Photodynamic therapy-based combinations with immunotherapy in colon cancer treatment

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General Discussion

CHAPTER 6

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Current Limitations and Optimization Strategies for PDT in Cancer Treatment

Currently, PDT is emerging as a low invasive and affordable cancer treatment [1]. Although great progress has been made in a variety of cancer types that appear at the skin surface, the potential of PDT has for solid tumors has not been fully exploited [2]. According to the mechanism of action of PDT, the optimization related to three essential elements (PS, light source and O_2) was (and is still) needed to improve the efficiency of photodynamic therapy [3]. Effective accumulation of PS at the tumor site is a prerequisite for PDT, but to date, only a few PS have entered clinical trials. Thus, more efforts in the field of PS development are needed [4]. On-going clinical trials point to the medicine value of existing secondary PS that so far have not been approved for clinical application. For example (NCT05374915), REM-001 supported PDT has shown promising results for the treatment of cutaneous metastatic breast cancer (CMBC) that is refractory or not amenable to radiotherapy or surgery. In addition, the development of novel third-generation PS that also optimize physicochemical properties is another focus of research [5]. As illustrated in **Chapter 1**, the development of third-generation PS is concentrated on targeted delivery strategies. This can be achieved by PS coupling to antibodies, engineering molecular couplings with specific structures, or introducing nanoparticles as delivery vehicles. An exampleis saratolacan®, a water-soluble silicone phthalocyanine derivative IRDye700DX (IR700) coupled to cetuximab. In this treatment, saratolacan® addresses the issue of reducing skin toxicity by targeting head and neck cancer cells with high levels of EGFR, reducing the distribution of ineffective PS, and achieving addictive anticancer response by combination PDT with targeted therapy [6]. Another strategy is to develop third-generation PS with characteristics like strong absorption bands in the near-infrared region of the spectrum or to use advanced laser technology (like interstitial irradiation, endoscopic balloon catheter, etc.) and functionalized carriers (such as Au nanostructures, upconverting NPs etc.) [7,8]. Of note, tissue hypoxia may limit the clinical efficacy of PDT. Therefore, the improvement of PDT efficiency by manipulating the conditions related to hypoxia is of interest. This point is discussed in **Chapter 4**.

The development of PDT in combination with existing therapies is also needed to increase the efficiency of anti-cancer effect of PDT in advanced cancer. Combining PDT with surgery has been proposed early on, namely image-guided surgery, in which the fluorescent and phototoxic properties of PS were exploited [9]. In addition, increasing evidence supports the view that combinatorial treatment holds great potential for mitigating advanced cancer progression through boosting immunogenic cell death (ICD). Related to this, PDT is reported can initiate ICD cascade and DAMPs release by generated reactive oxygen species (ROS) triggered ER stress, and thus, exploring new PDT combination strategies with ICD-boosted therapies (chemotherapeutics, radiation therapy and photothermal therapy (PTT)) or immunotherapy, hormonal therapy, etc., and underlying mechanisms can help to realize the potential of PDT as a mainstream cancer treatment option [10]. Basic nanomedicine research aims to address these issues, including optimizing the properties of PS (hydrophobicity, targeting, immune antigenicity, oxygen dependence, etc.), improving the efficiency of monotherapy, and providing tools for combination therapeutic strategies [11]. An overview of studies, including studies from our research group, on NP-supported PDT combination strategies is presented in **Chapter 4**.

Towards standardizing PDT protocols and achieving high response efficiency

In this thesis we explored the PDT effects of three PSs with different excitation bands in the "600-900 nm" region of the optical window (Figure 1). In contrast to the first-generation PS, Photofrin II, which has a 1-3 mm penetration depth at 630 nm, the tissue penetration of light is 50% higher at 650-690 nm and about twice at 700-850 nm [12-14]. VP (in **Chapter 2**) and FOSCAN (in **Chapter 3**) are clinically approved second-generation photosensitizers for PDT, while ICG (in **Chapter 5**) is the only FDA approved dye for clinical application in near infra-red (NIR) wavelength and has been shown possibility as PS for PDT application [15]. They have strong absorption peaks at 690 nm, 652 nm and 800 nm of the spectrum, respectively [16].

The kinetics of uptake and binding of three PS were studied in MC38 and CT26 colorectal cancer cells. It is well accepted that the subcellular localization (lysosomes, mitochondria and Golgi apparatus and endoplasmic reticulum) of PS has an impact with the efficacy of PDT and the ability to induce ICD. Generally, PS targeting mitochondria usually induce more effective tumor cell apoptosis by causing mitochondrial damage, whereas PS targeting ER is associated with the ability to induce ROS-mediated ER stress after PDT, producing ICD induction [17]. Osaki et al. demonstrated that VP is mainly located in or around mitochondria after 3 hours of incubation, while together our data regarding FOSCAN and ICG demonstrated that both have a strong accumulation in membrane transport system [18,19]. These findings partly explain why FOSCAN and ICG induces an increase in DAMPs. However, here we shown that PDT-generated dying tumor cells are phagocytosed by immature DC and promote their activation even when the PS not acting as an ICD inducer, a phenomenon that can be explained by the theory that necrotic cells are equally immunogenic. In addition, the efficiency of PDT supported by these three clinically approved PS with different excitation wavelengths was determined in MC38 mouse colorectal model. Although the treatment can effectively

control tumor growth but not completely ablate tumors, highlighting the limitations of PDT under clinic circumstances. Hence, combination of PDT and immunotherapy was chosen to compensate for the deficiencies of PDT.

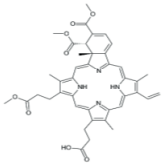
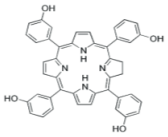
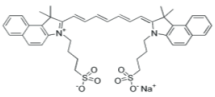
Name of photosensitizer (PS)	Vertepofin (VP)	Temoporfin (FOSCAN)	Indocyanine green (ICG)
			
Excitation wavelength	690 nm	652 nm	800 nm
Clinical application	Age-related macular degeneration	Advanced head and neck cancer	FDA-approved NIR imaging hye
Clinical trails	Brain tumors Solid pancreatic tumors Metastatic breast cancer Recurrent prostate cancer	Nasopharyngeal cancer Bile duct cancer Oropharyngeal cancer Non-small cell lung cancer	Not determined
PDT efficiency in vitro (MC-38, CT-26)	Good	Good	Good
ICD inducer	Yes (Need further tested)	No	Yes
Immunogenicity of PDT-treated cells	DC activation	DC activation	DC activation
PDT efficiency in vivo (MC-38 tumor mouse model)	Three in nine mice cured ($23\ mW/cm^2$ for $20\ J/cm^2$ irradiation at 24 h post 0.15 mg/kg PS i.v. injection)	Two in nine mice cured ($200\ mW/cm^2$ for $100\ J/cm^2$ irradiation at 3 h post 0.15 mg/kg PS i.v. injection)	No mice cured ($500\ mW/cm^2$ for $125\ J/cm^2$ irradiation at 4 h post 8 mg/kg PS i.v. injection)

Figure 1. Overview characteristics, biological functions and anticancer effects of selected PSs in this thesis.

PDT based Combination therapy effectively ablates tumors by promoting host immune responses

In **Chapter 2**, the potential of PDT-mediated combination therapy with STING agonist (ADU-S100) against colorectal cancer was investigated using MC38 and CT26 mouse colorectal models (Figure 2). When MC38 cells tumor-bearing mice were treated with PDT or ADU-S100 alone, only a small subset of tumor-bearing mice responsed to the therapy and leading to marginally pronlonged survival time. However, combination therapy significantly enhanced the anti-tumor immune effect and improved overall survival outcomes, with only one in eleven MC38 tumor-bearing mice eventually die due to high tumor load. All of these cured mice by therapeutic combination established the immune memory capability against homologous tumors rechallenge. In addition, local treatment of the tumor, either alone or in combination, could slow distant MC38 tumor growth through the abscopal effect of $CD8^+$ T cells (cytotoxic T lymphocytes) [20]. The same anti-cancer trend of combined therapy was observed in the CT26 cell model, but the response was much lower than in the MC38 cell model. This result emphasizes that the tumor cell type is a critical determinant for the additional anti-tumor effects of PDT in combination with ADU-S100; it highlights the critical impact of tumor heterogeneity for further studies. In addition, it will be valuable to study the underlying mechanisms of this

combination in more depth, especially the role of PS (ICD inducer or non-ICD inducer, vasular targeted of non-targeted) and inhibitory immune cells in the TME (Treg and MDSC, etc). In conclusion, our study establishes that immunomodulation of tumors by PDT and ADU-S100 combination can improve the survival in colorectal cancer in mice. We have thus provided a proof of principle for this combination strategy.

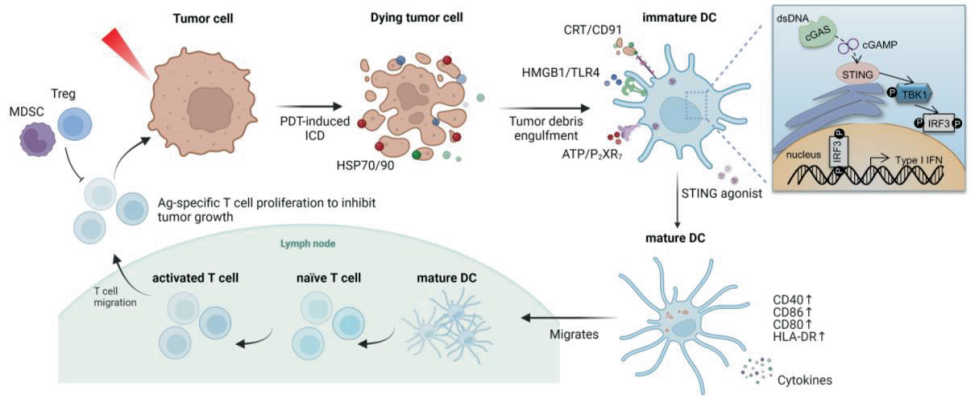


Figure 2. Anti-tumor T cell immunity after PDT in combination with immunomodulators in solid tumors. STING agonist is selected as an example of immune modulator. Exposure of tumor cells to PDT prior to immunotherapy induced immunogenic death and promoted the release of tumor-associated antigens, leading to greater sensitivity of tumor cells to immunotherapy.

In **Chapter 3,** the efficacy of PDT in combination with HBc VPLs was tested in the MC38 colorectal cancer cell model. There are several routes for delivery of vaccination regimen (nasal, intraperitoneal, or intratumoral). In our study we chose to inject HBc VPLs directly into the tumor site within one week after PDT. This choice was based on previous reports that intratumoral injection approach was found to be a better route of drug delivery in a variety of tumor types by reducing the drug diffusion from the tumor site [21,22]. No delay was observed on tumor growth when only HBc VPLs were administered to mice bearing MC38 tumors. However, significantly longer progression-free survival times and increased overall survival percentage was observed when PDT and HBc VPLs were administered together. In addition, the combination treatment cured mice developed long-term immune memory and protected them from homologous tumor cell attack. Wenjun et al. reported that HBc VLPs as natural nanoscale particles has the ability to be loaded in cargo (tumor targeting peptide and doxorubicin) [23]. Taken together, our study and those of others suggests that that the combination of PDT with modified immune-regulatory HBc VPLs is feasible to achieve an enhanced anti-tumor effect.

Hydrogel as Delivery Platform to Support Photodynamic Therapy in Combination with Immune Checkpoint Inhibitors

Anti-CTLA4 (ipilimumab) based therapies with PD-1/PD-L1 (e.g., pembrolizumab or nivolumab) are the backbone of tumor immunotherapy in the market today [24]. In

Chapter 5, we investigated the anticancer efficacy of PDT in combination with CTLA4 antibody in MC38 and CT26 cancer cell models using P407 hydrogel as a drug delivery system. We observed the excellent physicochemical properties of the hydrogel as a delivery platform, which satisfies the "single injection, slow-release, repeated irradiation" strategy. Its aim is to enhance the efficacy of PDT and reduce the systemic toxicity of ICI. As expected, the use of P407 hydrogel significantly reduced serum CTLA4 antibody levels, which is thought to be intrinsically correlated with the induction of immune related adverse events (irAEs). PDT followed by P407-supported CTLA4 antibody treatment effectively mitigated tumor growth and enhanced overall survival percentage on both tumor models. In addition, our data is in line with the statement that multiple rounds stimulation with PDT significantly enhanced the immune response by Zhouqi et al. and confirmed that simultaneous treatment with PDT and ICI of the P407 hydrogel modality further increased the survival rate in the two colon cancer models [25]. Our results indicate that the use of hydrogels is a very promising drug delivery system to support the combination of PDT and ICI.

Future Perspectives and Concluding Remarks

In this thesis, the work provides the experimental rationale to combine PDT with immunotherapy for treatment of colorectal cancer. The types of PS, the protocol of PDT, and the kinds of immunotherapeutic agents are all critical factors that affect the efficiency of combination therapy. Our data showed that PDT, although demonstrating potent tumor cell-killing ability *in vitro*, was not satisfactory as a stand-alone treatment modality *in vivo*. This is because cultured cells are monolayers that can be reached by visible light, while solid tumors are of a certain thickness, cancer cells that are located deeply in solid tumor do not receive enough irradiation during PDT, leading to an insufficient tumor ablative effect of PDT. Optimizing *in vitro* models (such as 3D spheroid tumor models) and constructing deep-seated *in vivo* tumor models (such as orthotopic implantation tumor models) can be employed to determine and optimize the anti-tumor efficiency of newly discovered PS.

PDT in combination with immune agonists (STING agonist or multi-TLR agonist) or immune checkpoint inhibitors (anti-CTLA4 antibody and anti-PDL1 antibody), showed excellent inhibitory effects against primary tumor growth, ability to counteract tumor cell re-attack, and attenuate growth of distal transplanted tumors (Figure 3). This observation underlines the potential of PDT, especially those capable of inducing ICD, as an adjuvant modality to provide tumor-associated antigens for immunotherapy, thereby boosting T cells immunity. Mechanistically, increased lymphocyte infiltration and an appropriate inflammatory state were observed in tumors and tumor lymph nodes after PDT combined with immunotherapy, and elevated levels of CD4 helper T cells and CD8 cytolytic T cells and increased serum IgG concentrations were repeatedly observed in the blood. In addition, all mice that were cured by the combination rejected tumor cell rechallenge and exhibited high levels of immune memory T cells.

Overall, photodynamic therapy holds great promise as an adjunct to immunotherapy to promote both local and systemic host immune responses. Although co-treatment of PDT with immunotherapy has shown better anti-tumor effect in (pre)clinical models,

more efforts are needed to elucidate in more detail the underlying mechanisms that improve treatment outcomes. This will likely provide reliable evidence to advance the translation of basic research to clinical applications. To date, clinical studies on this area of PDT combination immunotherapy are still scarce. Only one phase I clinical trial (NCT04836429) to investigate the efficacy and safety of PDT administered in combination with immune checkpoint inhibitor drugs in patients with non-small cell lung cancer is under recruitment (until July 2022). Nonetheless, the support of more fundamental disciplines will drive the exploration of the combination of PDT with immunotherapy in clinical trials of cancer patients.

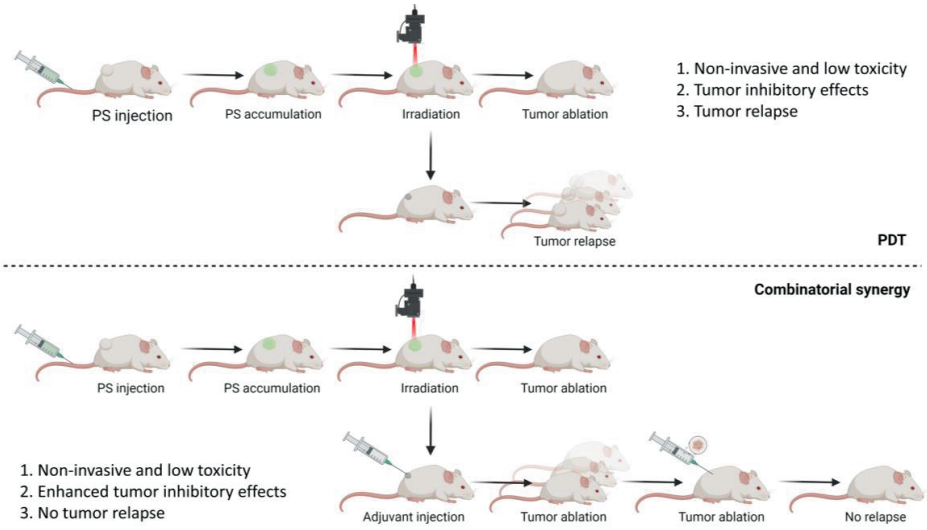


Figure 3. PDT in combination with immunotherapy increases the efficiency of cancer therapy.

In **Chapter 4**, a review is provided on the biological advantages of nanoparticles as drug delivery systems. In Chapter 5 thereafter, the feasibility of hydrogel as a combination therapy vehicle in two forms, PDT combined with single delivery of immune checkpoint antibodies, and co-delivery of PS and immune checkpoint antibodies. We found that the use of nanoparticles could further enhance the potential of combination therapy to achieve locally controlled release, reduce toxicities, and provide a platform for tailoring personalized treatment modalities to the patient's sensitivity to therapy. Of note, the tumor targeting effects of NPs is achieved through the enhanced permeability and retention (EPR) effect or active targeting strategy. Antibody drug conjugates based on active targeting strategies have been successfully used or in the clinic or are in clinical trials. The available data suggest that passive tumor targeting based on the EPR effect of nanodrug delivery systems in human cancers brings much less clinical value than the results obtained in animal models. A potential reason for this is that after surgery or radiation therapy, the reduced tumor volume falls far short of producing an EPR effect. Encouragingly, however, previous studies of our group have shown that NPs enter the tumor through the vascular system disrupted by PDT and are sequestered at the tumor site by blocked blood flow, providing a theoretical basis for PDT and NP-based

immunotherapy [26]. The interest in NP-based PDT and impact on effectiveness of combinational therapy is expected to remain high; the development of nanomedicine will further continue, especially regarding the further understanding of the nano-biological interactions and the immunological consequences of NPs (Figure 4). However, the inclusion of NPs in the combination of PDT with other therapies (of which the latter is already at the bottleneck of clinical translation), will make the clinical translation more difficult. The reasons for this are the increased complexity and the need to further consider issues such as safety, efficacy and scale-up production of NPs.

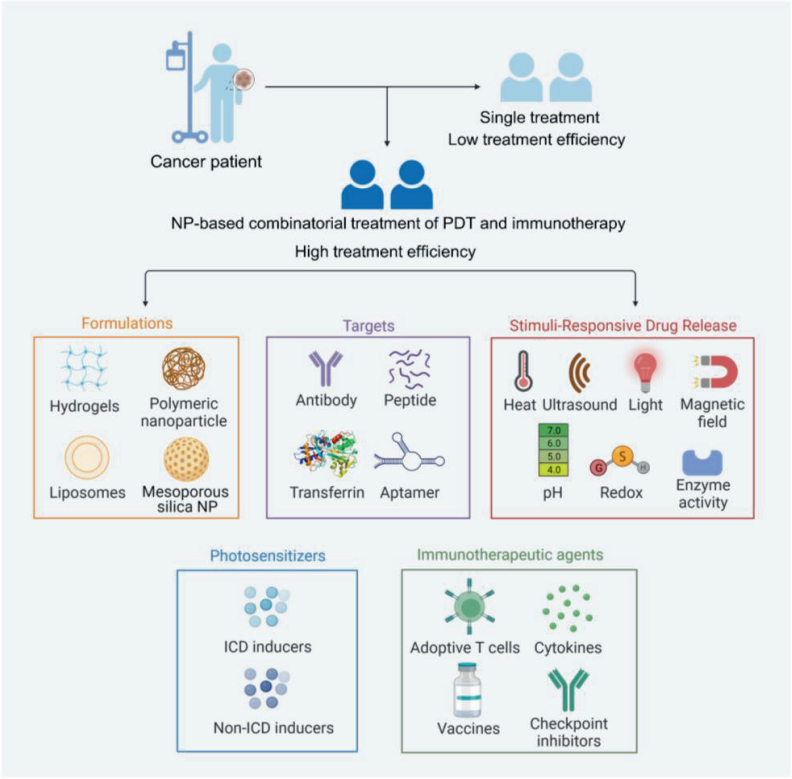


Figure 4. Design and rationale of NP-based PDT and immunotherapy combinatorial cancer treatment

In conclusion, the research described in this thesis was focused on discovering and validating the efficacy of photo-immunotherapy in colorectal tumor models. I hope that the obtained new insights for my research will increase the potential of PDT for treatment of patients with solid tumors by improving their survival and quality of life.

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