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A review on the scope of Photothermal Therapy-based nanomedicines in pre-clinical models of colorectal cancer

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Abstract

Oncological thermal ablation involves the use of hyperthermic temperatures to damage and treat solid cancers. Thermal ablation is being investigated as a method of treatment in colorectal cancers and has the potential to complement conventional anti-cancer treatments in managing local recurrence and metastatic disease. Photothermal therapy utilises photosensitive agents to generate local heat and induce thermal ablation. There is growing interest in developing nanotechnology platforms to deliver such photosensitive agents. An advantage of nanomedicines is their multifunctionality with the capability to deliver combinations of chemotherapeutics and cancer-imaging agents. To date, there have been no clinical studies evaluating photothermal therapy-based nanomedicines in colorectal cancers. This review presents the current scope of pre-clinical studies, investigating nanomedicines that have been developed for delivering multimodal photothermal therapy to colorectal cancers, with an emphasis on potential clinical applications.

Keywords

Nanoparticles; Targeted therapy; Photosensitive agent; Thermal ablation; Optical imaging

Introduction

Colorectal cancer (CRC) is one of the major causes of cancer-related mortalities worldwide. There is an increasing trend in CRC incidence, especially in more economically developed countries, attributed in part to lifestyle choices such as dietary and physical activity patterns [1,2]. Surgical intervention remains the cornerstone of CRC management with around 80% of new CRC patients presenting with localised disease amenable to curative resection [3]. Surgery is the first line of treatment, primarily for patients with stage I-III CRC. However, up to half of patients diagnosed with early stage CRC have recurrent disease after surgical resection and may also develop metastatic disease, typically in the liver or lungs [3,4]**.** Patients with metastatic and stage IV CRC are usually treated with chemotherapy as the first option, due to the unresectable nature of the cancer. If possible, chemotherapy is often used as an adjunct to surgery for downstaging or reducing the risk of recurrence. Treatments based on 5-fluorouracil, such as FOLFOX (Leucovorin, 5-fluorouracil and Oxaliplatin) and FOLFIRI (Leucovorin, 5-fluorouracil and Irinotecan), have been developed for patients with metastatic CRC. These treatment regimens have been found to improve treatment response, progression-free survival and overall survival rates [5–7]. More recently, treatment with FOLFOXIRI (Leucovorin, 5-fluorouracil, Oxaliplatin and Irinotecan) has been studied and found to be an effective and aggressive approach to treat metastatic CRC [8]. Targeting biological agents such as Bevacizumab, Cetuximab and Panitumumab have also been studied as part of treatment regimens, in combination with FOLFOX and FOLFIRI [9–14]. Studies on these combination treatment regimens, have demonstrated improved treatment response and survival rates in patients with metastatic CRC.

Despite the benefits of the current chemotherapeutic regimens, a recent review by Schuurhuizen *et al.*, highlighted the fundamental limitation of systemic chemotherapeutic and biological drugs, that are routinely used to treat late stage and metastatic colorectal disease. Whilst these treatment methods increase overall survival rates and improve prognosis, systemic toxic effects often have dire consequences on the patients' quality of life [15]. In current clinical practice, there remains a need for novel treatment methods, which can improve survival rates whilst minimising life-affecting adverse toxicities.

Thermal ablation and laser-induced thermotherapy are treatment methods, that may be able to address this unmet clinical need [16]**.** These treatments utilise hyperthermia to eradicate cancers and have recently been evaluated for treatment of metastatic CRC [17]. Clinical studies are currently being performed to evaluate the safety and efficacy of such treatment strategies [18,19]. Similar to the heat-mediated cytotoxicity observed in thermal ablation, Photothermal Therapy (PTT) is a non-invasive technique which involves the administration of a photosensitive agent, which can convert photonic energy into heat upon light irradiation [20]. With absorption of photonic energy from light, ground state electrons are elevated to an excited energy state. The excited electrons then relax and the released kinetic energy overheats the local environment, resulting in cell and tissue damage [21]. Hyperthermia can induce cell death through apoptosis, necrosis and necroptosis (programmed necrosis) [22,23]. Intracellular temperatures between 40°C to 47°C cause irreversible damage to cellular proteins and impair DNA function, often resulting in apoptotic cell death. In PTT, it is common for the intracellular temperature of cancer cells to reach 50°C, resulting in necrosis and rapid cell death [22,24]**.**

In recent years, there has been much interest in developing new nanomedicines for PTT, especially for the treatment of CRC. Nanomedicines and nanoparticle-based therapies have advantages over conventional treatments, which are hindered by low specificity and poor pharmacokinetics. This has led to the development of various nanostructures for biomedicinal applications [21,25]. Nanomedicines that can deliver combined payloads of chemotherapeutics and cancer-imaging agents are showing great promise for potential clinical application [26]. This review highlights the pre-clinical studies that have evaluated PTT-based nanomedicines as a method of treating and managing CRC. To date, no clinical studies have been reported, assessing PTT-based nanomedicines in CRC patients.

This review is categorised according to the different classes of materials and structures, that have been used to develop PTT mediating nanomedicines. The aim of this review is to explore the current state of PTT nanotechnologies in pre-clinical models of CRC. A summarised overview of all studies included in this review is given in Table 1.

Gold-based nanomedicines

Gold-based nanoparticles (AuNP) are attractive agents for PTT and form the basis of many anti-cancer platforms. They are efficient in converting light energy into heat and their structural dimensions permit the tailored tuning of AuNP particles to specific absorption spectra, including maximum absorption in the near-infrared (NIR) light region for optimal tissue penetration. Other benefits of AuNP are good biocompatibility, passive accumulation within tumours via the enhanced permeability and retention

effect, and the ability to functionalise their surfaces with targeting ligands and therapeutic molecules [27,28]. AuNP can also be used for optical imaging due to their capacity to absorb and scatter light in the visible and NIR regions [29].

O'Neal *et al.* conducted one of the earliest studies of AuNP mediated PTT in CRC. The authors coated PEGylated silica nanoshells with gold and evaluated efficacy of PTT *in vivo* in murine CT26 CRC tumour xenografts. Following the intravenous injection of gold-coated nanoshells, tumours were irradiated with NIR light. Control animals exposed to NIR light only, demonstrated ongoing rapid tumour growth. Treated tumours however all showed significant tumour regression and the animals appeared healthy and tumour free for more than 90 days post-treatment [30]. A subsequent study by Goodrich *et al.* found that CT26 tumour xenograft bearing animals treated with PEGylated gold nanorods (AuNR) and NIR irradiation, survived for longer than control animals (NIR light irradiation only or AuNR only). AuNR was found to selectively accumulate in the tumours and histological analysis confirmed no toxicity in vital organs. This study demonstrated the safety and efficacy of AuNR-mediated PTT in CRC tumours [31].

The versatility of nanomedicines, especially in theranostic applications, has driven clinical interest in combining PTT with chemotherapy. Combined treatments have previously been described as synergistically improving anti-cancer activity by reducing the systemic drug dose and unwanted side effects [32]. Guo *et al.* prepared multifunctional nanocarriers based on AuNR and chitosan and loaded them with the chemotherapeutic drug, cisplatin. They were able to use the inherent optical properties of AuNR to image LoVo CRC cells *in vitro* in real-time via dark-field optical microscopy.

Upon NIR irradiation, no cell death was observed at 1W/cm^2 but increasing the light output to 3W/cm^2 resulted in mass cell death. Cisplatin was found to synergistically accentuate the therapeutic effects [33]. This study successfully showcased the ability of nanoparticles to act as single platforms for drug delivery, cell imaging and PTT.

Kirui *et al.* conducted a series of experiments, investigating single chain A33-targeting antibody functionalised AuNP mediated PTT in CRC [34–36]**.** The cell surface antigen, A33, is overexpressed in CRC and may serve as a useful biomarker [37].The authors synthesised gold and iron oxide hybrid nanoparticles functionalised with the A33 antibody. Intracellular accumulation and PTT cytotoxicity following nanoparticle administration was significantly increased in SW1222 cells (A33 positive) as compared to HT29 cells (A33 negative) CRC cells *in vitro*. Apoptosis was the primary mode of cell death at 5.1W/cm^2, with necrosis observed at higher laser power [36]. In a follow up study, the authors designed AuNR functionalised with the A33 antibody. Similar to their previous study, selective uptake of the functionalised AuNR was observed in SW1222 cells and PTT mediated cytotoxicity was only observed in NIR irradiated AuNR treated SW1222 cells treated with functionalism AuNR [35]. The authors then conducted an *in vivo* analysis using the immuno-functionalised gold and iron oxide hybrid nanoparticles. Magnetic resonance (MR) imaging showed selective accumulation of the nanoparticle following intravenous injection in SW1222 tumour xenografts. PTT treatment of SW1222 CRC xenografts resulted in a reduction in the size of tumours. Histopathological examination revealed features of thermonecrosis as indicated by the loss of nucleus, cell shrinkage and coagulation in treated xenografts with minimal damage in control animals [34]. This study demonstrated targeted AuNP as an attractive adjunct for image-guided treatment in CRC, combining

the optical imaging properties of the nanoparticle with its ability to induce tumour death via PTT.

Lee *et al.* combined chemotherapy and PTT by developing Doxorubicin (DOX) loaded AuNP conjugated to anti-death receptor 4 (DR4) antibody. Animals bearing DOX-resistant DLD1 CRC tumour xenografts were treated with the AuNP and irradiated with NIR light. The greatest inhibition in tumour xenografts growth was observed in DR4-targeted DOX loaded AuNP and PTT treated animals. This was compared to free DOX alone, DOX loaded AuNP alone, and AuNP+NIR light treated animals. Combined PTT and chemotherapy from a single nanoparticle platform was found to be the most effective method of treatment. Packaging DOX into AuNP allowed the chemotherapeutic to be retained for longer in the cancer cells. Furthermore, the amount of DOX loaded into nanocarriers was 1.8% the amount used in DOX only treated animals, showing that lower doses of chemotherapeutics could be effectively administered with minimal side effects to treat multidrug resistant tumours [38]. Hosseinzadeh *et al.* also investigated combined cancer targeting chemotherapy and AuNP mediated PTT. The anti-cancer drug, SN38, was deposited onto the surface of AuNP via hyaluronic acid and electrostatic interactions and the AuNP were functionalised with MUC1 targeting aptamers. MUC1 positive (HT29 and SW480 CRC cell lines) and MUC1 negative (CHO) cell lines were treated with the nanoparticles *in vitro*. Uptake of nanoparticle and subsequent cytotoxicity in HT29 and SW480 cells was found to be highest in MUC1 aptamer SN38 loaded AuNP. Again, this was compared to free SN38 alone, non-aptamer SN38 loaded AuNP and PTT only treated cells. Furthermore, co-treatment of cells with MUC1 aptamer SN38 loaded AuNP and light irradiation limited their ability to migrate [39].

The ability to combine dual treatments into one nanocarrier and synergise anti-cancer effects has been evaluated in Photodynamic Therapy (PDT) and PTT dual therapy. Similar to PTT, PDT involves the administration of photosensitising agents, which upon excitation, transfer energy to oxygen and superoxides and produce reactive oxygen species that cause intracellular oxidative stress and cell death [40]. Seo *et al.* developed Methylene blue (MB) loaded AuNR for dual PDT-PTT therapy. CT26 CRC cells were treated with MB loaded AuNR and irradiated with NIR light *in vitro*. Cytotoxicity was enhanced in the nanocomposites, as compared to MB-PDT or AuNR-PTT alone. Interestingly, the NIR light used in this study was remote from the optimal absorption wavelength for MB (visible light region). However, the production of reactive oxygen species was still enhanced, which may be due to the transfer of energy from the excited electrons in AuNR to MB. Similar to previous studies the concentration of MB packaged into the nanocomposites was low but still resulted in a significant anti-cancer response [41].

The transmembrane protein, Mucin 1 (MUC1), is associated with increased proliferation and metastasis in CRC [42]. As described above, Hosseinzadeh *et al*. exploited the over expression of MUC1 in CRC to target nanoparticles to MUC1 positive cells. Similarly, Azhdarzadeh *et al.* synthesised gold coated superparamagnetic iron oxide nanoparticles (SPIONs) functionalised with MUC1 aptamers. SPIONs are popular theranostic agents, especially for MR imaging, with the gold coating conferring biocompatible and photothermal properties [43]. MR imaging confirmed the uptake of nanoparticles into MUC1 HT29 CRC cells. Upon NIR irradiation, nanoparticle treated cells showed significant cell death whilst control cells

(nanoparticle only and no light irradiation) remained viable [44]. White *et al.* evaluated PTT in anti-Mucin 5B antibody conjugated AuNP in liver metastatic models of CRC. Similar to MUC1, the overexpression of Mucin 5B (MUC5B) is associated with carcinogenesis and metastasis in gastric and CRC cancers [45]. Metastasis was modelled by implanting CC531 CRC cells into the liver of rats. Anti-MUC5B conjugated AuNP were injected through the tail veins and MR imaging confirmed the selective uptake of AuNP into hepatic metastatic deposits. Following light treatment, histological analysis confirmed large areas of tumour necrosis induced by PTT. No toxicity was observed in any of the major organs [46]. Recently, Parchur *et al.* reported a multimodal nanoparticle consisting of a gadolinium oxide shell coated on AuNR. Using a similar liver metastasis model, Gadolinium-mediated MR imaging confirmed a significant accumulation of AuNR into metastatic deposits. Histopathological analysis confirmed thermal damage in the tumour regions following fiber optic delivery of NIR light and photothermal ablation, with minimal damage to adjacent liver tissue [47]. Pathological hypoxia in solid cancers, plays an important role in aiding the progression and dissemination of cancer [48]. The expression of Carbonic Anhydrase IX (CAIX) is upregulated under hypoxic conditions, due to the highly induced activity of Hypoxia-inducible factor 1α [49]. In CRC cells, CAIX has been shown to promote the invasive potential and survival in cells. Plus, CAIX protein expression correlates with poor prognosis in solid tumours [50]. To exploit the importance of CAIX, Chen *et al.* developed AuNR constructs, conjugated to anti-CAIX antibody. Under hypoxic conditions, the binding and uptake of CAIX-targeted AuNR into HT29 CRC cells *in vitro* was higher, in comparison to non-targeted AuNR treated cells. Upon NIR irradiation, CAIX-targeted AuNR treated cells were found to experience significantly higher cell death, in comparison to control AuNR and untreated cells. Hyperspectral imaging *in vivo*,

confirmed a high accumulation of intravenously administered CAIX-targeted AuNR in HT29 xenografts, with penetration into the hypoxic areas of the tumours. *In vivo* NIR light irradiation of tumour xenografts, resulted in complete tumour regression in CAIX-targeted AuNR treated animals. Transient tumour regression followed by recurrence was observed in non-targeted AuNR treated animals [51].

The studies described above utilised CRC targeting antibodies and aptamers to direct and catalyse AuNP mediated PTT. Wang *et al.* constructed gold and silver hybrid nanorods conjugated to CRC specific phage fusion proteins. Selective uptake into SW620 CRC cells was confirmed via electron microscopy, and the gold and silver hybrid nanorods were efficient in inducing PTT mediated cell death [52].

Dye-based nanomedicines

NIR dyes are small organic molecules that can absorb light in the NIR region and are of interest in anti-cancer theranostic applications. NIR dyes have great versatility and can be used in a wide variety of clinical applications. This includes fluorescent imaging, PDT, and PTT [53]. IR780 is a lipophilic NIR heptamethine cyanine dye which has shown great promise in anti-cancer related applications [54]. Due to its hydrophobic nature, IR780 can be packaged into nanoparticles to improve uptake into cancer cells [54,55]. Peng *et al.* developed IR780 loaded micelles for NIR fluorescence and PTT. The micelles were also labelled with the radionuclide, rhenium-188 for nuclear imaging. HCT116 CRC cells treated with the IR780-micelles and irradiated with NIR exhibited significant cell death *in vitro*, as compared to IR780-micelles treated alone or NIR irradiation alone. Animals bearing HCT116 CRC tumour xenografts were fluorescently

imaged following the administration of the IR780-micelles and showed a high uptake at 96 hours. Following NIR light irradiation, substantial tumour regression was observed in animals treated with IR780-micelles as compared to controls. Photothermal ablation in treated tumours was confirmed by histopathological analysis, which showed large areas of thermonecrosis, irreversible tissue damage, increased apoptosis, and increased expression of heat shock proteins [55]. Shih *et al.* took this concept one step further by conjugating the EGFR targeting monoclonal antibody, Cetuximab, onto IR780 loaded micelles. NIR fluorescence showed high *in vitro* and *in vivo* uptake of the micelles into HCT116 CRC (high EGFR expression) cells as compared to SW620 CRC (low EGFR expression) cells. Following NIR irradiation, all HCT116 tumour xenografts treated with Cetuximab conjugated IR780-micellles had significant tumour regression. In comparison, SW620 tumour xenografts relapsed after 13 days [56].

Tsai *et al.* described a multiplatform and multifunctional nanoparticle composed with IR780 and SN38 as the core of the structure. The structure was PEGylated and was functionalised with the αvβ3 integrin targeting peptide, cRGD. The dual function of IR780 enabled non-invasive NIR fluorescent imaging to verify nanoparticle accumulation in tumour xenografts and NIR irradiation to generate heat for PTT. HCT116 CRC tumour xenografts treated with the nanoparticle and irradiated with NIR light exhibited a rapid increase in temperature, reaching 52°C in 60 seconds. Combining PTT with SN38 further enhanced tumour regression in a synergistic fashion [57]. Lin *et al.* designed and evaluated 'grenade'-like nanocages to deliver DOX to CRC cells. The nanocage comprised of DOX in the core with the NIR dye, ADS-780, on the surface. Following the administration of the DOX loaded nanocages to

HT29 CRC cells, NIR fluorescent imaging was used to track the uptake of the nanocages. Upon NIR light irradiation, cell death and tumour regression was observed *in vitro* and *in vivo* respectively. The thermal effects of ADS-780 also triggered the explosion of nanocages into smaller clusters and the acidic intracellular pH triggered the release of DOX from the clusters. NIR light and nanocage treated groups had a longer survival with histological analysis showing great anti-cancer effects. There were no signs of cytotoxicity in other major organs [58].

Indocyanine green (ICG) is a well-known NIR dye and its inherent fluorescent properties, have been utilised in many medical and surgical specialities [59]. ICG is also a photosensitising agent and can produce reactive oxygen species (ROS), as well as heat, upon light irradiation [60]. Chen *et al.* created ICG and DOX loaded nanoparticles and took advantage of ICG's ability to produce ROS and trigger the intracellular release of DOX. HCT116 CRC tumour xenograft treated mice exhibited enhanced cytotoxicity and tumour growth regression following treatment with DOX and ICG loaded nanoparticle with NIR light irradiation. Thermal imaging confirmed PTT, as tumour xenograft temperatures rose to around 50°C following light treatment. Hyperthermia also aided the increased accumulation of ICG in the tumour [61].

Serendipitously, Chinese traditional ink, a conventional writing material, was found to share similar optical and chemical properties as artificially synthesised NIR dyes. Wang *et al.* explored the photothermal effects of Chinese traditional ink in lymph node metastatic models of CRC. HCT116 CRC cells were subcutaneously implanted into mice and the animals that developed metastases in sentinel lymph nodes were used for experiments. Following the administration of the ink and NIR light irradiation,

thermal imaging was employed to map the location of the ink with temperatures in the sentinel lymph nodes reported to be around 60°C. Several days after PTT the lymph nodes were histopathologically examined and showed signs of necrosis and pyknosis. In contrast, intact morphology of cells with no anti-cancer effects were observed in control lymph nodes. Furthermore, Chinese traditional ink was found to be safe and biocompatible as indicated by no pathological damage to any of the major organs [62].

Carbon nanotubes-based nanomedicines

Carbon nanotubes (CNT) have attracted the interests of biomedical researchers due to their electrical, thermal and spectroscopic properties. CNT offer a potentially useful platform for developing photothermal agents due to their ability to efficiently convert NIR light energy into heat [63]. Tan *et al.* developed modified multiwalled CNT by functionalising CNT with the nanocomposite, polyhedral oligomeric silsesquioxane poly (carbonate-urea) urethane (POSS-PCU). POSS-PCU functionalising plays a similar role to PEGylation in that it improves biocompatibility, addresses inherent toxicity, and prolongs the degradation of nanomedicines in the systemic circulation [64]. In this study, the authors treated HT29 CRC cells with the POSS-PCU functionalised CNT and irradiated cells with NIR light. There was a 95% reduction in cell viability and negligible reduction in cell viability in cell cultures treated with the modified CNT, but not irradiated with NIR light [65]. Graham *et al.* also investigated multiwalled CNT by conjugating folic acid on to the surfaces. Folate receptor is known to be overexpressed in many cancers, including CRC, and has been exploited to target imaging agents and therapeutic compounds towards cancerous tissues [66]. Following incubation with the folic acid functionalised CNT and NIR light irradiation, cytotoxic analysis showed a significant reduction in cell viability in HCT116 and RKO CRC cells. Similar to the

previous study, no cell death was observed in cells treated with CNT, but not irradiated with NIR light [67]. Levi-Polyachenko *et al.* explored the hyperthermia inducing ability of CNT to improve the uptake and delivery of oxaliplatin and mitomycin C. The clinical relevance for this is its potential use in Hyperthermic Intraperitoneal Chemotherapy to treat peritoneal metastasis [68]. The authors loaded the chemotherapeutics into multiwalled CNT and incubated it with HCT116 and RKO CRC cells. There was a rapid accumulation of the chemotherapeutics into cells (tens of seconds), and the intracellular amount of anti-cancer drugs was found to be similar to cells incubated with the chemotherapeutics for two hours under non-hyperthermic conditions. Cell viability analysis showed a synergistic effect on cell death, mediated by combined CNT-PTT and oxaliplatin/mitomycin C toxicity [69].

Graphene oxide-based nanomedicines

Graphene is the fundamental building block in graphitic materials, and can be fashioned into many different geometrical structures. Graphene, graphene oxide, and other derivatives have been extensively explored for theranostic applications in nanomedicines due to their low cost, large molecular surface area, chemical and mechanical stability, low toxicity and biocompatibility. Graphene also exhibits high photonic energy absorption in the NIR range, making it a suitable candidate for PTT [70,71]. Fiorica *et al.* developed a hyaluronic acid based nanogel network with graphene oxide incorporated into the nano-structure. The anti-cancer drug, irinotecan, was loaded into the core of the nano-structure. The authors evaluated the multimodal activities of the nanogel in 3D spheroidal co-culture models of CRC comprised of HCT116 CRC cells and human dermal fibroblasts. The 3D models were directly

injected with the nanogel constructs, mimicking intratumoural injections. The nanogel was taken up by HCT116 cells, which may have been due to the recognition of hyaluronic acid by overexpressed cancer cell surface markers, such as CD44 [72]. Following NIR light irradiation, the destruction of both HCT116 CRC and healthy fibroblasts was observed in irinotecan loaded nanogel treated 3D models. Cytotoxicity was observed throughout the 3D model. This contrasted with necrosis alone being observed in the light irradiation spot on 3D models treated with non-irinotecan loaded nanogels. Viable HCT116 and fibroblasts were still present around the light irradiation spot [73]. Einafshar *et al.* used sheets of graphene oxide to produce biocompatible nanocarriers of SN38. The nanoparticles were comprised of SPIONs and were functionalised with cyclodextrins to improve aqueous solubility and confer biocompatibility. HT29 CRC cells were treated with the nanoparticle *in vitro*, and the results indicated a synergistic mode of cell death with combined graphene oxide-PTT and SN38-chemotherapy. This study also highlighted the effectiveness of designing nano-platforms to be used as dual carriers of chemotherapeutic and PTT agents [74]. Lu *et al.* developed a dual-targeting nanoparticle to deliver PTT and chemotherapy to CT26 CRC tumour xenografts. Magnetic nanoparticles were deposited on graphene oxide, which was then functionalised by PEGylation and Cetuximab was conjugated on to the nanoparticles. DOX was then loaded on to the nanoparticles. Magnetic guidance was employed to attract the nanoparticle towards the tumour xenografts, with Cetuximab binding to cells overexpressing EGFR. The nanoparticles were taken up via endocytosis, followed by the intracellular release of DOX. Following intravenous injection and application of magnetic field and NIR light irradiation, substantial regression in tumour growth was observed in treated animals. In comparison, the other animal groups (free DOX alone, DOX loaded nanoparticle alone, DOX loaded

nanoparticle + magnet alone) showed an initial regression in tumour growth but tumours relapsed after a few days. Histopathological analysis revealed large areas necrosis in tumour xenografts from treated animals [75].

Transition metals-based nanomedicines

The use of transition metals as oxides, hybrid structures, and alloys are being evaluated in an effort to create more efficient biofunctional nanomaterials and platforms with high photothermal effects [76]. Lee *et al.* reported the use of titanium oxide nanotubes (TONT) as mediators of PTT. In comparison to AuNP and CNT, TONT absorb more NIR light energy and convert light to heat more efficiently. *In vitro* cytotoxicity analysis found CT26 CRC to respond to TONT treatment and NIR light irradiation, with a significant reduction in cell viability. TONT was also found to be non-toxic when incubated with cells and devoid of NIR light irradiation [77]. Liu *et al.* developed a theranostic agent by combining MR imaging and PTT into a single nanoparticle. The nanoparticles were assembled using iron (Fe^{3+}), gallic acid and poly(vinylpyrrolidone). Due to the small size of the nanoparticles, they were found to preferentially accumulate in SW620 CRC tumour xenografts. The intratumoural acidic pH enhanced MR imaging and following NIR light irradiation, tumours were completely ablated due to the PTT mediating properties of Fe^{3+} [78]. Zhang *et al.* created a multimodal iron based hybrid anisotropic nanoparticle. Cell viability analysis showed cytotoxicity in nanoparticle and laser irradiation treated HT29 cells *in vitro*. Following the injection of the iron-nanoparticle into HT29 CRC tumour xenograft bearing animals, photoacoustic and MR imaging was used to track the uptake and dissemination of nanoparticles. Once the optimal time for intratumoural accumulation was identified, NIR light irradiation was applied to the tumour xenografts. Two days after irradiation,

tumour sizes in treated mice had regressed significantly. Histopathological analysis of tumour sections confirmed high therapeutic efficacy with apparent extensive necrosis [79].

In comparison to gold and other inorganic materials, copper is cheap, easily obtainable, biocompatible and possesses efficient photothermal properties. This makes it advantageous for the mass production of copper-based nanoparticles. Hessel *et al.* developed copper selenide (CuSe) nanocrystals, which exhibited strong NIR optical absorption. Cellular destruction was observed following the treatment of HCT116 CRC cells *in vitro*, with CuSe nanocrystals and NIR light irradiation [80]. Li *et al.* investigated PEGylated copper nanowires (CuNW) as PTT mediating agents. CuNW was found to efficiently generate heat upon NIR light irradiation. *In vitro* analysis showed CuNW intertwining around CT26 CRC cells and upon light irradiation heat was transmitted to cells, triggering thermal induced cell ablation. CT26 CRC tumour xenograft bearing animals were then injected with CuNW and irradiated with NIR light. Tumoural temperatures rose and tumour growth was suppressed. Necrosis was found to be the primary mode of cell death, as indicated by release of the damage associated molecular pattern molecule, HMGB1 [81].

Polymerically structured nanomedicines

Hong *et al.* evaluated porous silicon as a PTT mediating agent and found that, in combination with NIR light irradiation, CT26 CRC cells experienced substantial cytotoxicity *in vitro*. Porous silicon or NIR light irradiation alone did not influence cell viability. *In vivo* treatment of animal bearing CT26 CRC tumour xenografts with porous silicon and NIR light irradiation resulted in tumour regression with the animals

remaining healthy and tumour-free for more than 3 months. Furthermore, surrounding healthy tissue was found to be free from thermal damage [82]. Similarly, MacNeill *et al.* developed a novel chemically modified polymer nanoparticle. The nanoparticle was stable and non-toxic under non-NIR light conditions. Upon NIR light irradiation, the nanoparticle generated temperatures above 50°C *in vitro*. Photothermal ablation was seen in HCT116 and RKO CRC cells treated with the nanoparticle and NIR light irradiation [83]. Recently, polyaniline-based nanoparticles (PANP) have been developed with strong NIR optical absorption properties [84]. Zhou *et al.* investigated PANP and found that the cytotoxicity of PANP was dependant on NIR light irradiation *in vitro*. HCT116 CRC tumour xenograft bearing mice were treated with PANP and NIR light irradiation. Complete tumour regression was observed 5 days after treatment and tumours did not regrow [85].

Ideally, nanomedicines that have been developed for PTT should possess strong optical absorption in the NIR range (700nm – 2000nm, typically 800nm) of the electromagnetic spectrum [86]. Kelkar *et al.* developed a dual wavelength absorbing nanoparticle for PTT. The nanoparticle could absorb light at 450nm and 800nm. The rationale was that the shorter wavelength would be better suited to treat superficial lesions, whereas the longer wavelength could penetrate into deeper tumours. *In vitro* cell viability analysis in CT26 CRC cells showed that irradiation at both wavelengths resulted in a nanoparticle-dose dependant reduction in cell viability. Simultaneous application of both wavelengths was found to be better than blue (450nm) and NIR (800nm) wavelengths alone [87]. Yang *et al.* investigated SN38-loaded nanoporphyrin micelles. This tri-modal therapeutic platform could impart PTT, PDT and chemotherapeutic effects. Under non-light conditions, the micelles were stable, but

upon light irradiation they resulted in a controlled release of SN38. *In vitro* analysis showed a dose-dependent uptake and light-dependant reduction in cell viability in HT29 CRC cells. Due to the small size of the micelles, they were retained longer in tumours, as compared to the major organs in animal models. Complete tumour regression was observed in animals bearing HT29 CRC tumour xenografts treated with SN38-loaded nanoporphyrin micelles and light. Histopathological analysis showed signs of necrosis and pyknosis [88].

Potential clinical applications

As highlighted in this review, there are many classes, derivatives and highly modified multimodal nanomedicines that can deliver PTT to CRC. The studies evaluated in this review are pre-clinical in nature, showing great promise. However, there is still a long way to go before these techniques can be clinically applied. AuNP are the most favoured class of nanoparticles in PTT, due to their many inherent advantages in optical imaging, eliciting PTT and serving as effective nanocarriers. AuNP were the first class of nanoparticles to be evaluated for PTT in CRC, and are still being studied today. In addition, as summarised in Table 1, there are more AuNP-based PTT pre-clinical studies in CRC, in comparison to other classes of PTT mediating nanoparticles. Recently, Stern *et al.* published the findings of a small scale clinical study, evaluating the toxicity of AuNP in patients with prostate cancer. The primary endpoint of this study was patient safety, and the clinical safety profile of AuNP was found to be excellent and strongly supports further investigation [89]. Based upon the pre-clinical findings in this review and the aforementioned clinical study, there is ample evidence suggesting that AuNP-based PTT nanomedicine is a very attractive method of treatment in CRC,

and a strong candidate that would greatly benefit from early phase clinical evaluation in the near future [90].

Intraoperative PTT shows great clinical potential, as a treatment option for metastatic CRC. As PTT is similar to PDT, there are similarities in the clinical application of both treatment methods. In PDT, following the systemic administration of the photosensitising agent, light is delivered laparoscopically through optical fibers to the site of tumour growth. This method for practising intraoperative PDT, has shown to be the optimal and safest route for treating patients [91]. Without light mediated activation, photosensitising agents are unable to elicit cytotoxicity. It would therefore be practical, to employ the same technique for PTT. Due to the flexibility of optical fibres, light delivery can be localised and made accessible to any organ, making it ideal for precisely targeting metastatic CRC lesions.

Limitations of nanomedicines

The enhanced permeability and retention (EPR) effect is often described as a potential drawback in the clinical application of nanomedicines. Briefly, the EPR effect describes the extravasation of intravascular fluids from systemic circulation, carrying plasma proteins and macromolecules into the interstitial spaces within the tumour microenvironment. This is usually brought on by the leakiness and compromised structure of the tumour vasculature [92]. Coupled with the ineffective lymphatic filtration system in the tumour microenvironment, extravasated macromolecules are prevented from being removed back into systemic circulation [93]. The EPR effect is the basis on which nanomedicines are delivered to the tumour. Paradoxically, a major obstacle to this system, is the build-up of colloid osmotic pressure as a result of the EPR effect [94].

The continuous extravasation and accumulation of intravascular fluids, can increase the interstitial fluid pressure and impede the delivery of nanomedicines.

Off-target effects of non-targeted nanomedicines and challenged pharmacokinetics i.e. the inability to penetrate through multiple layers of tissues, can also result in the inefficient delivery of nanomedicines and treatment of tumours. There is on-going research to potentially improve the delivery of nanomedicines to tumours, which includes the use of magnetic nanoparticles, ultrasound and through the exploitation of tumour-specific physiology [95].

Conclusion

This review highlights the pre-clinical evaluations of nanomedicines for PTT in CRC. Thermal ablation is an effective method for treating solid cancers and via nanocarriers, nanoparticles, and similar platforms, it is possible to directly deliver photothermal agents to cancers. As detailed in this review, multimodal PTT-nanomedicines have been produced in various forms, which can deliver combinations of cancer-imaging agents and chemotherapeutics in addition to the photothermal agent (Figure 1). To date, the results are limited to pre-clinical (cancer cell line and tumour xenograft) models. Next steps are to investigate PTT-based nanomedicines for CRC, in a clinical setting. This would involve setting up phase I small scale pilot or feasibility studies, evaluating nanomedicine toxicity, safety of NIR light irradiation and the treatment efficacy of PTT-based nanomedicines. These clinical studies can be used to investigate this technique as either a stand-alone method of treatment, or combined with surgery. These vital studies are necessary, to demonstrate the clinical benefit of this technology in human CRC.

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Disclosure

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Figure captions

Figure 1 – Schematic diagram of a multimodal targeted PTT nanomedicine

Multimodal nanomedicines combine different therapies such as near-infrared (NIR) light absorbing nanoparticles to mediate Photothermal therapy (PTT) and conventional chemotherapeutic drugs i.e. Doxorubicin, SN38 and Oxaliplatin. Nanomedicines can be functionalised with superparamagnetic iron oxide nanoparticles (SPIONs) to facilitate magnetic resonance imaging and polyethylene glycol (PEG) to improve biocompatibility and prolong systemic circulation. Cancer-targeting ligands such as antibodies, aptamers and hyaluronic acid can be conjugated onto nanomedicines to improve the delivery and uptake of nanomedicines in cancers.

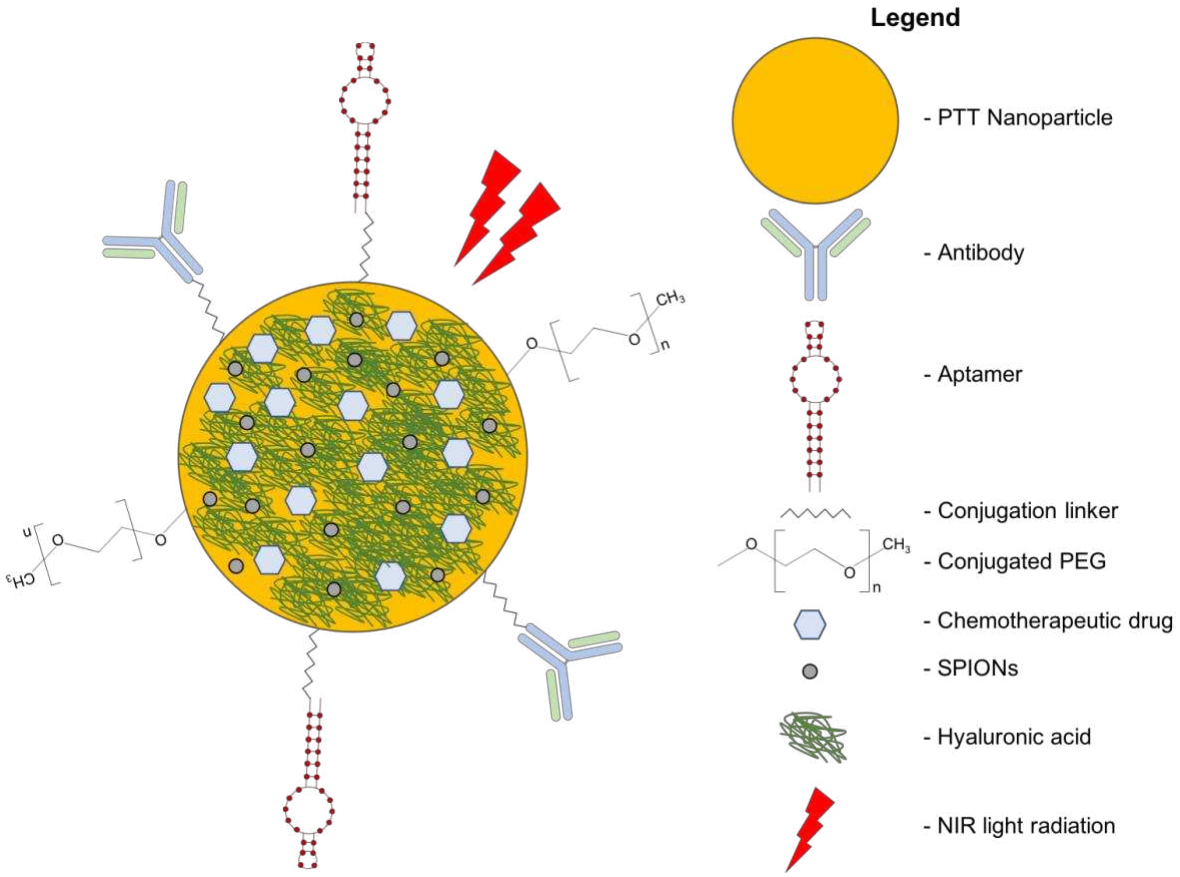
Table 1. A summary of the studies evaluating photothermal therapy nanomedicines in colorectal cancer

Class of nanomedicine	Reference	Pre-clinical model of colorectal cancer	Intended application of nanomedicine	Outcomes of treatment
Gold Nanoparticles	O'Neal 2004 [30]	CT26 tumour xenografts *in vivo*	PTT	Tumour growth regression
	Goodrich 2010 [31]	CT26 tumour xenografts *in vivo*	PTT	Treated animals were free of tumour growth
	Guo 2010 [33]	LoVo cells *in vitro*	PTT, cell imaging and deliver Cisplatin	Synergistic chemo- and PTT cytotoxicity
	Kirui 2010 [36]	SW1222 and HT29 cells *in vitro*	Targeted PTT	PTT induced cytotoxicity
	Kirui 2011 [35]	SW1222 and HT29 cells *in vitro*	Targeted PTT	PTT induced cytotoxicity
	Kirui 2013 [34]	SW1222 and HT29 tumour xenografts *in vivo*	Targeted PTT and tumour imaging	Tumour regression and confirmed tissue necrosis
	Lee 2014 [38]	DLD1 cells *in vitro* and tumour xenografts *in vivo*	Targeted PTT and deliver Doxorubicin	Synergistic chemo- and PTT tumour growth regression
	Hosseinzadeh 2017 [39]	HT29 and SW480 cells *in vitro*	Targeted PTT and deliver SN38	Synergistic chemo- and PTT cytotoxicity
	Seo 2014 [41]	CT26 cells *in vitro*	PTT and PDT	Synergistic PDT and PTT cytotoxicity
	Azhdarzadeh 2016 [44]	HT29 cells *in vitro*	Targeted PTT and cell imaging	PTT induced cytotoxicity
	White 2017 [46]	Liver metastasis of orthotopic CC531 deposits *in vivo*	Targeted PTT and tumour imaging	PTT induced confirmed tissue necrosis
	Parchur 2018 [47]	Liver metastasis of orthotopic CC531 deposits *in vivo*	PTT and tumour imaging	PTT induced confirmed tissue necrosis
	Chen 2018 [51]	HT29 cells *in vitro* and tumour xenografts *in vivo*	PTT and tumour imaging	PTT induced cytotoxicity and complete tumour regression
	Wang 2014 [52]	SW620 cells *in vitro*	Targeted PTT	PTT induced cytotoxicity
Dye-based nanoparticles	Peng 2011 [55]	HCT116 cells *in vitro* and tumour xenografts *in vivo*	PTT and tumour imaging	Tumour regression and confirmed tissue necrosis
	Shih 2017 [56]	HCT116 and SW620 cells *in vitro* and tumour xenografts *in vivo*	Targeted PTT	Tumour growth regression

	Tsai 2017 [57]	HCT116 tumour xenografts *in vivo*	PTT, cell imaging and deliver SN38	Synergistic chemo- and PTT tumour growth regression
	Lin 2018 [58]	HT29 cells *in vitro* and tumour xenografts *in vivo*	PTT, cell imaging and deliver Doxorubicin	Synergistic chemo- and PTT improvement in survival
	Chen 2017 [61]	HCT116 tumour xenografts *in vivo*	PTT and deliver Doxorubicin	Synergistic chemo- and PTT tumour growth regression
	Wang 2017 [62]	Lymph node metastasis of HCT116 tumour xenografts *in vivo*	PTT	Necrosis and pyknosis in lymph nodes
Carbon nanotubes	Tan 2012 [65]	HT29 cells *in vitro*	PTT	PTT induced cytotoxicity
	Graham 2013 [67]	HCT116 and RKO cells *in vitro*	Targeted PTT	PTT induced cytotoxicity
	Levi-Polyachenko 2009 [69]	HCT116 and RKO cells *in vitro*	PTT and deliver Oxaliplatin/Mitomycin C	Synergistic chemo- and PTT cytotoxicity
Graphene oxide nanoparticles	Fiorica 2017 [73]	3D co-culture model of HCT116 CRC cells and human dermal fibroblasts	Targeted PTT and deliver Irinotecan	Synergistic chemo- and PTT cytotoxicity
	Einafshar 2018 [74]	HT29 cells *in vitro*	PTT and deliver SN38	Synergistic chemo- and PTT cytotoxicity
	Lu 2018 [75]	CT26 tumour xenografts *in vivo*	Targeted PTT and deliver Doxorubicin	Tumour regression and confirmed tissue necrosis
Transition metals-based nanoparticles	Lee 2010 [77]	CT26 cells *in vitro*	PTT	PTT induced cytotoxicity
	Liu 2015 [78]	SW620 tumour xenografts *in vivo*	PTT and tumour imaging	Tumour growth regression
	Zhang 2016 [79]	HT29 cells *in vitro* and tumour xenografts *in vivo*	PTT and tumour imaging	PTT induced cytotoxicity, tumour growth regression and confirmed tissue necrosis
	Hessel 2011 [80]	HCT116 cells *in vitro*	PTT	PTT induced cytotoxicity
	Li 2016 [81]	CT26 cells *in vitro* and tumour xenografts *in vivo*	PTT	PTT induced cytotoxicity and tumour growth regression
Polymerically structured nanomedicines	Hong 2011 [82]	CT26 cells *in vitro* and tumour xenografts *in vivo*	PTT	PTT induced cytotoxicity and tumour growth regression
	MacNeill 2013 [83]	HCT116 and RKO cells *in vitro*	PTT	PTT induced cytotoxicity
	Zhou 2013 [85]	HCT116 cells *in vitro* and tumour xenografts *in vivo*	PTT	PTT induced cytotoxicity and tumour growth regression

	Kelkar 2016 [87]	CT26 cells *in vitro*	Dual wavelength PTT	Synergistic dual wavelength PTT induced cytotoxicity
	Yang 2017 [88]	HT29 cells *in vitro* and tumour xenografts *in vivo*	PTT, PDT, tumour imaging and deliver SN38	Synergistic PTT, PDT and chemo- induced cytotoxicity, tumour growth regression and confirmed tissue necrosis

Figure 1


Legend
- PTT Nanoparticle
- Antibody
- Aptamer
- Conjugation linker
- Conjugated PEG
- Chemotherapeutic drug
- SPIONs
- Hyaluronic acid
- NIR light radiation