes to drug resistance in colorectal CSCs, making it a critical target for therapeutic intervention.[79,80] Whereas EpCAM plays a role in regulating intercellular adhesion-related signal transduction, as well as in controlling cell migration, proliferation, and differentiation.[81] EpCAM can enhance the canonical WNT/β-catenin signalling pathway through intramembrane proteolysis and the subsequent nuclear translocation of its intracellular C-terminal domain. This activation facilitates crosstalk with key signalling pathways such as Notch, Hedgehog, and TGFβ/BMP, forming an integrated stem cell signalling network that regulates the expression of various functional CSC markers.[82–84]

6 CRC treatment modalities

Common screening techniques for CRC include colonoscopy, flexible sigmoidoscopy, digital rectal examination, and stool-based tests such as the fecal immunochemical test (FIT) and fecal occult blood tests (FOBTs), which detect the presence of blood or genetic material (*e.g.*, DNA) in the stool. Among these, colonoscopy remains the most widely adopted and cost-effective method, allowing for both the detection of CRC and the collection of biopsy samples for histopathological analysis.[85–87] Artificial intelligence technologies have been incorporated into colonoscopy procedures to enhance the detection and assessment of colorectal polyps.[88,89]

The use of computer-aided diagnostic (CAD) systems powered by deep learning has demonstrated promising improvements in accurately identifying polyp histology, with accuracy rates 63.8–71.8% to 82.7–84.2%.[87,88] Additionally, narrow-band imaging (NBI) in colonoscopy has been shown to enhance polyp detection compared to traditional white light colonoscopy, achieving an accuracy of 95% *versus* 74%.[87] When conventional colonoscopy is not feasible due to contraindications or patient refusal colon capsule endoscopy (CCE) serves as an alternative screening method for individuals at moderate risk of CRC. CCE involves swallowing a wireless, disposable capsule that captures multiple images of the colon, enabling a painless, radiation-free examination without the need for sedation or gas insufflation. While CCE shows potential as a non-invasive screening tool, it is less effective than colonoscopy in detecting smaller polyps and does not allow for therapeutic interventions. Therefore, it is not recommended as a first-line option for CRC screening or diagnosis.[89,90] Imaging modalities like computed tomography (CT) and magnetic resonance imaging (MRI) are utilized to assess tumor extent and identify the presence of metastases. Histological examination of tissue samples collected *via* biopsy or surgical resection is performed to confirm cancer diagnosis and determine its stage.[91]

The management of CRC is influenced by several factors, including the disease stage, tumor location, and the patient's overall health. Standard treatment approaches consist of surgery, chemotherapy, radiotherapy, and immunotherapy. Surgical removal of the tumor, either through open or laparoscopic procedures, remains the primary curative option. These treatments are generally most successful when CRC is diagnosed at an early stage, with survival rates reaching approximately 90%. In contrast, late-stage diagnosis, particularly stage 4, is associated with a significantly poorer prognosis, with survival rates around 15%. This underscores the urgent need for earlier detection and the development of more effective therapeutic strategies.[86,92,93]

PDT is a promising minimally invasive treatment approach that has the potential to enhance outcomes in CRC. This technique involves administering a photosensitizing agent that preferentially accumulates in cancer cells. When exposed to light of a specific wavelength, the photosensitizer is activated, generating ROS that induce targeted cell death and tumor destruction.[94]

PDT offers several benefits for treating colon cancer. Firstly, it is a minimally invasive procedure that can be performed during endoscopic interventions such as colonoscopy, enabling precise treatment directly at the tumor site. This targeted approach reduces harm to surrounding healthy tissues. Moreover, PDT enables multiple treatment sessions with minimal side effects. In contrast to conventional systemic therapies which are often associated with severe adverse effects PDT can be safely repeated without causing cumulative toxicity.[25,95]

7 Mechanisms of resistance in CRC-CSCs

Conventional adjuvant therapies typically focus on targeting differentiated cancer cells, frequently neglecting CSCs. This oversight plays a crucial role in therapy resistance and disease relapse, as it allows some cancer cells to evade treatment. The impact of CSCs on therapy resistance and recurrence is particularly notable in colorectal cancer, where these cells display abnormal activation of growth and survival signalling pathways, contributing to the persistence and resurgence of the disease. Studies involving cell cultures, animal models, and patient tissues highlight the crucial involvement of colorectal CSCs in treatment failure.[96] Resistance to therapy continues to be a significant obstacle in cancer treatment, with various mechanisms having been proposed over the past several decades.[97] These mechanisms involve mutations in crucial signalling molecules, leading to disrupted cell regulation, along with an overexpression of anti-apoptotic proteins that prevent cancer cell death. Additionally, the presence of dormant or treatment-resistant tumor cells further complicates therapy, while the heightened activity of drug efflux pumps enables cancer cells to expel therapeutic agents, reducing their effectiveness and promoting resistance. Additionally, the focus in recent years has shifted toward CSCs, a specific subset of cancer cells. The CSC theory suggests that cancer cells are organized hierarchically, adding complexity to treatment failure.[98,99] Cancer stem cells are believed to be the driving force behind cancer initiation and resistance to conventional chemotherapy and radiotherapy, potentially leading to recurrence even years after treatment ends.[97,100]

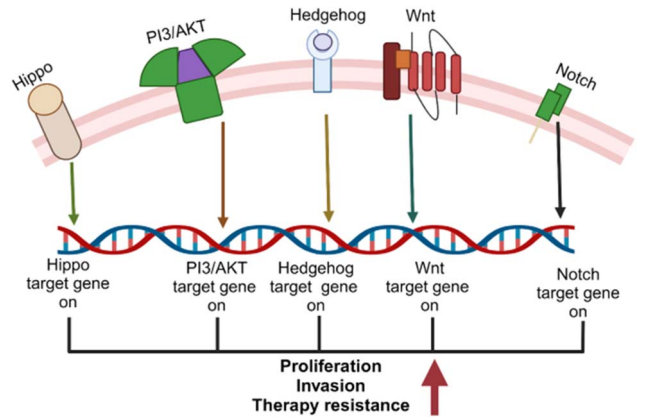

Fig. 2 Key signalling pathways – Wnt, Notch, Hedgehog, PI3K/AKT, and Hippo-regulate colorectal CSC populations. These pathways contribute to increased CSC proliferation, invasiveness, and notably, resistance to therapies.

Cancer stem cells in CRC contribute to therapy resistance by activating essential growth signalling pathways, such as Hedgehog, Notch, Wnt/β-catenin, Hippo, and PI3K/AKT (Fig. 2).

Table 2 Key signalling pathways and mechanisms of therapy resistance in CRC

Pathway	Mechanism of resistance in CRC	Therapeutic implication	Key study	Ref.
Wnt/β-catenin signalling	Mutations (APC truncations, β-catenin) lead to β-catenin accumulation, promoting cell growth and survival	Therapies targeting upstream factors may fail due to downstream mutations	Study showing mutations specific to CRC and therapy resistance	109
Exosomes and Wnt/β signalling	Exosomes from fibroblasts reprogram cancer cells into CSC-like traits, enhancing chemoresistance through Wnt signaling	Inhibiting exosomal Wnt signaling could improve chemosensitivity	Inhibition of exosomal Wnts increases sensitivity to drugs	110
Notch signalling	Higher Notch1 expression in colonospheres and resistant cells supports CSC maintenance and therapy resistance	Blocking Notch signaling (*e.g.*, with DAPT) reduces chemo-resistance and colonosphere formation	Notch1 inhibitors reduce cell growth in chemo-resistant cells	103
Hedgehog/GLI 1 signalling	GLI-1 expression drives 5-FU resistance and promotes CRC carcinogenesis	Hedgehog inhibitors (AY9944, GANT61) lower resistance to chemotherapy and decrease stem cell marker expression	Knockdown of GLI-1 reduces 5-FU resistance; Hedgehog inhibitors improve therapy response	111–114
Hipo/YAP signalling	YAP1 expression is linked to relapse, metastasis, and 5-FU resistance	YAP1 knockdown sensitizes resistant cells to 5-FU, reducing cancer relapse risk. metastasis, and 5-FU resistance	Studies show that high YAP1 levels are associated with poor outcomes and therapy resistance	115 and 116
PI3K/AKT signalling & MACC1	PI3K/AKT activation and MACC1 upregulation led to CSC-like	Targeting MACC1 and PI3K/AKT signalling enhances sensitivity to chemotherapy	PI3K inhibitors and MACC1 knockdown reduce chemoresistance in resistant colon cancer cells	117–119
MicroRNA dysregulation	miRNAs in CRC contribute to resistance by altering gene expression and promoting the CSC phenotype	Targeting specific miRNAs involved in drug resistance could reduce therapy resistance	Dysregulated miRNAs drive CRC chemo-resistances	101–104

Additional contributing factors include the dysregulation of microRNAs (miRNAs), the adoption of a quiescent or dormant state, metabolic reprogramming, and phenotypic plasticity. MicroRNAs play a crucial role in gene regulation, and their mis regulation can significantly promote a chemo-resistant phenotype in colorectal CSCs. For instance, certain miRNAs may suppress tumor-suppressor genes or enhance the expression of genes that promote cell survival and drug resistance. Meanwhile, the shift to a quiescent state allows CSCs to evade treatments targeting rapidly dividing cells, and metabolic reprogramming helps them adapt to various environmental stressors, ensuring their survival. Phenotypic plasticity further enhances the ability of these cells to switch between different cellular states, making them more resilient to standard therapies.[101–105] Furthermore, the activation of a quiescent state and a metabolic shift towards a more stable phenotype, combined with the ability to evade drug-induced DNA damage, collectively enhance the resilience of CSCs in CRC. These mechanisms, along with the activation of growth signalling pathways and microRNA dysregulation, contribute to the formidable resistance of CSCs to therapy, making them particularly challenging targets for treatment.[106–108] Table 2 below shows the key signaling pathways and underlying mechanisms driving therapy resistance in CRC, highlighting their contribution to CSC survival, tumor progression, and therapeutic failure, while also indicating potential targets for more effective treatments.

8 Mechanism of PDT in CRC treatment

PDT is a developing treatment modality for different cancer types. This approach involves a systematic procedure in which cancerous tissues are treated with a PS, a compound that becomes activated by light. The photosensitizer can be delivered either through topical application or intravenously, depending on the specific location of the tumor cells. Once administered, the PS accumulates in the cancer cells, and upon exposure to a particular wavelength of light, it generates reactive oxygen species that selectively destroy the malignant cells. This technique not only targets tumors but also minimizes damage to surrounding healthy tissue, making it a promising option in cancer treatment.[120,121] A photosensitizer is a compound that accumulates in specific cells or tissues and becomes activated only when exposed to light. The activation occurs through laser irradiation at a defined wavelength. When the PS absorbs photons, it transitions from its ground state to an excited state, called the singlet state. Alternatively, it may shift to a triplet state *via* a process known as intersystem crossing, where the electron's spin is altered. In the triplet state, the PS interrelates with molecular oxygen to produce ROS. These ROS play a crucial role in damaging cellular structures, ultimately resulting in the destruction of cancerous tissue. This process is essential in PDT, where the generation of ROS upon light activation not only targets tumor cells but also disrupts their microenvironment, enhancing the therapeutic efficacy against cancer (Fig. 3).[121,122]

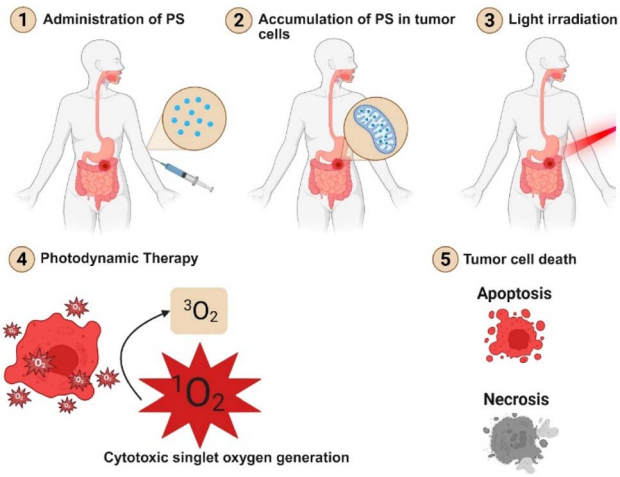

Fig. 3 Schematic illustration of the PDT mechanism in colon cancer treatment.

One significant benefit of using PDT is its ability to selectively destroy targeted cells while minimizing harm to surrounding healthy tissues. Although PSs are absorbed by all cells, they exhibit a tendency to accumulate more in diseased tissues and persist there longer, largely due to the enhanced permeability and retention (EPR) effect.[123] Four additional advantages of PDT compared to conventional treatments are its minimally invasive nature, its ability to reduce morbidity, and its capacity to preserve the anatomical and functional integrity of many cells. It also has minimal side effects, offers selective targeting with no risk of drug resistance, and has lower toxicity, allowing for repeated treatments.[124]

Currently, PDT for colorectal cancer frequently requires the use of high concentrations of phototoxic agents, which can result in negative side effects for patients. This situation underscores the necessity for the development of novel photosensitive nanomedicines that possess multifunctional capabilities, allowing for the integration of multiple chemotherapeutic agents. Such innovations could facilitate dual or even triple therapies at reduced systemic dosages, thereby significantly minimizing adverse effects. Research conducted by Shams *et al.* provides evidence in favor of this strategy, suggesting that enhanced therapeutic approaches can improve patient outcomes while mitigating the risks associated with traditional treatments.[125]

8.1 PDT potential against CSCs

PDT shows great potential in targeting colorectal CSCs due to its ability to selectively destroy cancer cells while sparing healthy tissue. Colorectal CSCs are a small subset of cancer cells within a tumor that possess self-renewal and differentiation capabilities, contributing to tumor growth, metastasis, and resistance to conventional therapies like chemotherapy and radiation. PDT, when combined with PSs that preferentially accumulate in CSCs, can target and disrupt specific pathways critical to their survival, offering a promising approach for preventing tumor recurrence and metastasis.[126] Advanced PSs, such as NPs and

liposomal carriers, play a crucial role in enhancing the specificity of PDT for targeting CSCs. These innovative drug delivery systems are designed to improve the accumulation of PSs in CSCs, increase the effectiveness of PDT, and reduce off-target effects, making them highly effective in overcoming the challenges associated with conventional therapies.[127–129] Like many conventional anticancer treatments, CSCs are also known to exhibit resistance to PDT. Therefore, it is crucial to understand the mechanisms underlying the CSC response to PDT and to identify the pathways through which these cells adapt and evade destruction. Gaining insights into CSC resistance to PDT could aid in the development of new strategies aimed at effectively eliminating CSCs. PDT has gained attention as a promising therapeutic modality for CRC and its stem cells, particularly in reducing recurrence and overcoming resistance.[130] PDT induces a sharp increase in intracellular ROS production, leading to extensive oxidation of biomolecules such as proteins, DNA, and lipids. This oxidative stress can directly influence stem cell fate. In particular, ROS-driven modifications like carbonylation may alter protein tertiary structures, promote protein–DNA and protein–protein cross-linking, and ultimately regulate the activity of critical CSC marker proteins, including OCT4 and SOX2.[131] ROS can directly damage DNA, causing point mutations in key genes such as proto-oncogenes (*e.g.*, Ras) and tumor suppressors (*e.g.*, p53). In particular, ROS-induced oxidation of deoxyguanosine can lead to mutagenic alterations at a single carbon atom.[132,133] Free radicals can disrupt cell membrane integrity by oxidizing lipid peroxides, ultimately inducing ferroptosis a unique form of cell death characterized by iron-dependent oxidative damage to lipids.[134]

CSCs have been characterized by the expression of specific markers, including TWIST1, CD105, LGR5, PROM1, and CD133. These markers play crucial roles in identifying CSCs, which are believed to contribute to tumor initiation, progression, and resistance to therapies. TWIST1 is associated with epithelial-to-mesenchymal transition, enhancing the metastatic potential of CSCs, while LGR5 and CD133 are commonly used as stem cell markers, indicating the cells' self-renewal capabilities. The presence of these markers not only aids in the identification of CSCs but also highlights their importance in the development and persistence of colorectal tumors.[135,136] The presence of these cells can adversely affect patient outcomes. For instance, nuclear expression of TWIST1 has been detected in samples from metastatic tumors, indicating its role in tumor progression. Additionally, heightened levels of CD105 expression are correlated with advanced TNM staging, suggesting that it may serve as a marker for more aggressive disease. Furthermore, CD133+ cells are associated with stemness, contributing to the cancer's ability to resist therapeutic interventions. Together, these factors underline the complexity of tumor biology and the challenges in effectively treating cancer.[137,138]

As with many conventional cancer therapies, CSCs can also develop resistance to PDT. Understanding how CSCs respond to PDT and identifying the mechanisms that enable their survival and adaptation are crucial. Gaining deeper insights into these resistance pathways may lead to the development of more effective strategies to completely eradicate CSCs.[139] Some research indicates that targeting colorectal CSCs may enhance patient outcomes. *In vitro* investigations have yielded encouraging outcomes for PDT and photochemical internalization techniques. For instance, Bostad *et al.* revealed that the photosensitizer TPCS2a was successfully taken up by CD133+ cells, where it concentrated in acidic vesicles. Upon exposure to light, this accumulation triggered cell death, highlighting the potential of TPCS2a as an effective agent in targeting specific cancer cell populations. This research underscores the importance of photosensitizer internalization in enhancing the efficacy of PDT in treating tumors.[140] Wei *et al.* also found that primary colorectal CD133+ CSCs accumulated four times more PpIX compared to CD133-cells; however, the increased PpIX accumulation did not lead to higher phototoxicity. CSCs exhibited greater resistance than non-CSCs and showed elevated VEGF secretion following PDT. Therefore, while colorectal CSCs can accumulate PpIX, they remain more resistant than non-CSCs.[141]

Recent studies have explored the impact of PDT using natural compounds on the stemness properties of colorectal CSCs. Cogno *et al.* examined three newly purified compounds derived from the stems and leaves of *H. pustulata* and *T. favicans*, plants rich in anthraquinones (AQs), which act as natural PSs. These PSs were activated by a multi-LED system at 420 ± 17 nm with a power density of 50 mW cm^{-2}. In monolayer cultures of SW480 colorectal cells, cell viability was reduced by 50% at 5 J cm^{-2} and by 75% at 10 J cm^{-2}. However, when cells were grown in sphere cultures, which promote stem cell characteristics, significant reductions in cell viability occurred only at higher light doses, suggesting that colorectal CSCs exhibit resistance to PDT when mediated by these natural compounds.[142] Wei *et al.* suggested that autophagy may serve as a defense mechanism for colorectal CSCs against PDT. PROM1/CD133+ colorectal cancer stem cells demonstrated a greater ability to form spheres and showed increased expression of genes associated with stemness, contributing to their resistance against 5-ALA-based PDT. This resistance was associated with an elevated production of microtubule-associated protein 1A/1B-light chain 3 (LC3-II) and enhanced autophagosome activity, indicating a strong autophagic response. Notably, when these CSCs were pre-treated with the autophagy inhibitor chloroquine (CQ) before undergoing PDT, there was a significant increase in the cytotoxic effects of the therapy, along with a decrease in sphere formation. This suggests that inhibiting autophagy may sensitize colorectal CSCs to PDT, potentially improving treatment outcomes.[143]

The WNT/β-catenin, Notch, and Hedgehog pathways play crucial roles in regulating CSC self-renewal.[144] Thus, targeting these signaling cascades at different levels offers a promising strategy to improve CSC eradication. For example, Karandish *et al.* employed iRGD-functionalized polymersomes to deliver napabucasin, a stemness inhibitor.[145] The iRGD-targeted polymersomes loaded with napabucasin were shown to reduce the viability of prostate and pancreatic CSCs while downregulating stemness markers like Notch-1 and Nanog, confirming their inhibitory potential. Likewise, Liu *et al.* designed NPU0126 NPs using PEG-PLA to deliver the MAPK inhibitor U0126 in hepatocellular carcinoma, achieving strong therapeutic efficacy with reduced toxicity, alongside marked suppression of sphere

formation and CD133+ CSC populations, thereby impairing self-renewal and stemness.[146] To improve CSC targeting, Miller-Kleinhenz *et al.* developed ultrasmall magnetic iron oxide NPs (IONPs) functionalized with iWnt and ATF24 peptides to target uPAR. When loaded with doxorubicin (DOX), these iWnt-ATF24-IONPs effectively suppressed WNT/β-catenin signaling, down-regulated uPAR and CSC markers, and inhibited both invasion and proliferation.[147]

In recent years, extensive preclinical studies on PDT for CRC have yielded important insights into its viability as a treatment option. There is increasing interest in combining PDT with other established treatment modalities, such as surgery and radiotherapy. This integrative approach aims to create a synergistic effect, whereby the strengths of each treatment can complement one another. By incorporating PDT alongside these conventional therapies, it may be possible to improve therapeutic efficacy and achieve better clinical outcomes for patients with CRC. This combination strategy not only enhances tumor targeting but also helps to mitigate treatment resistance, ultimately leading to more effective management of the disease.[148,149]

9 Preclinical studies in CRC

In vitro studies play a crucial role in laying the groundwork for PDT research in colorectal cancer. Nevertheless, additional investigations are necessary to validate these results in animal models and, ultimately, in clinical trials. It is crucial to demonstrate the effectiveness of the technique or drug in animal studies before progressing to clinical testing. The complex characteristics of *in vivo* tumor microenvironments, including factors like blood flow, immune responses, and tissue architecture, necessitate further research to comprehensively evaluate the feasibility and efficacy of PDT as a treatment for colon and rectal cancer. By addressing these complexities, researchers can better understand how PDT may be optimized for clinical applications, potentially improving outcomes for CRC patients.[150] Choosing a suitable animal model is essential for research, as it should accurately mimic human disease conditions. Among the various options, rats and mice are the most frequently utilized species in these studies. Their genetic, biological, and physiological similarities to humans make them ideal candidates for investigating disease mechanisms and testing therapeutic interventions. Additionally, the availability of well-established strains and genetic modifications further enhances their utility in preclinical research, enabling scientists to gain valuable insights into human health and disease.[151] Most preclinical investigations into the use of PDT (PDT) for colon and rectal cancer have focused on studying the phototoxic effects of PSs (PSs) on colorectal tumor cells cultured *in vitro*. One of the primary benefits of *in vitro* methods is their reliance

Table 3 Summary of preclinical studies on PDT for colorectal cancer

Model used	Photosensitizer	Key findings	Mechanism of action	Ref.
In vivo (SW480 colon cancer xenografts in nude mice)	Silicon phthalocyanine (Pc 4)	Effective induction of apoptosis in SW480 colon cancer xenografts. Tumor growth delayed by 9–15 days post-treatment	Caspase-9, caspase-3 activation, PARP cleavage, phosphorylation of p38 MAP kinase	153
In vitro and *in vivo* (HT29 human colon cancer cell line, HT29 tumor-bearing nude mice)	Photofrin and Pheophorbide a (Ph a)	Inhibition of HT29 cell growth in culture. Single PDT session led to 3-weeks delay in tumor growth	DNA fragmentation, PARP cleavage, mutant-type p53 expression, necrosis of tumor cells	154
	N-Acetyl chlorin e6 (NPe6)	Enhanced photodynamic efficacy with fractionated drug administration. Tumor control through vascular shutdown	Deeper tumor penetration, vascular shutdown	155
In vitro (HCT116 human colon carcinoma cell line)	Meso-diaryl-substituted tetrapyrrole derivatives	Greater *in vitro* photodynamic potency than *m*-THPC. Induced apoptosis and generated ROS and nitric oxide (NO*)	ROS generation, NO* induction, apoptosis	156
In vitro (SW480 colon cancer cells), *in vivo* (tumor-bearing mice)	Meso-*tetra* (carboxyphenyl) porphyrin (TCPP) NPs	Rapid internalization *via* clathrin-mediated endocytosis. Higher photocytotoxicity compared to free TCPP and TCPP-loaded PLGA NPs	Clathrin-mediated endocytosis, necrosis of tumor cells	157
In vitro (HCT116 human colorectal carcinoma cells), *in vivo* (BALB/c nude mice xenografts)	DH–II–24	Accumulated in mitochondria, lysosomes, and endoplasmic reticulum. Significant tumor growth suppression *in vivo*	Necrotic cell death, tumor vasculature destruction	158
In vitro (doxorubicin-sensitive and doxorubicin-resistant colon cancer cell lines)	Photofrin (Ph)	PDT induced oxidative stress in both cell lines. MDR cells exhibited a delayed response to oxidative stress	Oxidative stress, increased TBARS, decreased protein-associated –SH groups, elevated SOD1 activity	159
In vitro (A549 and DLD-1 cell lines)	Zinc sulfophthalocyanine (ZnPcS)(mix)	Significant reduction in cell viability and proliferation. No toxicity in absence of light	Targeting lysosomes and mitochondria, ROS generation	160

on human cells, eliminating the complexities associated with translating findings from animal models to human applications. These studies are pivotal for assessing the efficacy and specificity of different PSs in targeting and eradicating colorectal cancer cells.

By administering PSs to tumor cells and subsequently activating them with light, researchers can assess the resulting cytotoxic effects and determine the optimal parameters for PDT. This includes selecting the appropriate PS, its concentration, light dosage, and treatment duration. Additionally, these *in vitro* studies offer valuable insights into the mechanisms of PDT in colorectal cancer, such as cellular responses like apoptosis, necrosis, and reactive oxygen species (ROS) production. A thorough understanding of these processes is crucial for refining PDT protocols, ultimately contributing to the development of more effective cancer treatment strategies that enhance patient outcomes.[150] While monolayer cultures are useful for studying treatment effects, they do not adequately represent the complex and diverse characteristics of *in vivo* tumor environments. To overcome these limitations, researchers have created three-dimensional (3D) tumor models that more accurately mimic the tumor microenvironment. These 3D models enable extended studies of tumor cells and include vital features like cell–cell interactions, extracellular matrix components, and nutrient gradients, all of which are crucial for comprehending tumor behaviour. However, 3D tumor models are not without their own challenges. They still do not completely replicate the intricacies of *in vivo* tumor growth, metastatic processes, or interactions with the immune system. Moreover, the lack of vascular structures and immune cell infiltration can limit the applicability of findings from these models to clinical scenarios, highlighting the need for further advancements to enhance their relevance in cancer research.[152]

In summary as showed in Table 3, while *in vitro* studies have demonstrated the potential of PDT in targeting colorectal cancer cells, validating these findings in appropriate *in vivo* models is crucial for understanding the therapy's efficacy and translating it into clinical practice. Continued research in both settings will enhance our understanding and optimize PDT as a viable treatment option for CRC.

10 Clinical trials in CRC

Clinical trials have played a vital role in the safe and effective development of medical treatments since the landmark 1948 Medical Research Council study, which first demonstrated the efficacy of streptomycin in treating tuberculosis.[150] Clinical PDT for colorectal cancer involves the use of visible light in combination with a PS and oxygen to selectively destroy cancer cells in patients.[92,161] In the absence of standardized guidelines for PDT use in CRC patients, clinical trials have utilized diverse PDT parameters including variations in the choice and concentration of PS, light type and dose, treatment regimens, and integration with conventional therapies.[150] In clinical trials for CRC treatment, PDT is typically administered using optical fibers inserted through an endoscope to deliver light for PS activation. This targeted approach allows PDT to selectively destroy cancerous tissues in the colon while minimizing side effects and reducing systemic toxicity to surrounding healthy cells.[150,161]

Table 4 presents various clinical trials investigating the use of PDT in colorectal cancer. Most of these studies are pilot, phase I, or phase II trials. Phase I trials typically include small groups of patients with advanced-stage disease and primarily assess the safety and toxicity of PDT. In contrast, phase II trials involve larger patient cohorts and focus on evaluating the clinical effectiveness of the treatment.[150] Data from phase III clinical trials assessing the overall efficacy of PDT in CRC are limited. At present, no phase IV trials have been conducted since PDT is not yet an approved treatment for this specific cancer type. Nonetheless, existing clinical trials have consistently shown that PDT is effective in the clinical management of CRC.[162]

In a 1991 clinical study involving six patients with advanced rectal cancer at palliative stage I/II, PDT was administered using Porfimer sodium at a dose of 2 mg kg^{-1} delivered intravenously. The treatment was carried out using a 630 nm wavelength light with a fluence range of 50–200 J cm^{-2}. This approach aimed to provide symptom relief and improve the quality of life in patients with unresectable or recurrent rectal tumors.[163]

In 1994, a pilot study was conducted on eight patients with colorectal adenomas, where Hematoporphyrin Derivative (HpD) was administered at a dose of 2.5 mg kg^{-1} intravenously, followed by Porfimer sodium at 2 mg kg^{-1} i.v. The light used had a wavelength of 630 nm, a power output of 100 mW, and a total energy dose of 50 J.[164] In 1995, another pilot study included six patients with duodenal and colorectal polyps. The treatment involved oral administration of 5-aminolevulinic acid (5-ALA) at 30–60 mg kg^{-1} and intravenous Porfimer sodium at 2 mg kg^{-1}. The light source used had a wavelength of 628 nm and delivered either 50 or 100 J of energy.[165]

In 1998, a phase I trial involving 11 patients with various malignant tumors evaluated the use of *N*-aspartyl chlorin e6 (NPe6), administered intravenously at doses ranging from 0.5 to 3.5 mg kg^{-1}. The PDT was delivered at 664 nm with fluences between 25 and 100 J cm^{-2}.[166] In the same year, a larger pilot study was performed on 22 patients with tumors in the esophagus, duodenum, and rectum. This study utilized multiple PSs: *m*-THPC (temoporfin) at 0.15 mg kg^{-1} i.v., Porfimer sodium at 2 mg kg^{-1} i.v., and 5-ALA at 60 mg kg^{-1} administered orally. Illumination was provided at 650 nm (10–15 J cm^{-2}) for *m*-THPC, and at 628 nm with fluences of 50–150 J cm^{-2} for Porfimer sodium and 5-ALA.[167]

In 2002, another pilot study involving 51 patients with different malignant tumors used Radachlorin, administered intravenously at a dose of 0.8–1.2 mg kg^{-1}. The light source used was 662 nm with energy doses ranging between 100 and 500 J cm^{-2}.[168] In 2003, two small pilot studies were conducted: one on two patients with rectal cancer using HpD at 2.5 mg kg^{-1} i.v., with illumination at 627.8 nm and power levels between 150 and 280 mW and another on a single patient with anal intraepithelial neoplasia, using a 20% topical 5-ALA cream, followed by irradiation at 630 nm with a fluence rate of 125 mW cm^{-2} and a total energy dose of 125 J cm^{-2}.[169,170] These studies

Table 4 Summary of clinical trials investigating PDT for colorectal and related malignancies

Year	Phase	Patients	Photosensitizer	Dose	Light (nm)	Fluence	Ref.
1991	Pilot	6 (Advanced rectal)	Porfimer sodium	2 mg kg^{-1} i.v	630	50–200 J cm^{-2}	163
1994	Pilot	8 (Adenomas)	HpD + porfimer sodium	2.5 mg kg^{-1} + 2 mg kg^{-1} i.v	630	50 J	164
1995	Pilot	6 (Polyps)	5-ALA (oral) + porfimer sodium	30–60 mg kg^{-1} + 2 mg kg^{-1}	628	50 or 100 J	165
1998	Phase I	11 (Various tumors)	NPe6	0.5–3.5 mg kg^{-1} i.v	664	25–100 J cm^{-2}	166
1998	Pilot	22 (Esophagus, rectum)	*m*-THPC, porfimer sodium, 5-ALA	0.15 mg kg^{-1}, 2 mg kg^{-1}, 60 mg kg^{-1}	650, 628	10–150 J cm^{-2}	167
2002	Pilot	51 (Various malignancies)	Radachlorin	0.8–1.2 mg kg^{-1} i.v	662	100–500 J cm^{-2}	168
2003	Pilot	2 (Rectal CA), 1 (AIN)	HpD/5-ALA cream	2.5 mg kg^{-1}/20% topical	627.8/630	150–280 mW/125 J cm^{-2}	169 and 170
2004	Phase I	8 (Liver mets CRC)	*m*-THPBC/talaporfin sodium	1–6 mg kg^{-1}/40 mg m^{-2}	740/664	60 J cm^{-1}/100 J cm^{-1}	171
2005	Phase I	24 (Liver mets CRC)	*m*-THPBC	0.3–0.6 mg kg^{-1} i.v	740	60 J cm^{-1}	172
2006	Phase II	100 (Peritoneal mets)	Porfimer sodium	2.5 mg kg^{-1} i.v	532	150 mW cm^{-2}, 2.5 J cm^{-2}	173
2010	Phase II/III	8 (Anal cancer)	Porfimer sodium	1.2 mg kg^{-1} i.v	630	500 J cm^{-2} total	14
2014	Pilot	15 (AIN)	5-ALA cream/porfimer sodium	Topical/1.2 mg kg^{-1} i.v	630	75–100 J cm^{-2}	
2016	Phase II/III	23 (Advanced CRC)	Porfimer sodium	2 mg kg^{-1} i.v	630	200 J cm^{-2}	175
2019	Pilot	1 (Rectal adenocarcinoma)	Porphyrin-based	2 mg kg^{-1} i.v	630	120 J cm^{-2}, 100 mW cm^{-2}	176

collectively highlight the diversity of PSs and treatment parameters explored during the early stages of PDT research for gastrointestinal and anorectal tumors.

In 2004, a phase I clinical trial was conducted on eight patients with liver metastases from colorectal carcinoma. Two PSs were used: mono-L-aspartyl chlorin e6 (*m*-THPBC) administered intravenously at doses of 1, 3, or 6 mg kg^{-1}, and Talaporfin sodium at 40 mg m^{-2} i.v. Light activation was carried out at 740 nm with a fluence of 60 J cm^{-1} for *m*-THPBC, and at 664 nm with 100 J cm^{-1} for Talaporfin sodium.[171] In 2005, another phase I trial was performed on 24 patients with the same condition using *m*-THPBC at lower doses of 0.3–0.6 mg kg^{-1} intravenously, with light at 740 nm delivering 60 J cm^{-1}.[172]

In 2006, a phase II trial involving 100 patients with peritoneal carcinomatosis and sarcomatosis used Porfimer sodium at a dose of 2.5 mg kg^{-1} intravenously. PDT was conducted using a 532 nm light at a power density of 150 mW cm^{-2} and total energy of 2.5 J cm^{-2}.[173] Later, in 2010, a combined phase II/III study was conducted on eight patients with anal cancer, where Porfimer sodium was administered at 1.2 mg kg^{-1} i.v., followed by light application at 630 nm with a cumulative fluence of 300 J cm^{-2} plus an additional 200 J cm^{-2}.[14]

In 2014, a pilot study was performed on 15 patients with anal intraepithelial neoplasia. Treatment involved either topical application of 5-aminolevulinic acid (5-ALA) cream in two cycles (activated by 630 nm light delivering 75 J cm^{-2} each) or systemic administration of Porfimer sodium at 1.2 mg kg^{-1} i.v., followed by light at the same wavelength delivering 100 J cm^{-2}.[174] In 2016, a phase II/III clinical trial on 23 patients with advanced colorectal cancer used Porfimer sodium at 2 mg kg^{-1} intravenously, with light exposure at 630 nm and a fluence of 200 J cm^{-2}.[175] Finally, in 2019, a pilot study involving a single patient with rectal adenocarcinoma utilized a porphyrin-based photosensitizer at 2 mg kg^{-1} i.v. The treatment was activated with light at 630 nm, using a power density of 100 mW cm^{-2} and a total energy dose of 120 J cm^{-2}. These studies demonstrate the evolving application of PDT across a range of colorectal and gastrointestinal malignancies, with various PSs, light sources, and dosimetry parameters under investigation for optimal therapeutic outcomes.

11 Limitations of PDT in CRC

Despite the numerous advantages of PDT in treating CRC, its clinical application faces several challenges. These include poor water solubility of PS, limited selective uptake by tumor tissues, and the difficulty in effectively targeting deep-seated tumors due to the limited tissue penetration of the activating light.[162,177] Another limitation of PDT is its reduced effectiveness in treating hypoxic tumors. Since the cytotoxic action of PDT relies heavily on the presence of oxygen to generate reactive oxygen species, its efficacy is significantly compromised in low-oxygen (hypoxic) tumor environments.[11,95,178] The recurrence and progression of CRC have been linked to the presence of cancer stem cells, which exhibit strong resistance to PDT.[93,143] Consequently, supplementary therapeutic approaches may be necessary to effectively target advanced forms of CRC, addressing both

primary tumors and metastatic disease.[92] The hydrophobic nature of PS presents a significant challenge in PDT, as poorly soluble PSs tend to aggregate upon administration. This aggregation hinders their efficient uptake by cancer cells and decreases the generation of ROS, ultimately reducing the therapeutic effectiveness of PDT.[162] For optimal ROS generation and complete tumor destruction in PDT, it is essential to effectively deliver and concentrate high levels of PS drugs within the targeted tumor tissues.[179,180] However, clinical use of first- and second-generation PS drugs has often resulted in poor outcomes and limited effectiveness. This is largely due to the fact that only a small fraction of the administered PS can successfully cross biological barriers and passively accumulate in tumor cells, leading to insufficient ROS production and inadequate tumor destruction.[92,181] Furthermore, this passive accumulation may result in the buildup of photosensitizer drugs in healthy tissues, leading to undesirable side effects such as photosensitivity and damage to normal cells.[179,180] To address these challenges, third-generation PSs combined with NP carriers such as liposomes, dendrimers, polymeric NPs, and inorganic NPs are currently being explored. These nanocarriers improve the water solubility and cellular uptake of PSs, enabling more efficient and targeted delivery to tumor sites and enhancing the overall effectiveness of PDT in CRC.[8,108]

NP-based photosensitizer carriers hold significant promise for advancing colorectal cancer treatment and enhancing patient outcomes.[93] Near-infrared (NIR) light penetrates deeper into tissues more effectively than visible light. However, its longer wavelengths carry less energy, which can limit the strength of the photodynamic effect. To address this challenge, several studies have proposed using two-photon NIR photodynamic activation and up conversion-mediated photodynamic activation techniques.[182–184] In two-photon NIR photodynamic activation, PS is excited through the simultaneous absorption of two lower-energy photons within the near-infrared spectrum. The combined energy of these photons matches the PS's bandgap energy, enabling deeper tissue penetration and reducing photobleaching of the PS within the tissues.[183,184] Another approach uses up conversion NPs (UCNPs) to facilitate NIR photodynamic activation. These NPs can absorb multiple photons at a specific wavelength and convert them into a single photon of shorter wavelength through an anti-Stokes shift. The resulting higher-energy photon can then efficiently excite the PS for use in PDT.[183,185] In recent years, numerous UCNPs have been developed. For example, Gao *et al.* created UCNPs loaded with zinc phthalocyanine (ZnPc) as the photosensitizer and conjugated them with c(RGDyK) peptides to specifically target tumor vasculature. This design enabled deep-tissue activation of the photosensitizer through NIR light irradiation.[186]

Other studies have synthesized UCNPs loaded with various PSs, including Ce6, MC540, and AgBiS2. These formulations have demonstrated substantial tumor growth inhibition following PDT activated by high-wavelength light for upconversion.[185,187–189] Delivering light to CRC can often be challenging. To address this, Rodrigues *et al.* proposed an innovative approach by integrating a PDT module into an endoscopic capsule, enabling minimally invasive light delivery directly to the tumor site for effective PDT treatment.[190]

12 Enhancing PDT efficacy in CRC through synergistic combined therapies

Extensive research has shown that CRC exhibits considerable heterogeneity in its mutation profiles, posing significant challenges for existing treatment approaches. Consequently, conventional monotherapies often struggle to effectively eliminate colorectal cancer cells, resulting in less-than-ideal treatment outcomes. Additionally, these therapies can cause adverse side effects, complicating patient management and reducing overall treatment effectiveness. The diversity of mutations present within tumors highlights the urgent need for more personalized and targeted treatment approaches. By tailoring therapies to the unique genetic profiles of individual tumors, we can enhance the likelihood of successful treatment outcomes and improve the overall prognosis for patients with colorectal cancer.[16,111] Consequently, there is a growing focus on combination therapies that can achieve synergistic effects and overcome the drawbacks associated with single-agent treatments (Fig. 4). The integration of different therapeutic approaches shows significant potential, as it may enhance treatment efficacy while minimizing adverse side effects compared to monotherapy. By utilizing the unique benefits of various treatment modalities, this strategy seeks to effectively target CRC and improve overall patient outcomes. This multifaceted approach not only aims to attack the cancer from multiple angles but also fosters a more comprehensive response, potentially leading to better survival rates and quality of life for patients.[161,191]

PDT has shown the ability to trigger immunogenic cell death, activating the body immune responses and promoting antitumor immunity (Fig. 4). This feature makes PDT an attractive option for combination therapies with immunotherapies designed to boost the host's immune system. A highly

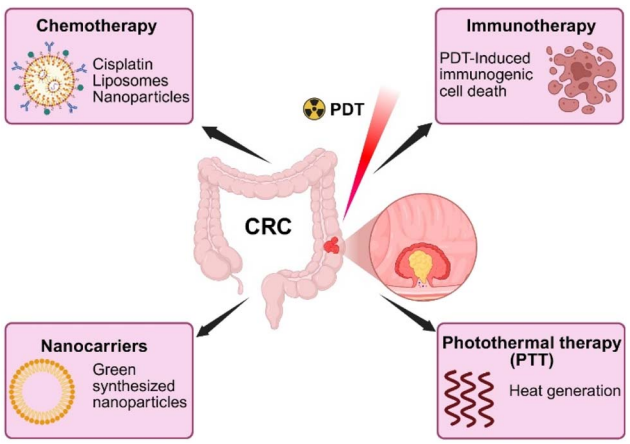

Fig. 4 Combined therapies enhance PDT efficacy in colorectal cancer treatment.

promising approach in cancer therapy involves the utilization of immune checkpoint inhibitors, which are antibodies designed to block immune checkpoints that dampen immune responses. This blockage allows for a more robust immune attack on cancer cells. By integrating PDT with these inhibitors, the objective is to enhance the immune response, thereby increasing the overall efficacy of cancer treatments. This combination aims to not only destroy tumor cells directly through PDT but also to activate the immune system to recognize and eliminate residual cancer cells, potentially leading to improved patient outcomes and reduced risk of recurrence. This synergistic approach could potentially overcome resistance mechanisms and lead to more durable treatment outcomes for patients.[192]

Recently, various NPs have emerged as innovative delivery systems designed to enhance the efficacy of PDT in conjunction with immune checkpoint inhibitors for tumor treatment. A notable example is the work by He *et al.* who engineered nanoscale coordination polymer (NCP) core–shell NPs. These NPs feature a core that encapsulates oxaliplatin, a chemotherapy agent, while the shell contains the photosensitizer pyrolipid. This strategic combination of oxaliplatin chemotherapy, PDT, and checkpoint blockade therapy has been shown to bolster antitumor immune responses and achieve significant therapeutic outcomes in metastatic colorectal cancer. By integrating these approaches, this method not only targets the tumor directly but also activates the immune system to recognize and eliminate cancer cells more effectively.[193] PDT based combination therapies have attracted significant attention across various cancers because of their strong therapeutic potential.[194] These effects include the induction of immunogenic cell death, generation of damage-associated molecular patterns and tumor-associated antigens, and modulation of the tumor microenvironment.[195] These processes collectively enhance the activation of antigen-presenting cells and promote T-cell infiltration. Therefore, combining PDT with immune checkpoint inhibitor (ICI) therapy can overcome the limitations of ICI treatment alone, boosting the antitumor immune response and improving overall immunotherapeutic efficacy.[196]

Programmed cell death protein 1 (PD-1) and its ligand, PD-L1, are key immune checkpoints that deliver inhibitory signals to suppress the host immune response.[197,198] Upregulation of PD-L1 in tumor cells is commonly linked to tumor progression, enhanced proliferation and invasion, activation of anti-apoptotic pathways, and inhibition of T-cell activity through interaction with PD-1.[199] Minimizing the immunosuppressive effects of PD-1/PD-L1 signaling is particularly important in cancer therapy. Recent studies have highlighted the potential of PDT to enhance immune responses by modulating immune checkpoints. For instance, Gurung *et al.* reported that PDT using the photosensitizer chlorin e6 can inhibit the PD-1/PD-L1 axis and boost $CD8^+$ T-cell activity through an as-yet-unclear mechanism. However, they also observed that, despite increased infiltration of these T cells in some mice, the cells were subsequently depleted, and a sustained antitumor immune response was not achieved.[200] Conversely, studies by Lobo *et al.* and Anand *et al.* indicate that PDT can lead to increased expression of CTLA-4 on tumor cells and elevated PD-L1 and PD-1 levels on lymphocytes in certain tumor models.[201,202] Nonetheless, this effect could potentially enhance the efficacy of ICI therapy, since the presence of pre-existing T cells within the tumor is critical for a response to anti-PD-1 treatment. Conversely, tumors lacking inducible PD-L1 expression may respond poorly to PD-1 blockade due to insufficient T-cell infiltration.[203] Additionally, PDT has been shown to induce elevated expression of CD80 on the surface of cells in certain tumors, and this overexpression can increase tumor immunogenicity.[204]

Xu *et al.* engineered multifunctional NPs, designated as UCNP–Ce6-R837, by concurrently incorporating UCNPs with chlorin e6 (Ce6) and imiquimod (R837), a Toll-like receptor-7 (TLR-7) agonist. When subjected to near-infrared (NIR) irradiation, these NPs demonstrated potent photodynamic therapeutic effects, effectively inducing cell destruction. Moreover, they significantly boosted antitumor immune responses in CT26 colorectal cancer cells, showcasing their potential for dual functionality in targeting tumor cells and enhancing immune activation. This innovative approach highlights the promising role of such NPs in advancing cancer treatment strategies.[205] Yuan *et al.* developed multifunctional NPs, known as mTHPC@VeC/T-RGD NPs, incorporating the photosensitizer mTHPC to enhance the effectiveness of PD-L1 blockade in colorectal cancer treatment. Their study investigated the ways in which PDT can improve the therapeutic outcomes of PD-L1 blockade when used in combination. By harnessing the synergistic effects of PDT, which facilitates localized destruction of tumor cells while also stimulating immune responses, the researchers aimed to gain deeper insights into the mechanisms that drive the enhanced efficacy of this combined treatment approach. This innovative strategy holds promise as a more effective therapeutic option for patients battling colorectal cancer, potentially leading to improved clinical outcomes.[192]

The integration of chemotherapy with surgical interventions has been shown to markedly enhance survival rates for patients diagnosed with metastatic CRC. Despite these benefits, chemotherapy often brings a range of side effects that can considerably diminish the patients' quality of life. These side effects may include nausea, fatigue, hair loss, and increased susceptibility to infections, which can lead to treatment interruptions and a negative impact on the overall well-being of patients. Therefore, while the combination of these treatments offers a potential for improved outcomes, it also necessitates careful management of the associated adverse effects to support patients throughout their treatment journey.[206]

The integration of PDT with chemotherapy has emerged as a promising strategy for treating CRC. This combined approach offers multiple benefits. First, PDT enables the precise targeting and elimination of cancer cells at the treatment site, reducing the reliance on extensive chemotherapy, which often damages healthy tissues and can cause severe systemic side effects. Additionally, the cytotoxic effects of PDT can increase the efficacy of chemotherapy by making cancer cells more sensitive to chemotherapeutic agents, leading to better tumor response rates and helping to address issues of drug resistance. In this

context, Su *et al.* developed an innovative nanoplatform for combined chemo-PDT designed to modify the redox balance and induce endoplasmic reticulum stress specifically in CRC cells. This approach aims to enhance therapeutic outcomes by exploiting the vulnerabilities of cancer cells, making them more susceptible to treatment while minimizing harm to surrounding healthy tissue. This innovative system combines the chemotherapeutic agent brigatinib with the photosensitizer chlorin e6 (Ce6) within a TPGS-based nanosystem. By integrating these components, the platform seeks to maximize therapeutic efficacy while minimizing adverse effects, highlighting the potential of this synergistic approach in cancer treatment.[207] Hashemkhani *et al.* suggested a novel approach that employs cetuximab-conjugated Ag_2S quantum dots loaded with aminolevulinic acid (ALA) and 5-fluorouracil. This strategy aims to achieve tumor-specific targeting in combination therapy, integrating PDT with conventional chemotherapy for colorectal cancer cell lines that express the epidermal growth factor receptor (EGFR). By harnessing the targeted delivery capabilities of cetuximab, the Ag2S quantum dots can selectively accumulate in EGFR-positive tumors, enhancing the efficacy of both ALA-mediated PDT and 5-fluorouracil chemotherapy. This innovative combination not only aims to improve therapeutic outcomes but also to reduce systemic side effects by sparing healthy tissues.[208] Chen *et al.* introduced a novel therapeutic approach for colorectal cancer that utilizes a combination of porphyrin-grafted lipids and a triad of camptothecin and floxuridine microbubbles, which are activated through ultrasound. This strategy seeks to merge the chemotherapeutic effects of camptothecin and floxuridine with the PDT provided by the porphyrin-grafted lipids. By integrating these modalities, the therapy aims to effectively address the issue of multidrug resistance commonly encountered in CRC, enhancing treatment efficacy and potentially improving patient outcomes.[209] The integration of PTT and PDT has shown promise in the treatment of CRC by generating cytotoxic ROS and inducing hyperthermia when PSs are activated by light. This combined approach enhances the therapeutic effect by not only damaging cancer cells directly through oxidative stress but also by raising the temperature of the tumor microenvironment, which can further weaken cancer cell viability and improve treatment efficacy. By utilizing both mechanisms, this strategy aims to overcome some of the limitations associated with traditional therapies and provide a more effective solution for combating CRC.[93] Seo *et al.* designed NPs composed of gold nanorods coated with silica (SiO_2) and loaded with methylene blue to facilitate a synergistic therapeutic strategy for colorectal cancer. This innovative system combines PDT and PTT, enhancing the overall effectiveness of treatment. The methylene blue serves as a photosensitizer that generates reactive oxygen species upon light activation, contributing to tumor cell destruction, while the gold nanorods provide localized heating when exposed to near-infrared light. This dual-action approach not only targets cancer cells more effectively but also minimizes damage to surrounding healthy tissues, representing a promising advancement in cancer therapy.[210] In another study by Wang *et al.*, researchers developed hyaluronic acid-coated polydopamine NPs conjugated with chlorin e6 (Ce6) for targeted cancer therapy that integrates PDT and PTT. This innovative formulation capitalized on the properties of hyaluronic acid to enhance the targeting of cancer cells. The resulting synergistic effects led to increased accumulation of the NPs within tumors, which in turn resulted in more effective tumor growth inhibition and enhanced phototoxic effects in mice bearing HCT-116 tumors. This study underscores the potential of combining different therapeutic modalities in a single NP platform to improve cancer treatment outcomes.[211] Yang *et al.* created photonic micelles with a size of less than 100 nm, encapsulating SN-38 for a comprehensive trimodal cancer treatment strategy that combines photothermal, photodynamic, and chemotherapy modalities. This innovative approach demonstrated significantly improved antitumor efficacy *in vivo*, particularly in nude mice implanted with HT-29 colon cancer xenografts, when compared to single treatment methods. By leveraging multiple therapeutic mechanisms simultaneously, this strategy not only enhances the effectiveness of cancer treatment but also addresses the limitations of traditional therapies, potentially leading to better patient outcomes in combating tumor growth and progression.[212]

13 Conclusion and future suggestions

Colorectal cancer remains a leading cause of cancer-related morbidity and mortality worldwide, necessitating innovative therapeutic strategies to overcome treatment resistance and enhance patient outcomes. PDT has emerged as a promising approach, leveraging the selective targeting of tumor cells while minimizing damage to surrounding healthy tissue. The integration of advanced PSs and nanocarrier systems has shown potential in improving the efficacy of PDT, particularly in addressing the challenges posed by CSCs, which are key players in tumor recurrence and treatment resistance. Despite the encouraging preclinical results, further research is essential to fully realize the clinical potential of PDT. Initiating well-designed clinical trials to evaluate the safety and efficacy of PDT in diverse CRC patient populations is crucial, particularly for different stages and molecular subtypes of the disease. Additionally, further exploration of nanocarrier systems for delivering PSs and combination therapies could optimize their design for enhanced targeting and reduced off-target effects. Identifying biomarkers that predict responses to PDT, especially in the context of CSCs, will help tailor treatments to individual patients based on their tumor characteristics. Investigating additional combination strategies that integrate PDT with chemotherapy, radiotherapy, or novel agents may maximize treatment efficacy and minimize resistance. Lastly, conducting in-depth studies on the molecular mechanisms underlying PDT effects on CSCs and the tumor microenvironment is essential for understanding how to overcome resistance and improve treatment outcomes. By addressing these areas, future research can contribute to developing more effective and personalized treatment approaches for colorectal cancer, ultimately enhancing patient care and survival rates.

Author contributions

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Conflicts of interest

The authors declare no conflicts of interest.

Data availability

No data was used for the research described in the article.

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