Chapter

Targeted Photodynamic Therapy as Potential Treatment Modality for the Eradication of Colon Cancer

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Abstract

Photodynamic therapy (PDT) can be used to treat colorectal cancer (CRC). When a photosensitizer (PS) drug is administered to a patient, it can either passively or actively accumulate within a tumor site and once exposed to a specific wavelength of light, it is excited to produce reactive oxygen species (ROS), resulting in tumor destruction. However, the efficacy of ROS generation for tumor damage is highly dependent on the uptake of the PS in tumor cells. Thus, PS targeted uptake and delivery in CRC tumor cells is a crucial factor in PDT cancer drug absorption studies. Generally, within non-targeted drug delivery mechanisms, only minor amounts of PS passively accumulate in tumor sites and the remainder distributes into healthy tissues, causing unwanted side effects. To improve the efficacy of PDT research is currently focused on the development of specific receptor based photosynthetic nanocarrier platform drugs, which promote the active uptake and absorption of PS drugs in CRC tumor sites only, avoiding unwanted side effects, as well as treatment enhancement. This chapter will focus on current actively targeted PS nanoparticle drug delivery systems, which have been investigated for the PDT treatment of CRC cancer.

Keywords: colorectal cancer, photodynamic therapy, photosensitizer, nanoparticles, targeted drug delivery

1. Introduction: colorectal cancer (CRC)

There are over a million new cases of colorectal cancer (CRC) being diagnosed worldwide each year [1]. CRC is known to be the third most commonly diagnosed cancer malignancy worldwide and is the fourth most frequent cause of cancer related cell deaths, with around 0.6 million deaths annually [2].

CRC is an uncontrolled growth that originates within polyps that are found in the inner lining of either the colon or rectum [3]. The intestinal wall of the colon and rectum is made up of many layers [3]. CRC polyp growth formation begins within the innermost mucosal layers of either the colon or rectum and these polyps can grow outward through some or all of these intestinal layers [1]. When CRC primary polyp cells growth spreads from the inner to the outer intestinal walls, they can grow into

blood or lymph vessels and so spread to other parts of the body forming secondary cancer metastasizes [1]. Adenocarcinomas polyps originate within intestinal cells that produce mucus to lubricate the inside of either the colon or rectum and this is the most common form of CRC, with approximately 96% of cases, being diagnosed annually [3]. Other less common types of CRC tumors that can originate in colorectal tissues or cells include: lymphomas, sarcomas, gastrointestinal carcinoid or stromal tumors [3].

The risk of developing of CRC is often attributed to either a variety of environmental factors or genetic predispositions. Approximately 25% of diagnosed CRC cases can be attributed to inherited syndromes, while the remaining 75% cases are due to external environmental contributing factors [4, 5]. The most common CRC inherited syndromes include familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancers (HNPCC) [4]. While common triggering environmental factors include: diets which are low in fiber and high in fat and red meat, low physical activity, obesity, heavy alcohol consumption, cigarette smoking and deskbound seated occupations [5].

Even though numerous advances have been made in relation to early diagnosis and treatment of CRC, tumor reoccurrence and metastatic spread are two critical factors which affect the survival rate of patients [6]. Dependent on the stage at which they have been diagnosed, approximately 25% of patients with CRC at time of diagnosis have metastases (due to late detection) and 50% of patients diagnosed with CRC will develop metastases, either at presentation or during follow-up [7, 8].

2. Resistance of CRC to conventional treatments

The most common conventional treatments for CRC include surgical resection, chemotherapy or radiation therapy [9]. These treatments are either used in combination or alone depending on the stage at which the disease has been detected and diagnosed [9].

In early stages (0 to I) of CRC diagnosis, the most common treatment practice is surgical resection of the CRC polyps, without any further need for treatment [10]. In stages II to III of CRC detection, surgical resection with lymph node dissection to examine for presence of cancer cell spread, is standard practice [11]. Patients with stage IV CRC disease often require chemotherapy and/or radiation therapy combined with surgery to treat the disease [12].

Typical standard CRC chemotherapy treatment regimens include; FOLFOX: leucovorin, 5-fluorouracil (5-FU), and oxaliplatin (Eloxatin), FOLFIRI: leucovorin, 5-FU, and irinotecan (Camptosar), CAPEOX or CAPOX: capecitabine (Xeloda) and oxaliplatin, FOLFOXIRI: leucovorin, 5-FU, oxaliplatin, and irinotecan, one of the previous combinations, plus either a drug that targets VEGF, (bevacizumab [Avastin], ziv-aflibercept [Zaltrap], or ramucirumab [Cyramza]), or a drug that targets EGFR (cetuximab [Erbitux] or panitumumab [Vectibix]) or 5-FU and leucovorin, with or without a targeted drug, Capecitabine, with or without a targeted drug, Irinotecan, with or without a targeted drug, Cetuximab alone, Panitumumab alone, Regorafenib (Stivarga) alone, Trifluridine and tipiracil (Lonsurf) [13].

Thus, 5-FU-based chemotherapy remains the mainstay of therapy for patients with CRC, however in recent year's chemotherapy drugs such as oxaliplatin, irinotecan and capecitabine have been developed and generally conventional chemotherapy treatment for advanced CRC combines 5-FU and leucovorin with oxaliplatin or irinotecan [14]. The greatest strides over recent years in chemotherapy treatments have been combining these drugs with monoclonal antibodies such as Bevacizumab and Cetuximab in order to target vascular endothelial growth factor (VEGF) or epidermal growth factor receptor (EGFR) which are

respectively overexpressed in CRC cells [14, 15]. Angiogenesis, plays an important role in CRC tumor development and metastasis, is partly mediated by vascular endothelial growth factor (VEGF), thus by combining chemotherapeutic drugs with Bevacizumab monoclonal antibodies, VEGF overexpressed receptors can be targeted to enhance drug uptake and so improve treatment [14, 15]. Likewise, since EGFR plays and important role in tumorigenesis, it is often found to be overexpressed in a high percentage of patients with late-stage colorectal cancer and by combining chemotherapeutic drugs with Cetuximab monoclonal antibodies, chemotherapeutic drug targeting and uptake can be promoted [14, 15]. Moreover, by utilizing chemotherapy monoclonal antibody treatments for CRC, resistance to EGFR inhibitors may be partially mediated, by activating VEGF-dependent signaling, and so drug delivery strategies that combine anti-EGFR and anti-VEGF agents appear promising [15].

Overall, the choice of these various chemotherapy treatment regimens for CRC depends on various factors such as previous treatments received, if the regime is no longer working and the patients overall health [13]. For some patients with certain genetic marker changes in their CRC cells another treatment option after chemotherapy to be considered is immunotherapy with pembrolizumab [13].

Nevertheless, despite the improved CRC response rates with these various advanced strategies, the overall survival rate for metastatic CRC remains only slightly over 12% [18]. One of the major causes for this poor survival rate is due to the fact that nearly half of all metastatic CRC patients are resistant to 5-FU-based chemotherapies, which demises their overall treatment and recovery [14]. The reason for the development of chemotherapeutic drug resistance in CRC cells is that they have the ability to enhance DNA repair mechanisms, deregulate signaling pathways, as well as increase drug metabolism [16]. Generally, 90% CRC patients report drug resistance to chemotherapies, resulting in poor treatment due to oncogene mutations, which deregulate signaling pathways [16]. This deregulation of signaling pathways, results in increased aerobic glycolysis, fatty acid synthesis, and glutamine metabolism causing a decrease in chemotherapeutic drug induced apoptosis [17]. Moreover, drug efflux transporter proteins are often found to be overexpressed in drug-resistant CRC cells, which decrease the successful uptake of chemotherapeutic drugs in cancer cells [6, 18]. Thus, if metastases has occurred, chemotherapy will probably not be curative and so only help in improving prognosis via tumor shrinkage [19]. Thus continuous research is required into CRC in order to unravel these multiple drug resistance mechanisms and so develop improved treatment regimens with better outcomes [18, 19].

Radiation therapy is usually utilized pre-CRC surgical resection in stages II to IV, depending on the degree of metastasis, to shrink un-respectable tumors or to try and help control the cancer that has spread to other parts of the body [11, 20]. However, radiation therapy has numerous unwanted side effects in patients receiving such treatments, which include: nausea, stool leakage, fatigue, sexual problems, skin irritation, rectal irritation and diarrhea [21]. Moreover, some CRC patients have noted resistance to radiation therapy, whereby in response to radiation DNA damage, Ataxia Telangiectasia Mutated (ATM) genes and anti-apoptotic factors phosphatases of regenerating liver-3 (PRL-3) become activated in cancer cells and so begin to regulate cancer cell pro-survival and resistance [22]. Additionally, these genes have been noted to be overexpressed in CRC patients whom have previously received radiation therapy and their cancer has reoccurred, shortening their survival [23].

Moreover, in addition to resistance to conventional treatments, the metastasis spread of CRC is of major concern. Primary CRC tumors are highly prone to TGF-β, PIK3CA, and TP53 gene mutations and since these genes are responsible for clonal

expansion and invasiveness, the metastatic cellular potential of CRC to spread is high [24]. Lastly, another important factor in CRCs resistance to conventional therapies and metastasis, is the presence of cancer stem cells, since these cells have the ability to go by undetected (due to their slow growth) and so enhances CRC treatment resistance, as well as allows this type of cancer to initiate new tumor growth and so metastasize [6].

Thus, currently conventional treatments are not very successful at curing CRC and patients are at high risk of developing secondary cancers, due to the ease at which this cancer can migrate through the blood and lymphatic systems to other parts of the body, such as the liver, lungs and digestive system [8, 25]. Thus, there is dire need to investigate other alternative therapies for the treatment of CRC.

3. Photodynamic therapy an unconventional treatment for CRC

Photodynamic therapy (PDT) is a promising unconventional treatment method for CRC (**Figure 1**) [8].

PDT treatment is a coordinated process, which begins with the intravenous administration of a photosensitizer (PS) drug [8]. Once the PS drug enters the blood stream it is then either passively or actively absorbed in tumor site, depending on the PS drug delivery mechanism that is involved [26]. Within standard conventional PS drug delivery mechanisms the advantage is that the PS drug tend to preferentially localize in diseased tissue via the enhanced permeability retention (EPR) effect and so is passively absorbed, promoting PDT induced tumor destruction with only slight healthy tissue damage [26]. However, current research studies are focused on improving PS passive drug uptake via chemical or functional modifications in order to promote a more specific and actively targeted PS delivery in cancer cells only, so that photosensitivity, localized healthy tissue destruction and other additional unwanted side effects can possibly be eliminated [26]. Since, PS drugs are light absorbing molecules their activation is achieved when they are

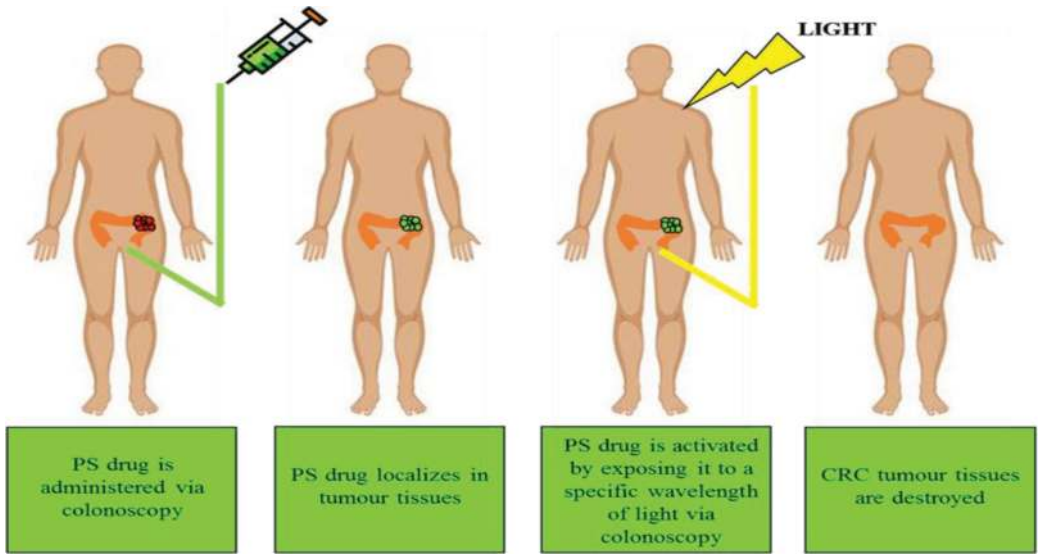

Figure 1.
PDT treatment of CRC. PS drugs are administered to a patients CRC tumor site via a colonoscopy endoscope, whereby they localize in targeted tumor cells. Laser light irradiation is then also administered to the target tumor site via a colonoscopy endoscope, whereby it penetrates the large intestines tissues/tumor and activates the PS. The PS then undergoes a photoreaction to produce ROS and/or singlet oxygen, which in turn induces cytotoxic cell death in CRC tumor tissues.

exposed to laser irradiation light at a specific wavelength [26]. Thus when a tumor (which contains the localized PS drug) is exposed to laser irradiation light, the PS absorbs the photons and becomes excited [2]. This excitation promotes the PS from a ground state to a higher level of energy known as a singlet state [27]. This singlet state is very short lived and PSs return to their ground state rapidly after losing their energy to fluorescence or internal heat conversion [27]. However, the singlet state PS may also convert to a triplet state via intersystem crossing, resulting in an electron spin change, which if reacted with molecular oxygen (as found in cells), it will give rise to free reactive oxygen species (ROS), which can result in tumor destruction [27].

Since the colon can be easily accessed via the rectum opening of the large intestine using an endoscope, this form of oncological PDT treatment for CRC tumors is possible [8]. Studies by Hodgkinson et al. [2] and Kawczyk-Krupta et al. [8] have noted that the PDT treatment of CRC which are inoperable, have only slightly advanced lesions/polyps or massive advanced tumors is a safe and feasible treatment option. Thus, colonoscopy endoscopes are used to directly deliver PS drugs to target tumor regions, as well as administer the required wavelength of laser irradiation light to activate a PS drug [2, 8].

The overall ability of PDT to successfully destroy cancer cells depends of the efficacy of ROS production in target cells. ROS can be produced via two different types of photoreactions (**Figure 2**) [26]. Within photoreaction type I, the PS drug reacts with surrounding cellular biomolecules via a hydrogen atom electron transfer to form free radicals, which react with cellular molecular oxygen, generating ROS, which in turn induces oxidative stress in target cells and so destroys them [27]. Whereby within photoreaction type II, the PS drug reacts directly with molecular oxygen in the cell to form singlet oxygen species, which are able to oxidize various substrates within target cells and so induce cell death [27]. When ROS and singlet oxygen species are generated from a PDT reaction, they are cytotoxic and so oxidize various substrates in a tumor cell inducing stress that triggers various cell death pathways such as apoptosis, autophagy or necrosis. Both types of photoreactions may occur simultaneously, however type I reactions generally favor apoptotic death in tumor cells [28]. Additionally, the effectivity of both photoreaction pathways depends on the type of

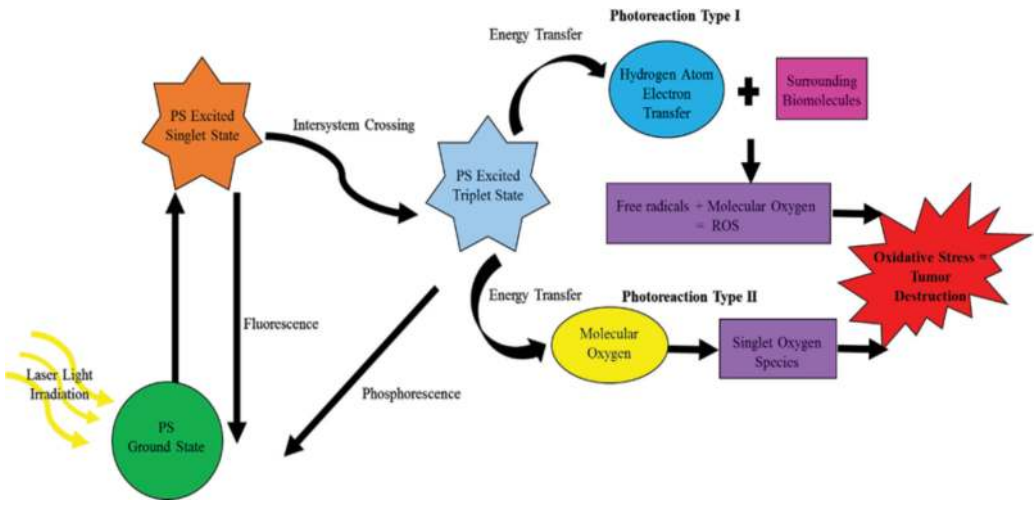

Figure 2.
PDT photophysical and photochemical mechanism of action for PS drug activation in tumor cells. When a PS drug is activated at a specific wavelength of light it becomes excited and so reacts with either molecular oxygen or other substrates within the surrounding areas of a cell, generating ROS, which in turn induces oxidative stress in cells triggering various cell death pathways and overall tumor destruction.

PS drug administered, where is localized in the tumor cell, as well as the amount of molecular oxygen present within the tumors microenvironment [29].

In the absence of laser irradiation light the PS drug remains inactive and so is not phototoxic in the body, therefore PDT can provide an alternative method to eradicate target tumor cells (since it is a localized treatment), while avoiding systematic toxicity and unwanted side effects when compared to conventional therapies (which affect healthy cells and tumor tissues) [26]. Thus, the major advantage of PDT over conventional therapies is that PS drugs tend to preferentially localize and be passively absorbed in tumor cells due to the enhanced permeability retention (EPR) effect and so their selective uptake can be achieved, allowing only minimal damage to healthy surrounding cells to occur during treatment [30]. Therefore, PDT can provide an alternative for the treatment of CRC, since it can avoid systematic toxicity, is minimally invasive, has a low morbidity rate, has the ability to preserve the anatomical function of healthy tissues, has minimal side effects, has no drug resistance and allows for repeated treatments [31].

However, in relation to PS drug delivery mechanisms CRC PDT research has now begun to focus on more selective passive (e.g. nanocarriers) and active (e.g. antigen–antibody targeting) uptake delivery mechanisms in tumor cells in order to further improve the efficacy of treatment [26]. These actively targeted PDT PS drug delivery mechanisms ensure preciously targeted PS drug delivery and localization in CRC only so that no damage occurs to normal healthy surrounding tissues [26].

4. PS drugs for CRC

PS drugs generate cytotoxic ROS or singlet oxygen species when they become activated at a particular wavelength, which in turn induces physical or chemical damage in target tumor cells [28]. In relation to the activation of PS drugs for effective PDT, it is important that they have a high molecular absorption coefficient within the red spectrum of light (650–780 nm), as to ensure maximum light absorption for PS excitation (as some endogenous human body pigments can absorb light), warrant minimal patient photosensitivity before treatment, as well as guarantee deep tissue penetration in target tumor sites [32, 33].

PSs are generally categorized into four different groups dependent on their functional capabilities. First generation PSs are one of the first types of PSs to be developed in PDT applications and they are stable, however have been shown to induce photosensitivity in patients and are activated within the lower red regions of light and so have a poor laser light tissue depth excitation range (e.g. haematoporphyrin derivatives) [33]. Second generation PSs have been further researched in PDT applications and since they are activated within the higher red regions of light, they have reported far less patient photosensitivity, with far deeper tissue laser light excitation (e.g. phthalocyanines, benzoporphyrins, purpurins, hypercin and chlorines [34]. Third generation PSs are currently the most promising PS drugs which are currently being researched within PDT cancer treatments [10]. Third generation PSs comprise of second generation PS drugs which have been chemically modified, functionalized or bound to nanoparticles (in order to promote their passive uptake) or active targeting biomolecules (such as aptamers, peptides, monoclonal antibodies, in order to promote their specific uptake in cancer cells only) [33]. In relation to current research, third generation PSs are reporting enhanced uptake in cancer cells with some of the most promising PDT treatment outcomes in CRC patients [33]. Lastly, most recent research has also begun to develop fourth-generation PS, which consist of second-generation PS encapsulated in a nanoparticle delivery system so its of third generation, however it is additionally co-encapsulated with a

Photosensitizer	Remarks	Ref.
***In vitro* PDT CRC research**		
3,4,5-trimethoxyphenyl, 3-hydroxyphenyl,4-hydroxyphenyl and sulfonamide phenyl porphyrin derivatives	Significant apoptotic cell death within HCT-116 CRC cells.	[37]
5,15-diaryltetrapyrrole derivatives porphyrin derivatives	Significant apoptotic cell death within HCT-116 CRC cells, with high yields of ROS being noted.	[38]
5-aminolevulinic acid	Enhanced PS uptake and improved PDT was noted within SW-480, HT-29 and CaCO-2 CRC cell lines.	[39]
5-aminolevulinic acid (ALA)	After PDT prognostic factor S100 protein concentration was reduced by 27% in SW480 and by 30% in SW620 CRC cell lines.	[40]
5-aminolevulinic acid (ALA)	Following PDT treatment autophagy cell death in human SW620 colon carcinoma cells was observed.	[41]
Chlorin e6 (Ce6)	CRC *in vitro* SW620 cells noted PDT induced apoptotic cell death.	[42]
Gallium phthalocyanine	CaCO-2 CRC cell line reported PDT induced cytotoxic effects.	[43]
Glycoconjugated chlorin (H2TFPC-SGlc)	MKN28, MKN45, HT29 and HCT116 CRC cell lines noted suppressed cell growth and apoptotic cell death post-PDT.	[44]
Hypericin	High doses induced massive ROS generation and severe ER stress, which then led apoptotic cell death while low doses triggered protective autophagy and promoted cell proliferation.	[45]
Indocyanine green	Effective ROS generation was observed with apoptotic cell death within *in vitro* cultured colon cancer cells at high PS concentrations.	[46]
Lysosome localizing Chlorin e6 (Ce6) ATX-S10Na(II)	Within CRC HCT116 cells, early apoptosis via Bax- and p53-dependent proteins was noted post-PDT.	[47]
Meta-tetra (hydroxyphenyl) chlorine (mTHPC)	Liposomal PS sub cellular localized localization in Colo-201 CRC cells was noted with significant cytotoxic apoptotic PDT induced cell death.	[48]
Meta-tetrahydroxyphenylchlorin	PS reported and effective PDT dose dependent effectivity in inhibiting cell proliferation, decreasing migration ability and colon formation within SW620 CRC cell lines.	[3]
Palmatine hydrochloride (PaH)	PDT showed significant photocytotoxicity on HT-29 cells and apoptotic cell death increased significantly in PS concentration-dependent and light dose-dependent manner.	[49]
Pheophorbide-a methyl ester (PPME)	HT-29 CRC cell line noted significant apoptotic cell death post-PDT treatment.	[50]
Photofrin II (Ph II) and hypericin (Hyp)	Combination of both PS post-PDT noted more effective cell death within doxorubicin-resistant LoVo DX CRC cell lines by reducing the multidrug resistance efflux protein P-glycoprotein (P-gp) and so promoted improved cytotoxic cell death.	[51]

Photosensitizer	Remarks	Ref.
Porfimer sodium (PII) and 2-[1-hexyloxyethyl]-2-devinylpyropheophorbide-a (HPPH)	PDT controlled metastatic tumor growth in murine colon 26-HA cells and enhanced anti tumor immunity.	[52]
Protoporphyrin IX (PpIX)	Enhanced the apoptosis in HCT116 CRC cell line	[53]
Sulfonated zinc phthalocyanine ($ZnPcS_{mix}$)	Within CRC DLD-1 and CaCo-2 cells the PS localized in multiple organelles and noted significant apoptotic PDT induced cell death.	[54]
Tetra-α-(4-carboxyphenoxy) phthalocyanine zinc	Noted interaction between p38 MAPK and caspase-9 regulated mitochondria-PDT mediated apoptosis in LoVo human colon carcinoma cells.	[55]
δ-aminolevulinic acid (ALA)	CRC cell lines SW480 and SW620 were treated in sublethal doses with ALA PDT in hypoxia-like conditions with cobalt chloride and noted decreases release of VEGF and significant tumor inhibition.	[56]
***In vivo* PDT CRC research**		
Bacteriochlorin analogues: 3-(1′-butyloxy) ethyl-3-deacetyl-bacteriopurpurin-18-N-butylimide methyl ester	High tumor uptake and long-term cure within BALB/c mice bearing Colon 26 tumors.	[57]
Hydrophilic bacteriochlorin (F2 BOH)	PDT enabled long-term cures of BALB/c mice with subcutaneously implanted CT26 tumors, and the cured mice rejected tumor re-inoculation 1 year after the treatment.	[58]
Metalloporphyrin Ga-4cisPtTPyP (5,10,15,20-tetrakis{cis-diammine-chloro-platinum(II)}(4-pyridyl)-porphyrinato gallium(III) hydroxide tetranitrate)	High tumor accumulation and almost completely inhibited tumor growth over 2 weeks in BALB/c mice bearing Colon 26 tumors.	[59]
Photosan-II (PS-II) and chloroquine	Significantly reduced the tumor size in a xenograft mice model and induced apoptotic and autophagy cell death within *in vitro* SW620 and HCT116 cells.	[60]
Porphyrazine platform with gadolinium (III) cation chelated by tetrapyrrole macrocycles (GdPz1 and GdPz2)	Selective *in vivo* accumulation within murine colon carcinoma CT26 models was observed, with significant inhibition of tumor growth.	[61]
Redaporfin	Single dose was well tolerated by male BALB/c mice with subcutaneously implanted colon (CT26) tumors and PDT led to the complete tumor regression in 83% of the mice.	[62]

Table 1.
Current PDT studies which utilize different types of PS for the in vitro, in vivo or clinical treatment of CRC.

small-molecular inhibitor system capable of blocking any tumor survival pathways post PDT, in order to halt possible tumor reoccurrence [35]. However, in relation to fourth-generation PSS this form of PDT treatment research is limited to only being able to target and inhibit VEGFs, in order to promote PS drugs uptake and so deter the neovascularization of tumors, preventing CRC tumor metastatic spread and reoccurrence [35].

At the moment clinically FDA approved first and second generation PSs for PDT oncology include: Porfimer sodium (Photofrin), 5-Aminolevulinic acid

(Levulan), Methyl aminolevulinate (Metvixia), Meta tetra(hydroxyphenyl) chlorin (Foscan), N-aspartyl chlorin e6 (NPe6, Laserphyrin), Benzoporphyrin derivative monoacid ring A (Visudyne) and N-hexyl ester of ALA (Cysview) [32–35]. Whereas, first- and second-generation PSs, which are currently under clinical trials include; Hypocrellin A, Pheophorbide-a, Chlorin e6, Methylene Blue, Hypericin, Phthalocyanine, Rose Bengal, HPPH: 2-(1-Hexyl-oxyethyl)-2-devinyl pyropheophorbide-alpha [30, 34, 36]. However, in relation to third and fourth generation PSs, none to date have received clinical approval for PDT CRC treatments and so remain a commanding area of research focus [26]. **Table 1** shows various research studies currently that have been performed with different types of PSs for the PDT treatment of CRC.

To date only one single successful clinical study from 2016, utilizing Photofrin II (Ph II) PS PDT drug on 23 young patients with advanced CRC, noted improved clinical symptoms and reduce complications post-PDT treatment [63]. These findings suggest that more research is required to develop better PS drugs to withstand clinical trials.

5. PDT CRC clinical challenges

Despite the many positive features of CRC PDT, within clinical settings this form of treatment has experienced some drawbacks in relation to PS drug solubility, mode of delivery and selective tumor uptake [64, 65].

In order to ensure the maximum levels of ROS are generated during a PDT treatment, as to ensure complete tumor destruction, the highest possible concentrations of PS drugs must be able to be successfully delivered and localize in target tumor tissues [27]. Within PDT clinical settings using first and second generation PS drugs, poor outcomes and effectiveness has been noted, as only minor amounts of PS drugs are able to overcome the human bodies biological barriers and so passively accumulate (due to the EPR effect) in tumor cells, generating very low levels ROS and tumor destruction [2, 31]. Additionally, due to this passivation process sometimes PS drugs can accumulate in healthy tissues inducing unwanted PDT side effects such as patients' photosensitivity and damage to normal tissues [26].

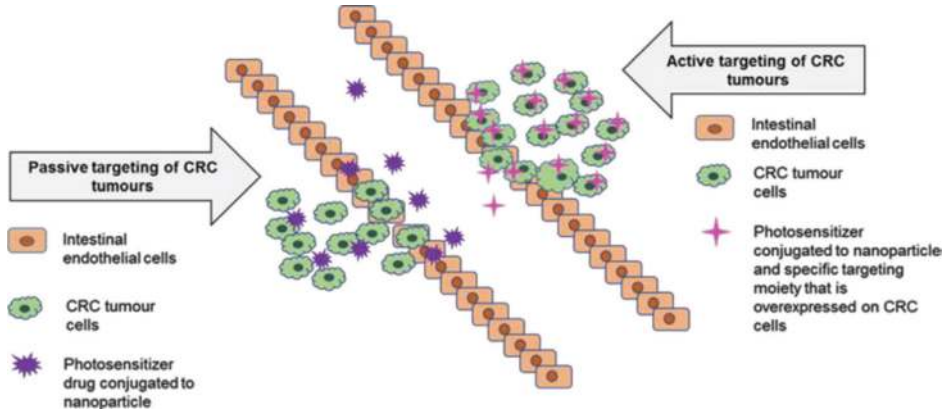

Figure 3.
Passive PS NP drug delivery versus active targeting moiety conjugated PS NP drug delivery, which shows targeted and enhanced CRC tumor PS drug uptake for more effective PDT treatment outcomes.

Another issue sometimes noted in clinical settings is that PS drugs have limited solubility and so tend to aggregate during administration, limiting their overall uptake and effectivity [2]. Moreover, a PS drugs concentrated subcellular localization in a tumors mitochondria, lysosomes, endoplasmic reticulum, plasma membrane etc., is of utmost importance since ROS have only a very short half-life and so will only induce effective cell death in tumor cells if they are proximately localized within these organelles [29, 32].

Thus, shortcomings such as poor solubility, bioavailability, maximum ROS generation and tumor subcellular localization targeting need to be overcome in order to ensure the effectivity of PDT [26]. Nevertheless third generation PS drug nanoparticle (NP) drug carriers are currently being investigated to ensure PS drug solubility and improved passive uptake, with functionalized active targeting abilities (e.g. overexpressed peptides), as to ensure specific uptake in tumor cells only to enhance the overall efficacy of PDT (**Figure 3**) [29, 32].

6. Nanoparticles for enhanced passive PS drug delivery and PDT

Passive PS uptake makes use of the drugs physiochemical factors, as well as the morphological and physiological differences between tumor and tissues (i.e. EPR effect) to allow PSs to accumulate in tumor sites [30].

There has been great interest in combining PS drugs with NPs in order to overcome some of the challenges conventional PS drug delivery mechanisms experience in clinical settings [65]. This is because NPs can enhance PS drug passive uptake, promote solubility, stability and limit non-specific toxicity [66]. Additionally, NPs can mimic biological molecules and so when combined with PSs, they go by unnoticed by immune barriers, remaining in tacked and so improved passivation of PS drug uptake in tumors [33]. Examples of nanoparticle platforms to assist in the passivation PS drug delivery for PDT CRC treatment include: liposomes, polymers, micelles, dendrimers, silica, nanoemulsion, nanotubes and nanogels [67]. Moreover, these NP platforms (especially polymeric NPs) have the additional benefit of protecting PS drugs against chemical and enzymatic gastrointestinal tract degradation, and so increase the drugs stability and cellular uptake within the intestinal epithelium [68, 69]. Various studies listing the effective passive PS drug delivery in CRC tumors utilizing NP carrier platforms have been listed in **Table 2**.

***In vitro* and *in vivo* PDT CRC research**

Photosensitizer	Nanoparticle	Remarks	Ref.
5-(4-aminophenyl)-10,15,20-triphenylchlorin and 5-(4-carboxyphenyl)-10,15,20-triphenylchlorin	Chitosan	Drugs localized in endocytic vesicles of HCT116/LUC human colon carcinoma cells and within tumor-bearing mice, showed strong PDT treatment.	[70]
5,10,15,20-Tetrakis(4-hydroxy-phenyl)-21H, 23H-porphine (pTHPP)	Polyhydroxyalkanoates (PHAs)	*In vitro* photocytotoxicity in human colon adenocarcinoma cell line HT-29 revealed time and concentration dependent cell death.	[71]
5-aminolevulinic acid	Co polymer methoxy poly(ethylene glycol)-chitosan	Enhanced delivery and PDT phototoxicity.	[72]
5-aminolevulinic acid (ALA)	Chitosan	Enhanced cellular absorption in Caco-2CRC cells.	[73]
5-flurouraci (5-FU)	Solid lipid	Enhanced delivery and PDT phototoxicity, within CRC cells and chemo resistant stem-like cells.	[74]

***In vitro* and *in vivo* PDT CRC research**			
Photosensitizer	**Nanoparticle**	**Remarks**	**Ref.**
Chlorin e6 (Ce6)	Methoxy poly(ethylene glycol) (MePEG)	Showed enhanced cellular uptake, phototoxicity, and ROS generation within *in vitro* CRC cells and reported improved tumor tissue penetration and accumulation within *in vivo* animal studies.	[75]
Chlorin e6 (Ce6)	Polymeric carrier polyvinyl alcohol (PVA)	Noted higher uptake in murine colon carcinoma CT26 tumors models with significant tumor regression and necrotic cell death.	[76]
Chlorin e6 (Ce6)	Doxorubicin (DOX)-loaded micelles with mPEG lipoic acid (LA)	PS and anticancer drug are colocalized in within *in vitro* CT-26 and HCT-116 CRC cells. Dual therapy induced apoptotic cell death and inhibited tumor growth in CT-26 tumor bearing mouse model	[77]
Curcumin and 5-fluorouracil	Chitosan	CRC HT29 cell line had a 3-fold increase in anticancer effects.	[78]
Cyanine IR-780	Solid lipid and flavonoid derivatives for electroporation	Showed improved uptake and demonstrated the ability to act as an anticancer PDT modality to eliminate LoVo and CHO-K1 CRC cells in vitro	[79]
Diaryl-porphyrin (PMMA@ PorVa)	Core-shell poly-methyl methacrylate	Human colon carcinoma cell line HCT116 noted PDT induced apoptotic cell death.	[80]
Dimeric zinc(II) phthalocyanine	Alkyne-modified mesoporous silica	Exhibited high intracellular fluorescence in human colon adenocarcinoma HT29 cells with notable photocytotoxicity	[81]
Hypericin	Pluronic P123 (P123)	*In vitro* Caco-2 and HT-29 intestinal colon carcinoma cells noted 90% photocytotoxic cell death.	[82]
Indocyanine green (icg)	Super carbonate apatite (sCA)	*In vitro* and *in vivo* HT29 CRC tumors exhibited drastic and highly significant tumor growth retardation.	[83]
IR780 iodide	Pluronic coated gold	Show enhanced phototherapeutic and photothermal activity with no dark cytotoxicity within *in vitro* murine colon carcinoma cells (C26).	[84]
Meso-tetra (carboxyphenyl) porphyrin (TCPP)	Poly(D,L-lactide-*co*-glycolide) (PLGA)	Improved uptake of PS, with enhanced phototoxicity within *in vitro* SW480CRC cells and dramatic tumor-inhibiting efficacy in four-week-old female athymic mice.	[16]
Meta-tetra (hydroxyphenyl) chlorine (mTHPC)	Liposomal formulation FosPeg®	Improved PS absorption with enhanced phototoxicity and cell death in HT29 cell lines.	[85]
Oxaliplatin	Chitosan micelles	Eliminated bulk CRC cell populations and stem-like cells both *in vitro* and *in vivo*.	[86]
Photoprotoporphyrin IX dimethyl ester (PppIX-DME)	Polyethylene glycol and polylactic acid block copolymer (PN-Por)	Noted improved uptake and sustained release within *in vitro* Colon-26 carcinoma and efficient tumor deposition was found in C26 tumor-bearing mice with a significant and highly effective PDT anti-tumor effect.	[87]
Porfimer sodium (PII) and 2-[1-hexyloxyethyl]-2-devinylpyropheophorbide-a (HPPH)	Polymeric tubule-forming phospholipid, DC PC with PEGylated lipid	Enhanced tumor accumulation and superior therapeutic efficacy in HT29 tumor mouse xenographs and Colon-26 bearing BALB/c mice showed no tumor reoccurrence up to 100 days.	[88]
Porphyrin	Nano micelles and SN-38 (7-ethyl-10-hydroxycamptothecin) chemotherapeutic drug	Synergistic chemo drug and PS dramatically enhanced *in vivo* antitumor PDT efficacy over single treatment in nude mice bearing HT-29 colon cancer xenograft.	[89]

In vitro and *in vivo* PDT CRC research			
Photosensitizer	**Nanoparticle**	**Remarks**	**Ref.**
Protoporphyrin IX (PpIX)	Non-biodegradable silica	Improved PS accumulation in both HCT-116 cell lines and tumor bearing mice, with enhanced ROS generation.	[90]
SN-38-Cyclodextrin Complexation	Chlorin-core star-shaped block copolymer (CSBC) micelles	Combination of PS and chemotherapy nanocarrier showed 60% tumor regression in HT-29 human CRC xenograft model, after three applications.	[91]
Zinc phthalocyanine	Liposomal	CRC CT26 tumor models which received PDT and sonodynamic therapy tumors shrank by 20% after 120 days.	[92]
Zinc phthalocyanine	Titanium dioxide	Improved uptake and enhanced theranostics of PDT within *in vitro* colorectal adenocarcinoma (HT29) cells.	[93]
Zinc protoporphyrin (ZnPP)	N-(2-hydroxypropyl) methacrylamide copolymer with PEG	Nanodrug caused necrosis and disappearance of >70% of tumors in colon cancer mouse models.	[94]
Zinc(II) phthalocyanine	Tetronic® 1107 polymeric poloxamine micelles (T1107)	Improved uptake and enhanced PDT apoptotic cell death within *in vitro* 2D and 3D murine colon adenocarcinoma CT26 cells.	[95]

Table 2.
Passive Targeting PDT PS drug delivery mechanisms within in vitro and in vivo CRC.

7. Active targeting biomolecules for enhanced PS drug delivery and PDT

Active PDT PS drug delivery involves the conjugation of the PS drug to specific ligands or biomolecules moieties, which are complementary to overexpressed cancer cell receptors and so via a molecular recognition process PS drug uptake in target tumor cells is enhanced [31]. These moieties include monoclonal antibodies (mAb), proteins (e.g. transferrin), nucleic acids (aptamers), small molecules (folic acid), polymers (hyaluronic acid) and peptides (proteins), which are over-expressed on CRC tumor cells only [31, 96]. These specific ligands or biomolecules moieties, which are conjugated to a PS NP drug delivery system, have a specific affinity for receptors that are over-expressed on CRC tumor cells and their vascular, but not on normal cells [34]. This facilitates enhanced PSs retention in tumor target sites only, improving the efficacy of PDT and localizing its treatments effectiveness to killing CRC tumors only [34]. Common protein receptors in CRC cells which have been noted to be overexpressed and so can be utilized for possible PS active drug targeting include: epidermal growth factor receptor (EGFR), fibroblast growth factor receptor (FGFR), epithelial cell-adhesion molecule (EpCAM), carbonic anhydrase IX (CA IX), peroxisome proliferator-activated receptor γ (PPARγ), cyclooxygenase-2 (COX-2), cholesterol and low-density lipoprotein, estrogen receptors, cholecystokinin A receptors, lectin saccharide receptors, anti-DR5 antibody, as well as cluster of differentiation 44, 133, 166 and 24 (CD44+, CD 133+, CD166+ and CD24+) [13, 97–99]. Recent research approaches to enhance PS NP drug delivery by actively targeting CRC tumors using various moieties and so increase the efficacy of PDT have been listed in **Table 3**.

***In vitro* and *in vivo* PDT CRC research**			
Photosensitizer	**Active drug delivery system**	**Remarks**	**Ref.**
5-Fluorouracil	Eudragit S100 coated citrus pectin nanoparticles	Eudragit S100 is a pH responsive enteric polymer and citrus pectin is a ligand receptor for galectin-3. Targeted drug delivery was found both *in vitro* and *in vivo* with enhanced PDT cytotoxic effects.	[100]
Chlorin e6 (Ce6)	Site specific immune-conjugates (murine monoclonal antibody 17.1A)	Cationic electric charge of photoimmune-conjugate enhanced PS delivery and showed a 90% phototoxic effect within *in vitro* HT-29CRC cells.	[46]
Chlorin e6 (Ce6)	Phototoxic DNA aptamers were bound to unique short O-glycan-peptide signatures	Drug conjugate reported >500-fold increase in toxicity upon light activation in HT-29CRC cells and was not cytotoxic towards cell types without O-glycan-peptide markers.	[101]
Chlorin e6 (Ce6) and indium	Biotinylated to target biotin receptors	Colon carcinoma *in vitro* CT26 cell lines showed targeted uptake with enhanced apoptotic cell death.	[102]
Chlorin e6 (Ce6)	Hyaluronic acid conjugated to 5β-cholanic acid (5β-CA) to target CD44 ligands	Effective tumor targeting noted with tumor growth being significantly suppressed and inhibited by 9.61 ± 1.09-fold in human colon HT29 cell line and murine tumor model.	[103]
Chlorin e6 (Ce6)	Hyaluronic acid nanoparticle to target CD 44 receptors	Enhanced uptake in human colon cancer xenograft model was observed with significant tumor destruction.	[104]
Chlorin e6 (Ce6)	Glycoconjugated chlorin (G-chlorin)	PDT induced significant targeted immunogenic apoptotic cell death in a syngeneic CT26 mouse tumor model (allograft model)	[105]
Hypericin	Histone deacetylase inhibitor sodium phenylbutyrate (NaPB)	Reported significant increase in tumor suppressor CDKN1A gene in CRC model with enhanced uptake and PDT effects.	[106]
IR780 iodide	Self-assembled transferrin-IR780 for direct Transferrin-receptor (TfR) targeting	Within Murine CT26 colon carcinoma cells and CT26 tumor-bearing mice notable targeting and tumor suppression was observed.	[107]
Meso-tetraphenyl chlorin disulfonate (TPCS2a)	IM7-saporin immunotoxin CD44 targeting receptor	Drug carrier was successfully transported into *in vitro* WiDr CRC cells via photochemical internalization (PCI) and resulted in 90% cytotoxic response.	[108]
Meta-tetra(hydroxyphenyl) chlorin (mTHPC)	Bevacizumab (Avastin™), an anti-VEGF neutralizing monoclonal antibody	PDT PS with Avastin™ and monoclonal antibody in murine model, reported even lowered expression of VEGF in tumors with improved tumor killing efficacy than when compared to anti-angiogenic chemotherapeutic Avastin™ and monoclonal antibody treatment alone (which indirectly kills cells by via vascular damage), suggesting that PDT PS contributed to overall combined treatment approach by directly killing cells via ROS generation as well, and so improved CRC cell death.	[109]
None	Photothermal gold coated superparamagnetic iron oxide nanoparticles conjugated with thiol modified MUC-1 aptamers	Photothermal therapy of colon cancer cells exhibited notable cell death.	[110]

In vitro and *in vivo* PDT CRC research			
Photosensitizer	**Active drug delivery system**	**Remarks**	**Ref.**
Pyropheophorbide-a (PPa) protoporphyrin	ATP-binding cassette subfamily G2 (ABCG2) porphyrin-based targeted PDT.	PS drug delivery was improved within *in vitro* HT29 cells show high levels of ABCG2 expression with significant PDT induced cell damage.	[111]
Pyropheophorbide-a methyl ester (PPME)	Peroxisomal proliferator-activated receptor gamma (PPARγ) ligand troglitazone	Enhanced uptake in DLD-1 CRC *in vitro* cells, with significant growth retardation and apoptotic cell death in a PDT dose-dependent manner.	[112]
Verteporfin succinimidyl ester	Single chain variable fragments (scFvs), antibody fragments	Improved uptake and within *in vitro* and *in vivo* PDT applications it effectively killed tumor LoVo (CEA+, HER2-) cells.	[44]
Zinc phthalocyanine (C11Pc)	HER2 receptor or jacalin, a lectin specific for carbohydrate T antigen on PEG Gold nanoparticles	HT-29 CRC cells reported enhanced targeted PDT with 80–90% cell death being noted.	[113]

Table 3.
Active Targeting PDT PS drug delivery mechanisms within in vitro and in vivo CRC.

8. Conclusion

From this chapter it can be observed that PDT is most definitely a highly effective and alternative therapeutic treatment for CRC [8]. However, conventional PS drug delivery applications have numerous limitations in relation to solubility and poor tumor subcellular localization specificity [26]. Nevertheless, NP PS drug delivery systems which are surface functionalized with various tumor-targeting moieties can help overcoming some of these limitations be passively, as well as actively enhancing PS drug uptake.

In this chapter, we have shown that there are many positive and promising research studies being conducted *in vitro* and *in vivo*, for the use of PDT in CRC treatment (**Table 1**). We have also evidenced the remarkable potential of passivation NP PS drug carrier platforms (**Table 2**) and specific receptor based PS drug active targeting (**Table 3**), in order to promote the selective absorption of PS drugs in target CRC tumor sites only and so avoid unwanted side effects, as well as overall enhance the PDT treatment of CRC. However, it must be noted that the research studies which have been reported in **Tables 2** and **3** are within early stages of *in vitro* and *in vivo* research and no clinical trials have been performed as of yet. Thus, researchers need to start further exploring specific functionalized NP PS drug delivery platforms for the targeted drug delivery of PSs and effective PDT treatment of CRC within pre-clinical and clinical trials in order to develop optimized standards for this form of CRC therapy [8]. The findings from these studies should drive the application of targeted PDT PS drug delivery to the forefront of oncological interventions as a possible treatment modality for the eradication of CRC.

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Conflict of interest

The authors confirm that this chapter has no conflict of interest.

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