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Recent Progresses in Phototherapy-synergized Cancer Immunotherapy

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Abstract. Cancer immunotherapy have recently gained much attention in the search of cancer cure due to its ability to “train” the immune system in seeking out and removing residual tumor cells. However, relying on cancer immunotherapy alone may fail to ablate primary tumors. Phototherapy is a promising modality that offers an elegant solution to eradicate tumors through the simple application of light irradiation and meanwhile triggers immune responses by immunogenic cell death to enhance antitumor immunity. Uniting phototherapy with cancer immunotherapy have been found to achieved synergistic outcomes, promote cancer regression and even attain immunologic memory. This review summarizes the recent studies on utilizing phototherapy and immunotherapy combinatorial approach to treat cancer. A variety of nanoplatfroms have been investigated in combination with immunoadjuvants and immune checkpoint blockades such as CTLA4 and PD-L1 pathways. Higher levels of proinflammatory cytokines, improved migration of dendritic cells and an increased ratio of tumor-infiltrating cytotoxic T cells against regulatory T cells are revealed in many studies, offering a glimpse into the mechanistic principles. Future advancements targeting different cancer checkpoints that could offer better immunosuppression coupled with other phototherapeutic strategies remains to be explored in accomplishing complete elimination of cancer.

1. Introduction

The search for a cure in cancer has been the constant focus for many decades. Currently, available options of treatment include surgery, chemotherapy and radiotherapy. With a better understanding of cancer immunology recently, therapies such as hormonal and immunotherapy are gaining more recognition by many as another treatment modality.^[1] Immunotherapy by broad definition is the use of the body's natural defenses to fight against a disease, especially uncontrolled cancer.^[2] It is accomplished by restoring or attempting to boost the elements of the immune system back to its former non-diseased state.^[2] Examples of immunotherapies include, chimeric antigen receptor (CAR) T cell therapy,^[3] monoclonal antibodies,^[4] oncolytic virus therapy^[5] and cancer vaccines.^[6] In cancer vaccines, immunoadjuvants are the main components that attribute to the mounting of an immune response. Commonly used immunoadjuvants such as granulocyte-macrophage colony-stimulating factor (GM-CSF), ovalbumin (OVA) and oligonucleotides containing cytosine-guanine motifs (CpG) have been examined as a means of tumor prevention and treatment.^[7]

Recently, certain immune pathways known as “immune checkpoint” have entered the limelight in cancer immunotherapy.^[2b, 8] The first to gain recognition was the cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) pathway, a receptor constitutively expressed on regulatory T (Treg) cells.^[9] In lymph nodes, Treg cells are responsible for maintaining immunosuppression which could lead to unchecked tumor cell growth.^[9] The binding of anti-CTLA4 antibody to the CTLA4 receptor on Treg cells enables bypassing of this immune checkpoint, reducing the immunosuppression of T cell activation and restoring proliferation.^[10] Anti-CTLA4 immunotherapy has been reported with success in a number of studies and one such antibody, ipilimumab has further gained **Food and Drug Administration (FDA)** approval.^[1d, 10-11] Shortly thereafter, the programmed death 1 (PD-1) pathway was discovered to modulate the immunoresponse towards cancer.^[12] Unlike CTLA4 which is

confined to T cells, PD-1 is expressed in effector T cells and hence, occurs further downstream and is predominantly present in peripheral tissues.^[13] The programmed death-ligand 1 (PD-L1), a ligand to PD-1 expressed in many types cancer has been shown to inhibit activation of T cell receptor mediated positive signaling, negatively impacting cytokine secretion and survival.^[8a, 14] The PD-1 pathway has achieved similar success as CTLA4 pathway with two biological monoclonal antibodies, pembrolizumab and nivolumab gaining FDA approvals.^[1d] More recently, indoleamine-2,3 dioxygenase (IDO) was discovered as another potential immune checkpoint modulator.^[8b] IDO is an intracellular enzyme overexpressed in the tumor microenvironment of many cancers.^[8b] The enzyme catabolizes amino acid tryptophan into kynurenines which eventually “starves” cytotoxic T cells while at the same time, activates Treg cells.^[15] Targeting this enzyme has led to the development of multiple IDO inhibitors, providing another strategy for immune checkpoint blockade.^[15a]

Though immunotherapy could enable the induction of a systemic antitumor immunity, it is relatively ineffective in ablating solid primary tumors.^[16] Moreover, in immune checkpoint blockade therapy, it does not prime the immune system to specifically respond and target the tumor cells but only exerts its therapeutic function on its associated pathway. Not all tumors may express ligands binding to CTLA4 and PD-1 for example and hence, a successful outcome depends greatly on predicting prospectively through biomarkers to ascertain if the host’s tumor is likely to respond positively.^[8a] Engaging a more specific immune response with potentials of establishing immunologic memory is highly desirable and work towards this goal has resulted in the development of cancer vaccines.^[1b, 6a] However, because current vaccines focuses on predefined tumor antigens,^[17] slight nuances in antigens specificity between different tumors and between patients can dampen specific antitumor response.^[18] Another form of vaccine known as whole cancer cell vaccine circumvents this challenge by using minimally modified lysates derived from tumor tissues which would theoretically

overcome the slight variations in antigens profile of different tumors.^[19] However, the complex manufacturing process and difficulties in characterization coupled with uncertainties in dosage requirements led to limited clinical success.^[20] Furthermore, tumor antigens are hardly immunogenic enough and thus cancer vaccines critically requires the inclusion of an immunoadjuvant to enhance the immune response towards the antigen of interest.^[21]

In phototherapy, harnessing the absorption of light for effecting a therapeutic response is the central notion behind this treatment modality.^[22] In this technique, near-infrared light (NIR) wavelength of region between 650 to 1350 nm, known for its transparency to biological tissues is applied to phototherapy agents which absorb this region of wavelength.^[23] Absorption of this wavelength by the agent is then converted to heat by internal conversion in the case of photothermal therapy (PTT) while the release of reactive oxygen species (ROS) by intersystem crossing relaxation is the main mechanism behind photodynamic therapy (PDT).^[24] In general, PTT leads to cell death by necrosis, though depending on the temperature while PDT typically induces cell apoptosis.^[25] The original idea of phototherapy (PDT and PTT) was for the destruction of the tumor. However, it is now realized that a more important phenomenon arising from phototherapy is immunogenic cell death (ICD), that releases damaged-associated molecular patterns (DAMPs)^[26] thereby increasing the immunogenicity of the tumor microenvironment.^[6b, 27] DAMPs are normally located intracellularly and do not present itself as a danger signal until its release by sudden or uncontrolled cell death and/or damage.^[26b] The release of DAMPs then activates the immune system, specifically by inducing the maturation of dendritic cells (DCs) which eventually migrates to the lymph node where cross-presentation of the antigens to naïve T cells prime them into **cluster of differentiation 8⁺ (CD8⁺)** cytotoxic T cells.^[28] In addition, certain cytokines like **interferon- γ (IFN- γ), tumor necrosis factor- α (TNF- α) and various**

interleukins (IL) such as IL-2, IL-12 and IL-6 were also reported to increase, contributing to immunostimulation.^[18, 29]

In the past few years, research groups have started to realize the potential benefits of phototherapy when introduced to augment some of the inherent shortfalls of immunotherapy. As shown in Figure 1, the general principles that allow phototherapy to successfully boost the effects of immunotherapy could be summarized as follow: 1) application of phototherapy enabled effective eradication of primary tumors by ICD; 2) subsequent release of tumor-specific antigens then serves as the substrates for an *in situ* autovaccine;^[30] 3) DAMPs concurrently released from phototherapy are able to stimulate immune responses, boosting the inherently weak immunostimulatory properties of native tumor antigens;^[28] and 4) proinflammatory cytokines designed to activate the immune system are also elevated. Immunotherapy then plays its part either through 1) increasing immunogenicity of the tumor microenvironment (utilizing immunoadjuvants), eventually recruiting more DCs or 2) decreasing immunoregulatory suppression (immune checkpoint blockade therapy), thereby increasing the number of tumor-infiltrating cytotoxic CD8⁺ T cells as well as effector memory T cells. In the end, this combinatorial therapy effectively removes the primary tumor, clears up residual tumor cells and targets metastatic tumor sites. Immunologic memory could potentially be attained, preventing tumor recurrence and promising a chance for a complete recovery. Synergistic treatment is a type of combinatorial treatment that has the synergistic effects of two or more combinational treatment modalities, which often provides improved therapeutic efficiencies than traditional combinatorial treatment. Therefore, combination of phototherapy with immunotherapy endows unique synergistic mechanisms to boost immune activation for cancer therapy. In this review, recent studies examining the therapeutic effects on cancer by combining PTT and/or PDT with the application of immunotherapies are discussed. We first cover the combinatorial approaches between PTT-synergized

immunotherapy treatments by immunoadjuvants and immune checkpoint blockade followed by the same combinatorial approaches utilizing PDT. Lastly, a conclusion and future outlook is summarized at the end.

Figure 1. An overview of phototherapy and immunotherapy combinatorial treatment.

2. PTT-synergized Immunotherapy

Despite the many immunological stimulants released by PTT and PDT, almost all studies have shown that phototherapy agents itself could not arrest tumor growth in distant sites and sometimes fail to reduce the primary tumor itself.^[18, 29c, 31] At the same time, immunotherapies faced certain challenges as discussed and therefore, a logical strategy adopted was to combine both treatments. In an example by Liu and coworkers, the immunological responses induced by **single-walled carbon nanotubes (SWCNT)**-based photothermal ablation failed to adequately inhibit the growth of a secondary tumor.^[32] The introduction an anti-CTLA4 antibody after thermal ablation of the primary tumor greatly improved the efficacy as well as exhibiting abscopal immune responses^[32]. Another immune checkpoint pathway against PD-L1 and IDO was also documented to boost photothermal mediated antitumor immunity.^[33] Other common strategies used biologically based immunoadjuvants and such examples include unmethylated CpG,^[18, 31b] OVA^[29c] and glycated chitosan (GC)^[31c] etc. Hence, many photothermal agents together with the immunoadjuvants have been applied as an endogenous vaccination, namely laser immunotherapy (LIT).^[29b, 34] Chen et al. introduced the term *in situ* autologous cancer vaccine (inCVAX) for treatments by the combination of PTT and immunoadjuvant GC targeting the primary tumor.^[35] The advantage of this whole tumor therapeutic vaccine is its ability to treat different cancer tumors as the source of antigens originating from the tumors themselves and so any slight nuances in the antigen profiles are captured accordingly.^[35-36] To unite the merits of each methods, Liu et al. employed both checkpoint inhibition, an immunoadjuvant together with PTT in an attempt to trigger a synergistic outcome for cancer immunotherapy.^[37] In the next section of this review, some of the notable strategies deployed and successes in the application of hyperthermia by photothermal agents as a cancer immunotherapeutic tool are highlighted.

2.1. PTT-synergized Immunoadjuvant Therapy

In the past few years, various groups have reported synergism in treating cancer metastasis by applying PTT in combination with other immunotherapeutics. Particularly, PTT in conjunction with an immunoadjuvant has emerged as a popular strategy for enhancing immune response against the growth of a secondary tumor. In an interesting example reported by Lu and coworkers, CpG and copper sulfide nanoparticles were used as a pair in the treatment against a primary and distant tumor.^[18] Briefly, copper sulfide nanoparticles were assembled in a hollow core coated with chitosan and CpG (HCuSNPs-CpG) (Figure 2a). This conjugated structure was injected intratumorally and better retention of CpG was achieved compared to its free form (Figure 2b). Upon excitation at 900 nm, the structure breaks down into the immunoadjuvant, Chi-CpG-NPs and the photothermal agent, SCuSNPs. The released Chi-CpG-NPs gets phagocytized and binding of CpG to Toll-like receptor-9 activated plasmacytoid dendritic cells (pDCs) to increase IFN- α secretion. Simultaneously, SCuSNPs generated heat to ablate primary tumor cells and release tumor-specific antigens that will be specifically captured by myeloid dendritic cells (mDCs) where in the presence of IFN- α , attained maturity. Subsequently, migration of matured mDCs into the lymph node cross-prime cytotoxic T-cells into antigen specific CD8⁺ T cells, triggering the start of adaptive immunity (Figure 2c). Evaluation of these nanoparticles in an EMT6 tumor bearing mouse model revealed significantly higher levels of IFN- γ and impeded tumor growth in the treated primary tumor as well as the distant secondary tumor (Figure 2d and 2e).^[18] Cancer metastasis is a widely known challenge leading to poor cancer prognosis and the authors have showcased the synergistic effect of photothermal assisted immunotherapy in regressing untreated distant tumors.

Figure 2. (a) A scheme illustrating the assembly of HCuSNPs-CpG conjugates and nanoparticles rearrangement upon near-infrared light-triggered disintegration. (b) Fluorescence imaging of (1) free CpG; (2) HCuSNPs-fCpG; or (3) HCuSNPs-fCpG plus laser at 1 and 4 h. (c) A diagram depicting the mechanism of HCuSNPs-CpG conjugates in photothermal assisted immunotherapy for primary and distant tumor. (d) IFN- γ levels in tumor of mice with different treatments. (e) Tumor growth curves of distant tumor in EMT6 tumor-bearing mice after various treatments. Reproduced with permission.^[18] Copyright 2014 American Chemical Society.

Besides copper sulfide, gold nanoparticles are another attractive photothermal agents.^[31b, 38] The popularity of gold nanoparticles can be attributed to their easy synthesis, targeting capability and favorable biocompatibility.^[38c, 38d] In addition, gold nanoparticles display easy functionalization capability, allowing conjugation to different biological species^[39] including proteins^[40] and oligonucleotides^[31b, 41] *via* simple chemistry. For instance, Jon et al. conjugated thiol-modified CpG to gold nanospheres, creating photothermic and immunologic nanoparticles.^[38d] The desired wavelength absorption and efficiency of the nanoparticles can also be achieved easily by fine-tuning the size.^[42] Notably, gold nanorods have the inherent advantage where the difference in its width and length allows a broader plasmon range of 600

to 1100 nm and above.^[38c, 43] In a study, Nishikawa et al. prepared an injectable shear resistant hydrogel consisting of gold nanorods and nanospheres linked by CpG oligonucleotides.^[31b] NIR irradiation to the administered hydrogel stimulated the expression of **heat shock protein (HSP) 70 and** mRNA, while leading to tumor volume reduction in the primary and distant sites, achieving survival rates of 80% in C57BL/6 mice inoculated with EG7-OVA cells.^[31b] Gold nanostars have also been proven to induce strong photothermal efficiency due to its “sharp branches”^[33b, 44] and used in combination with immunotherapy.^[33b] Aside from gold nanoparticles, an ancient dye in the form of mixed valence iron hexacyanoferrate known as Prussian blue, has also recently been recognized as a photothermal agent^[45] for immunotherapy combinatorial approach.^[46]

While metallic nanoparticles are the popular choice as their optical properties can be altered through modification of their size and morphologies, organic compounds have also established themselves as potential photothermal agents.^[31a, 47] For instance, the biopolymer melanin, found naturally in human skin is appreciated as a photothermal agent with strengths in excellent biocompatibility and toxicity. Utilizing the photothermal conversion property of melanin, Gu et al. designed and developed a melanin-contained microneedle (MN) patch for thermally enhanced cancer immunotherapy. The MN patch was fabricated within a micromold, forming a hyaluronic acid-based MNs with the encapsulation of melanin, B16F10 whole tumor lysate and adjuvant GM-CSF.^[29a] Their administration format was accomplished through the skin insertion of the intradermal MN patch and NIR irradiation at 808 nm to raise the local temperature to approximately 42 °C during each round of laser exposure (Figure 3a). The mild local temperature did not influence the release of tumor lysate protein and GM-CSF from MN, while enabling the release of inflammatory cytokines and immunogenic substrates such as extracellular HSPs and ROS, as well as increased the blood and lymphatic flow to facilitate the migration of antigen presenting cells (APCs), T cells and

natural killer (NK) cells, leading to the activation of immune response (Figure 3b-d). As such, the melanin-mediated MN in combination with NIR laser irradiation resulted in a 9.8-fold increase of infiltrating CD8⁺ T cells in the tumor and an 8-fold increase in **immunoglobulin (IgG)** levels of the immunized mice (Figure 3e), affording effective immunotherapy (Figure 3f). Indeed, when an additional study involving two injection sites (left and right flank) was performed, sole treatment to the left flank conferred protection to distant sites as well, e.g. right flank (Figure 3g) demonstrating its capability to be used as a therapeutic vaccination for other tumor models.^[29a]

Figure 3. (a) Diagram illustration of the principles in adaptive immunity pathway mediated by the MN transdermal patch. (b) Surface temperature changes at increasing laser power. (c) *In vitro* measurement of tumor lysate proteins. (d) *In vitro* activation of DCs at varying levels of NIR irradiation. (e) Cartoon representation of MN patch treatment. (f) IgG levels in serum

of mice. (g) Tumor volume growth curves between left and right flank in mice with different treatments. Reproduced with permission.^[29a] Copyright 2017, The American Association for the Advancement of Science.

Besides polymeric compounds, small molecule dyes like Indocyanine Green (ICG) is another widely used photothermal agent having gain the FDA approval as a contrast agent and thus offers quicker access to clinical trials.^[48] Being a small molecule, this agent is usually encapsulated in some form for protection and better retention. A unique encapsulation method reported by Yan and coworkers required only facile mixing of ICG and OVA, omitting the need of harsh reaction conditions. OVA itself is also the source of the immunoadjuvant and activates the specific immunity as proposed in Figure 4a.^[29c] This approach achieved an 80.8% antigen loading efficiency and 19.2% ICG content. Interestingly, integrating ICG into OVA does not alter the photothermal conversion efficiency of ICG, providing a viable vehicle for delivering of ICG (Figure 4b). The OVA-ICG nanovaccine was evaluated *in-vitro* with immature DC 2.4 cells and resulted in elevated secretions of IL-6 and TNF- α , among other biomarkers indicating positive immunostimulation (Figure 4c). This nanovaccine was used both therapeutically and prophylactically. In the therapeutic model, intratumoral injection followed by 808 nm laser impingement onto the B16 tumor region exhibited complete tumor growth suppression among other control treatments. The authors then further tested the vaccine in a prophylactic model. Initial immunization of the OVA-ICG nanovaccine twice before B16 melanoma cells transplantation was thought to allow recognition of melanoma-specific antigen OVA by APC cells in the subcutaneous tissues, generating immunologic memory. Subsequently, when B16 tumor cells were reintroduced, it led to impressive tumor prevention ability in the mice compared to the phosphate buffered saline (PBS) control group (Figure 4d and 4e), suggesting the combined modality's potential as a nanovaccine.

Figure 4. (a) A scheme illustrating the construct of OVA-ICG nanovaccine and the main pathway leading to specific tumor immunity. (b) Temperature increase profiles of 808 nm irradiation on different solutions. (c) Amount of TNF- α secreted by DC 2.4 cells after various treatments. (d) Experimental design for prophylactic vaccination. (e) Images of tumor sizes of prophylactic vaccination against PBS control. Reproduced with permission.^[29c] Copyright 2018, John Wiley and Sons.

2.2. PTT-synergized Immune Checkpoint Blockade Therapy

Though immunoadjuvants are an effective tool to elicit an immune response with photothermal assistance, an alternative strategy encompassed the manipulation of immune checkpoints, mainly by inhibition with a specific antibody. One study which targeted the PD-L1 receptor that is frequently overexpressed in many cancer cells was performed by Vo-Dinh and coworkers. In their experimental setup, C57BL/6 mice implanted with MB49 bladder cancer cells on both flanks were injected intravenously with gold nanostars, then irradiated with an 808 nm laser one day later. Following irradiation, anti-PD-L1 antibody was injected

intraperitoneally to only the right flank. This two-pronged modality was referred to as synergistic immuno photothermal nanotherapy or SYMPHONY which resulted in survival rates of 40% (regression of right and left flank tumors) after 49 days compared to 0% for the control or anti-PD-L1 antibody monotherapy.^[33b] In another study, Li et al. prepared a hydrogel (termed as FK@IQ-4T1) incorporating of a tumor penetrable peptide and used it as a personalized cancer vaccine (PVAX) for postsurgical immunotherapy.^[49] This hydrogel acted as a “vessel” to encapsulate fixed tumor cells that had been co-loaded with ICG and JQ1 (a BRD4 small molecule inhibitor suppressing PD-L1 expression). NIR laser irradiation triggered the release of antigen and JQ1, leading to the activation of PVAX, which promoted the maturation of DCs and elicited tumor infiltration of cytotoxic T cells. The intratumoral injection of FK@IQ-4T1 successfully achieved a complete regression of implanted distant tumors over a 59-day experimental period and inducing immunologic memory post 30 days after the last vaccination, clearly highlighting its potential to prevent tumor recurrence and metastasis. This positive result was attributed to the increase in matured DCs (~62% higher) and elevated levels of proinflammatory cytokines in the serum.^[49]

Figure 5. (a) Structure of PEGylated SWNTs. (b) Temperature increase curves in tumors at different NIR irradiation intervals. (c) Graphic representation on the mechanism of hypothesized anti-tumor immune response. (d) Ratio of tumor-infiltrating CD4⁺ and CD8⁺ cells over Treg cells. (e) Lung photographs depicting the metastatic sites. **Reproduced with permission.**^[32] Copyright 2014, John Wiley and Sons.

Aside from PD-L1 immune checkpoint, antibody binding against CTLA4 receptor has also been exploited in photothermal based cancer immunotherapy.^[32, 46] Combining anti-CTLA4 antibody with PTT enables the mounting of a stronger immune response by downregulating the level of Treg cells in the tumor draining lymph node.^[50] In a study conducted by Liu et al, SWNCTs grafted with a layer of amphiphilic polyethylene glycol (PEG) was employed as the photothermal agent (Figure 5a). PEGylation endowed excellent

stability of SWNCTs in various physiological solutions and effected a temperature change close to 53 °C when subjected to 808 nm NIR laser irradiation (Figure 5b). As depicted in Figure 5c, upon thermal ablation by PEGlyated SWNCTs, tumor-associated antigens are released along with danger signals such as HSPs and inflammatory cytokines to induce dendritic cells maturation, eventually recruiting tumor-specific CD8⁺ T cells.^[32] Concurrently, anti-CTLA4 blockade therapy effectively reduces the immune suppressive activity of Treg cells, increasing the ratios of CD4⁺ and CD8⁺ cells to Treg cells (Figure 5d). Notably, the high ratio against Treg cells is only observed when anti-CTLA4 is combined with PTT and not surgery, clearly deducing the important immunological role played by PTT. Lastly, CD20⁺ tumor-infiltrating B cells may also assist in processing and presentation of tumor-specific antigens.^[51] Taken together, this enabled adaptive immunity to occur further downstream. As expected, the combined treatment markedly improved tumor regression. The researchers further simulated lung metastasis by intravenous injection of 4T1 cells into the mice one day before treatment on the primary tumor. Impressively, 57% of the mice survived 50 days post combination therapy with PTT in contrast to 25% that received surgery instead. This was also evidenced by the far fewer metastatic sites in the photograph shown in Figure 5e.

Inspired by the combinatorial treatment's ability to inhibit cancer metastasis, Liu et al. attempted to create nanovaccine properties with this two-pronged treatment modality with the proposed immunostimulatory pathway as described in Figure 6a and the protocol overview in Figure 6b. In their study, they developed a poly(lactic-co-glycolic acid) (PLGA)-based nanoparticle encapsulating ICG and the immunoadjuvant, imiquimod (R837), a Toll-like-receptor-7 agonist. Formation of the nanoparticle did not alter the absorbance characteristics of ICG while the sustained release of its contents resulted in increased DCs maturation and elevated levels of proinflammatory cytokines (Figure 6c and 6d). It is noteworthy to point out

that the cytokine secretions are not only higher but also lasted longer which could be an important factor to trigger antitumor responses. A series of systematic experiments were then performed in two cancer mice models (4T1 and CT26) harboring tumors on both flanks. Remarkably, upon administration of the various treatment combinations to eliminate the first tumor, the researchers achieved a complete remission of the second tumor in the CT26 mouse model and almost full remission in the 4T1 model, evidently proving its clinical utility. The high ratio of CD8⁺ and CD4⁺ over Treg cells could also likely be responsible for the cell mediated antitumor effect. In another set of experiments, the mice were rechallenged with a second round of tumor inoculation 40 days after removal of the first tumor (Figure 6b). Unsurprisingly, the mice attained systemic immune memory as evidenced by the inhibited growth of the secondary tumor and a higher percentage of effector memory T cells (CD3⁺ CD8⁺ CD62L-CD44⁺) when treated with the PLGA-R837-ICG nanoparticles compared to the other control groups (Figure 6e).^[37] Furthermore, this combination when delivered systemically rather than intratumorally still retained its excellent anticancer activity, allowing clinical flexibility in treatment delivery.

Figure 6. (a) Schematic illustration of the pathway by PLGA-ICG-R837-based PTT and anti-CTLA4 combination therapy in eliciting tumor-specific long-term immunity. (b) Overview of the tumor inoculation and photothermal-immunotherapy procedures. (c) Absorbance characteristics of free and encapsulated ICG. (d) Levels of IL-12p70 at various time points. (e) Percentage of effector memory T cells upon rechallenge at Day 40. **Reproduced with permission.**^[37] Copyright 2016, Nature Publishing Group.

2.3. Considerations in Photothermic Temperatures

The release of DAMPs,^[52] in particular, various forms of heat shock proteins, promote immunostimulatory effects and has increasingly been shown to occur at temperatures ranging from 39 to 45 °C.^[31b, 53] One mechanism for the elevated levels of HSPs was proposed by

Fiering et al where they believed the milder heat causes partial cell damage and increased HSPs production as part of the cell physiological response but when subjected to higher temperatures, rapid necrosis would hamper this process.^[53b] Besides chemical signals, the modest rise in temperature encourages more interstitial blood and lymphatic circulation, facilitating migrations of APCs and T cells.^[54] As a result, in contrast to direct cytotoxic approach, achieving immunostimulation by PTT requires careful and controlled heating. Consequently, the thermal dose (a function of temperature and response time) plays a crucial role in acquiring the desired level of immunostimulation.^[53a] However, understanding of thermal dose in cancer immunotherapy is still poorly understood as research into this area is still in its infancy.^[53a] Although studies on photothermal immunotherapy have demonstrated that controlled heating to no more than 45 °C is an important parameter, there are numerous reports from many groups reporting antitumor immunity at temperatures beyond 45 °C. For instance, Gong et al used conventional PTT (up to 50 °C) involving a fluorophore-loaded liposome containing IR-7 as the photothermal agent and an immunoadjuvant, unmethylated CpG motif.^[29b] The outcome of the treatment on a CT26 colon cancer mouse model demonstrated tumor reduction at the primary site (right flank) and also distant site (left flank).^[29b] Notably, Lu et al also observed success in eliciting CD8⁺ cytotoxic T lymphocytes response with higher levels of IFN- γ and IL-2 in the spleen.^[18] The treatment was able to reduce the primary tumor and distant tumors as well in a BALB/c mice bearing the EMT6 tumor.^[18] Using the same BALB/c EMT6 tumor bearing mouse, another group by Xing et al achieved similar success with hyperthermic temperature greater than 50 °C with an immunologic photothermal agent consisting of SWNCTs and GC as a surfactant mixture.^[31c] In another cancer mouse model challenged with B16 melanoma cells, Yan et al investigated OVA as a nanovehicle to transport antigens and ICG to the tumor region.^[29c] The authors applied hypothermic temperature of 60 °C and reported success in treating the mice not only

against primary tumors but also prophylactically when challenged with tumor cells after a nine-day immunization procedure.^[29c] More research needs to be conducted to extensively understand the exact principles behind photothermal assisted immunostimulation.

3. PDT Cancer Immunotherapy

Apart from PTT, PDT has also been shown to trigger antitumor immune response as well. The mechanism is largely similar to PTT whereby generation of DAMPs stimulate the immune system. Currently, ablating primary tumor by current standards of treatments including chemotherapy, surgery and radiotherapy could not stimulate sufficient host's immunity response. Consequently, tumor recurrence and even metastasis would be a common and challenging problem for doctors. With the production of immunoadjuvants (arising from PDT) to gather the host's innate and adaptive immune capability, higher successes have been achieved by many groups in tumor ablation and reducing recurrence rates. Tumor-specific antigens and DAMPs produced in the remnants of the ablated tumor are internalized by DCs allowing their maturation and cross presentation to T cells, establishing the start of immunological memory against the tumor and various groups have succeeded in this attempt.^[55] Fong et al. managed to eradicate 70% of the tumors in a group of CT26 BALB/c mice model and reported long term immunity of over a year by BAM-SiPc-mediated vascular PDT.^[55a] Yang et al. created a prophylactic vaccine tumor lysate by DTPP-mediated PDT *in vitro* and observed marked increase in CD4⁺/CD8⁺ cell ratios, NK cell percentages, IFN- γ and IL-1 levels in serum and lymphocyte congregation at the tumor peripheral.^[55b] Marek et al. designed an affibody (Z_{EGFR:03115}) conjugated to the phthalocyanine dye, IR700DX for targeting against glioblastomas tumor cells in the brain and successfully inhibited tumor growth compared to control groups.^[55c] Korbelyik and Merchant's study on a squamous cell carcinoma SCCVII mouse model indicated not only higher levels of HSP 70 production but also glucocorticoids hormone that promoted macrophage stimulation.^[55d] Several other

groups have also stated that PDT could be used as an immunoadjuvant to enhance serum cytokines, increase ratio of tumor infiltrating immune cells and mice acquiring long term adaptive immunity.^[55e-g]

Currently, PDT is an option of treatment for some types of cancer.^[56] The improvements in second and third generation photosensitizers^[57] along with reported successes have allowed PDT to gain recognition as a viable strategy for cancer therapy^[56] and more clinical trials are underway in exploring the feasibility of PDT in cancer.^[58] PDT was first developed as a prophylactic vaccine by Gollnick and Henderson in 2002^[59] while Korbelik and Cecic based their approach as a therapeutic vaccine.^[60] In contrast to standard PDT treatment where the photosensitizer is injected intravenously and NIR light is shone onto the tumor area *in situ* after a fixed incubation period, a fraction of the resected tumor is subjected to PDT *ex vivo* to produce the tumor lysate for therapeutic vaccination.^[6a, 61] The treated lysate would then be administered either to the same patient (autologous) or to another individual (allogenic).^[6a] This form of vaccine was utilised by Liu et al in their investigation of a graphene oxide (GO) based PDT. A schematic diagram of the photodynamic agent is represented in Figure 7a. Basically, GO coated with photochlor(2-[1-hexyloxyethyl]-2-devinyl pyropheophorbide- α (HPPH) photosensitizer is conjugated to a tumor targeting HK peptide. The peptide has a high binding affinity to integrin $\alpha\beta 6$ which is a common receptor expressed in a variety of tumors. Initial experiments confirmed increasing singlet oxygen generation at increasing concentrations and that conjugation to the HK peptide greatly improved tumor uptake, suggesting its suitability as a PDT agent (Figure 7b and 7c). The GO(HPPH)-PEG-HK PDT agent successfully induced higher levels of DCs maturation as observed by the marked increase of CD40 and CD70 surface expression in the tumor draining lymph nodes. In addition, cytokine level of IFN- γ and the ratio of CD8⁺ versus CD4⁺ cells were also increased, implying the activation of antitumor immunity (Figure 7d). In the later part of their study, the

PDT agent was then applied *ex vivo* to a resected portion of the 4T1 tumor, generating necrotic 4T1 tumor cells where it was administered intravenously back into the mice. Interestingly, the activation of DCs was only significantly increased by GO(HPPH)-PEG-HK induced necrosis and not by other chemotherapeutic drugs 5-fluorouracil and docetaxel (Figure 7e). To investigate how this therapy would inhibit lung metastasis and its ability to trigger an immune response based on activation by immunological memory, tumor inoculation was carried out 20 days after the first vaccination. Aside to near-infrared fluorescence imaging, the authors also utilized single-photon emission computed tomography (SPECT)/CT imaging as an orthogonal technique to confirm the infiltration of CD8⁺ T cells (Figure 7f). Inspiringly, the number of detected metastatic lesions was far lesser than the control as seen in Figure 7g. This work represented an example of an effective strategy to completely eliminate residual tumor cells, target distant metastatic sites and prevent future recurrence.

Figure 7. (a) Schematic representation of the GO(HPPH)-PEG-HK nanoparticle. (b) Assessment of singlet oxygen levels. (c) 4T1 tumor fluorescence intensity at different time points. (d) Difference in levels of CD4^+ and CD8^+ cells in control and PDT treated mice. (e) Percentage levels of mature DCs by different treatments. (f) SPECT/CT imaging depicting the level of infiltration of CD8^+ T cells in tumor (dashed circle). (g) Photograph of metastatic sites in the lungs. Reproduced with permission.^[62] Copyright 2017, American Chemical Society.

Though Liu et al accomplished activating host immunity solely by PDT, various other groups have reported synergism in combinatorial approaches using PDT and at least one other immunotherapeutic tool, similar to PTT. The following section attempts to summarize some of the methods and combinatorial strategies explored by different groups in achieving

stronger and longer lasting immunotherapeutic effects for the purpose of anticancer immunotherapy.

3.1. PDT-synergized Immunoadjuvant Therapy

Calreticulin, a Ca^{2+} binding protein is a well-known DAMP generated in pre-apoptotic cells.^[63] Normally located within the lumen of the endoplasmic reticulum, it gets translocated to the cell surface during an apoptotic event and serves as a phagocytic signal.^[64] This immunogenic property enabled its replacement of previously discussed immunoadjuvants such as OVA and CpG.^[65] For instance, Korbelik et al established calreticulin as an effective immunoadjuvant augmenting the antitumor effect of PDT. However, this effect was not observable in immune-deficient NOD-SCID mice, providing an indirect confirmation.^[65a] Zou et al also demonstrated calreticulin's role as an immunoadjuvant and its ability to work synergistically with tumor associated antigens.^[65b] Synthetic long peptides (SLP) have been found to exert an immunotherapeutic effect and are currently being developed as cancer vaccine for some types of cancer.^[66] In a study by Ossendorp et al, combining SLP with PDT enhanced CD8^{+} T cell response to the tumor and effectively eradicated distant secondary tumor in their tumor mouse model.^[67] Their study discovered PDT assisted SLP vaccination cured over 30% of the primary and distant tumors compared to around 20% for SLP vaccination alone and 0% for PDT alone and control groups, indicating systemic immunity in the mice was achieved.^[67] Besides the uncommon immunoadjuvants, more mainstream ones like OVA have also successfully improved the antitumor immune response and have been reported by various groups.^[55g, 68]

3.2. PDT-synergized Immune Checkpoint Blockade Therapy

Li et al has shown that PDT-induced cancer immunotherapy can be further enhanced by the silencing of RNAi-mediated PD-L1.^[69] In this paper, the authors proposed an acid-

activatable multifunctional micelleplex nanoplatfrom. The micelleplexes (POP micelle) were comprised of three components and upon contact with an acidic microenvironment of pH 6.2, will disintegrate to release its contents which are siRNA specific to PD-L1 and the active PDT sensitizer, Pheophorbide a (PPa) as illustrated in Figure 8a. Subsequent irradiation at 671 nm with pH 6.0 condition generated ROS 5.6-fold more efficient than at pH 7.4 and this capability was verified by its hemolytic ability under acidic conditions (Figure 8b). The POP micelles are thought to induce adaptive immune response through HSP70 and NF- κ B expression, while also triggering proinflammatory cytokines secretion and recruited proliferating, tumor-infiltrating CD8⁺ T cells (Figure 8c). While applying PDT or PD-L1 siRNA silencing alone had inhibited approximately 73% and 65% of the respective tumor growth, combining the two modalities achieved complete tumor elimination (Figure 8d). Moreover, the combined treatment had notably increased the tumor-free mouse survival percentage to 75% in a period of 26 days, far superior to PDT alone at 25%. In addition, the number of metastatic lesions were significantly reduced compared to PDT-treatment or PD-L1-silenced tumor cells (Figure 8e).^[69] The utilization of PD-L1-blockade siRNA was also recently reported by Cai et al in which they adopted a similar releasing mechanism relying on the acidic tumor microenvironment to disassemble the micelleplex.^[70] The conclusion from their findings affirmed similar antitumor response improvement, reducing the levels of Treg cells while substantially elevating the proportion of tumor-infiltrating CD8⁺ T cells. Furthermore, higher percentages of effector memory T cells were measured during rechallenge of the tumor, suggesting long lasting anticancer immunity.^[70] Clearly, this highlighted the important role played by PDT in ensuring the capability of PD-L1 gene silencing in cancer immunotherapy.

Figure 8. (a) Illustration of acid-activatable micelleplexes loaded with siRNA targeting PD-L1 utilizing PDT-induced cancer immunotherapy pathway. (b) Erythrocytes lysis capability at different pH. (c) Percentage levels of Ki67-positive CD8⁺ T cells. (d) Tumor volume changes of mice given different treatments. (e) Quantitative comparison of lung metastatic

sites by different treatments. Reproduced with permission.^[69] Copyright 2016, American Chemical Society.

Another strategy to target PD-L1 is through binding with an anti-PD-L1 antibody. Lin et al designed a multimodal nanoscale coordination nanopolymer (NCP) loaded with oxaliplatin and the photosensitizer pyrolipid (NCP@pyrolipid) to augment the antitumor efficacy mediated by anti-PD-L1 immunotherapy (Figure 9a).^[71] The approach combined chemotherapy and PDT to ablate tumors and provoke an immune response by inducing ICD that causes exposure of calreticulin to membrane surfaces of pre-apoptotic cells where it serves as an “eat me” signal, promoting migration of dendritic cells (Figure 9b). Besides calreticulin, mice injected with NCP@pyrolipid exhibited higher levels of proinflammatory cytokines TNF- α , IL-6 and IFN- γ in their sera and significantly slowed tumor growth in two different tumor mice models. With the inclusion of anti-PD-L1 therapy, the abscopal effect studied pointed to both the primary and distant tumor growths having been successfully halted (Figure 9c). The general principle behind this enhancement effect was alluded to the increased infiltration of CD8⁺ T cells (Figure 9d) and that the infiltrated CD8⁺ T cells consist of a greater proportion of antigen-specific IFN- γ producing T cells (Figure 9e). As hypothesized by the authors, anti-PD-L1 checkpoint blockade therapy is an excellent tool to target tumors but only if the microenvironment is immunogenic enough. To increase the immunogenicity, the combination of chemotherapy by oxaliplatin and PDT by pyrolipid provided an efficient solution that complemented well with anti-PD-L1 therapy.

Figure 9. (a) Representation of the NCP@pyrolipid nanoparticle. (b) Schematic illustrating the antitumor immunity from combined treatment with PDT, chemotherapy and anti-PD-L1 immunotherapy. (c) Growth curves for primary and distant tumors of a MC38 tumor-bearing mouse model. (d) Immunofluorescence staining image of infiltrated CD8 T cells (shown in red). Scale bar, 50 μm . (e) ELISpot analysis of tumor antigen-specific T cells in the spleen. Reproduced with permission.^[71] Copyright 2016, Nature Publishing Group.

In another similar study by Lin et al, PDT in conjunction with anti-PD-L1 antibody therapy not only successfully eradicated 4T1 primary breast tumor but also significantly inhibited metastasis in the lungs.^[72] This combination treatment has also been reported by another group to stimulate recruitment of CD8⁺ T cells, suppress the growth of a secondary tumor and decreased the diffusion of 4T1 lung metastasis.^[73] The implementation of PD-L1 therapy with PDT continues to be a popular strategy for “educating” the immune system probably owing to the widespread expression of PD-L1 as found in many types of cancers.^{[14,}

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The other well-known checkpoint frequently exploited is CTLA4 expressed in Treg cells. Inhibiting this receptor with an anti-CTLA4 antibody has been shown to restore immunity as

an anti-cancer therapy.^[11] The combination of anti-CTLA4 with PDT has been hypothesized by some groups to gain even greater therapeutic effects.^[75] Liu et al designed an upconversion nanoparticle (UCNP) based on 20% Yb and 2% Er-doped NaYF₄ nanoparticles coated with PEG and loaded with the photosensitizer Chlorin e6 (Ce6) and an adjuvant, R837 for targeting tumors in deeper tissues (Figure 10a). Compared to free Ce6, the presence of UCNP resulted in more efficient singlet oxygen generation (Figure 10b). The UCNP-Ce6-R837 nanoparticle would theoretically effect the release of tumor-associated antigens, which gets amplified by R837, then activates the immune system by DCs maturation and promoted secretion of cytokines IL-12p40, IFN- γ and TNF- α in the sera of CT26 tumor mice model (Figure 10c). The highlight of their study combined PDT utilizing UCNP-Ce6-R837 and anti-CTLA4 antibody injection. The combination yielded certain immunotherapeutic effects such as the increased ratio of CD8⁺ to Treg cells in distant tumors, release of proinflammatory cytokines, IFN- γ and TNF- α in the sera of mice and a greater proportion of effector memory T cells in the spleen compared to respective individual treatments, indicating successful synergism (Figure 10d and 10e). Remarkably, the combined therapy fully inhibited the growth of secondary tumors and it is interesting to note that pre-injection of anti-CTLA4 prior to secondary tumor inoculation alone is sufficient to induce excellent long-term immune memory protection effect in the mice (Figure 10f).^[75a]

Figure 10. (a) Fabrication design of UCNP-Ce6-R837 nanoparticles. (b) SOSG evaluation of ROS generation. (c) Overview and mechanism of PDT and anti-CTLA4 combined treatment (d) Ratio of CD8⁺ cells to Treg cells in distant tumor on day 15 of various treatments. (e) Percentage of effector memory CD8⁺ T cells in spleen (left) and levels of IFN-γ (right) in the sera of mice that undergone different treatments. (f) Tumor volume curves of mice treated with different conditions. Reproduced with permission.^[75a] Copyright 2017, American Chemical Society.

Immune suppression by IDO enzyme has rapidly gained recognition as another promising alternative pathway to focus for augmenting the immune system.^[8b, 15b] By employing an IDO inhibitor, 1-methyltryptophan (1MT)^[76] linked to a caspase-responsive peptide connected to the photosensitizer PpIX *via* a PEG segment and palmitic acid as

described in Figure 11a, Zhang et al reported remarkable CT26 primary tumor growth regression in mice treated with intravenous injections of the PpIX-1MT nanoparticles. The designed nanoparticles were able to generate increasing amounts of ROS with time, overcoming the issue of self-quenching caused by aggregation (Figure 11b) while caspase-3 cleavage sustained a steady release of 1MT over 50 h to approximately 83% (Figure 11c). PDT is known to evoke ICD and a common cellular response is the translocation of calreticulin to the cell surface. This was detected by flow cytometry as displayed in Figure 11d. Along with calreticulin exposure, immune-associated factors analyzed with western blot also indicated activation of the immune system during the PDT process (Figure 11e) while flow cytometry revealed a greater ratio of CD8⁺ T cells against CD4⁺ T cells in the sera and spleen. These data verified that targeting IDO in combination with PDT is a viable strategy to activate the immune response.^[77] The group further tested their PpIX-1MT nanoparticles in a CT26 metastatic mouse model. Compared to the other treatments, mice given PpIX-1MT did not show any luminesce, indicating absence of tumor sites (Figure 11f) while treatment of the primary tumor was also highly successful (Figure 11g). Combining PDT and IDO inhibition have also improved the eradication of CT26 and MC38 treated primary tumors as well as secondary distant tumors as published by Lin et al.^[78]

Figure 11. (a) Schematic showing the molecular structure and micellar assembly of the chimeric peptide PpIX-1MT along with its mechanism of action inside tumor cells. (b) Quantitative analysis of *in vitro* ROS generation. (c) Kinetic release profile of 1MT. (d) Fluorescence-activated cell sorting analysis of **calreticulin** exposure on CT26 cell surface. (e) Western blot analysis of various cytokines in the tumor and lung. (f) Analysis of lung metastasis sites in mice upon various therapies (red arrow represent an individual tumor node). (g) Respective tumor growth curves of different groups. **Reproduced with permission.**^[77] Copyright 2018, American Chemical Society.

3.3. Other PDT combinatorial approaches

Acid ceramidase is an important enzyme regulating the levels of ceramide and sphingosine and plays a crucial role in cell apoptosis.^[79] Overexpression of acid ceramidase has been found in some cancers cells, conferring apoptotic resistance on them.^[80] Hence, developing acid ceramidase inhibitors such as LCL521 have been studied as a possible immunosuppressive agent.^[81] The use of LCL521 in combination with PDT studied by Korbelik et al suppressed Treg cells and myeloid derived suppressor cells (MDSCs) populations in the tumor draining lymph node compared to standard PDT treatment as analyzed by flow cytometry.^[82] The suppression however, was not seen in spleen and this critical observation suggests that application of LCL521 allows PDT to realize its full immunostimulatory potential in recruitment of macrophages and CD8⁺ cytotoxic T cells.^[82] It is well known that Treg cells strongly dampen the immune activation which make them a significant target in cancer immunotherapy.^[10] In addition to the blocking CTLA4 receptors as abovementioned, another strategy has been developed to directly and specifically deplete the local Treg cells by taking advantage of the targeting specificity of antibodies. For example, conjugating Ce6 photosensitizer to anti-CD25 antibody allowed targeted PDT against Treg cells while minimally affecting neighboring cell populations or tissues.^[83] In this study, an intratumoral injection of the Ce6-anti-CD25 resulted in localized depletion of Treg cells and consequently, improved the infiltration of CD8⁺ T cells. The authors further examined the level of the infiltrated CD8⁺ T cells by measurement of its cytokines secretion especially IFN- γ , and discovered a five-fold higher level of IFN- γ for mice with depleted Treg cells.^[83] Kobayashi et al also reported success in Treg immunodepletion with only IR700 conjugated F(ab)₂ fragments generated from an anti-CD25 antibody.^[84] Working along similar cell immunodepletion mechanism, the use of anti-GR1 antibody to target another population of immunosuppressing cells, namely, MDSCs cells provides another avenue to inhibit negative

immunoregulatory effects that restricts the strength and duration of antitumor immune response.^[85]

4. Summary and Future Outlook

Cancer immunotherapy, particularly anti-cancer vaccines and immune checkpoint blockade may have shown some clinical efficacy as a treatment by itself.^[1b, 2b] However, limitations exist including but not limited to eradication of primary tumors, inconsistent results between different patients and difficulty in identifying relevant biomarkers. Various combination therapies became a popular choice to overcome the shortfalls and have shown promise to improve the treatment outcome.^[1d] One popular modality is phototherapy that has emerged as an excellent tool to complement immunotherapies for a more efficacious cancer treatment regime. This review described the recent progresses in utilizing PTT and PDT in combination with immunotherapies such as immunoadjuvants and/or immune checkpoint blockade *via* administration of either antibodies or gene silencing aimed at inhibiting and disrupting these immune checkpoint pathways. Currently, CTLA4 and PD-1/PD-L1 pathways along with inhibiting IDO enzymes in combination with phototherapy have demonstrated therapeutic synergism for various tumor mice models. The inclusion of phototherapy has strongly enhanced prophylactic vaccination, clearing of secondary or residual tumors, reducing the occurrence metastasis and last but not least, induction of long-term immunologic memory. **In addition**, direct suppression and deletion of Treg cells **using phototherapy** are mentioned as another alternative **methods to promote immunotherapy**. Many studies as discussed in this review have affirmed this synergism and some of the important ones are summarized in the table below (Table 1). **Noted that the treatment timeframe in most of the current studies were quite short (< 30 days in mice), which should be due to the fact that the tumor volumes in the control mice without treatments were too large and the mice could not survive longer. Therefore, monitoring the long-term survival rates and tumor recurrence in the**

treated mice have also be performed, confirming the successes in achieving long-term and systemic treatment efficacies of cancer photoimmunotherapy. While, because of the reduced effectiveness of immunologic memory, the long-term treatment efficacies for rechallenged tumors remain to be questionable.

Table 1. Summary of studies utilizing phototherapy and immunotherapy combinatorial treatments

Photo-therapy	Phototherapy Agent	Immunother-apy	Immunotherapy Agent	Cancer Model	Therapeutic Outcome	Ref
PTT	Gold	PD-L1 Blockade	Anti-PD-L1 antibody	MB49	Delayed and inhibited tumor growth	[33b]
PTT	Copper sulfide, melanin, ICG, IR7, carbon nanotubes.	Immuno-adjutant	CpG, OVA, GC, GM-CSF	B16, 4T1, EG7-OVA, EMT6	Inhibited tumor growth to almost complete tumor elimination	[18, 31b] [29c] [29b] [31c]
PTT	Carbon nanotubes	CTLA4 blockade	Anti-CTLA4 antibody	CT26	Inhibited tumor growth	[32]
PTT	ICG	CTLA4 blockade and Immunoadj-uvant	Anti-CTLA4 antibody and R837	4T1, CT26	Almost complete inhibition	[37]
PDT	HPPH	None	Not Applicable	4T1	Inhibited tumor growth	[62]
PDT	Bremachlorin, ICG	Immuno-adjutant	SLPs, OVA, CpG	TC1 RMA, EG7-OVA	Inhibited tumor outgrowth	[55g] [67] [57]
PDT	PPa, MTPP	PD-L1 blockade	PD-L1 gene silencing	B16F10	Complete elimination	[69]
PDT	Pyrolipid, IRDye700, TBP	PD-L1 blockade	Anti-PD-L1 antibody	MC38, CT26, 4T1	Inhibited tumor growth to complete elimination	[71] [72] [73]
PDT	Bremachlorin	Anti-CTLA4 blockade	Anti-CTLA4 antibody	MC38, CT26	Significant to almost complete elimination	[75b]
PDT	Ce6	Anti-CTLA4 blockade and immuno adjuvant	Anti-CTLA4 and R837	CT26	Complete elimination	[75a]
PDT	PpIX, H ₄ TBC	IDO inhibition	1MT	CT26, MC38	Almost complete elimination	[77] [78]
PDT	Ce6, Temoporfin	Treg cell depletion	LCL521, Ce6 conjugated anti-CD25 antibody	SCCVII, B16F10	Inhibited tumor growth	[82] [83]

PDT	Temoporfin	Mreg cell depletion	Anti-GR1 antibody	SCCVII	Inhibited tumor growth	[85]
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Table 1: Summary of studies utilizing phototherapy and immunotherapy combinatorial treatments

However, with all the benefits discussed, there are still some challenges in phototherapy that limits its full synergistic potential with cancer immunotherapy. One major challenge is the limited penetration depth of NIR light for deep seated tumors such as tumors in breast.^[38a, 86] As a result, phototherapy is mostly limited to surface tissues where PDT is sometimes offered as a choice of treatment for certain skin carcinoma. Unless major advancement progresses in this area, phototherapy may find it difficult to penetrate into clinical studies. Currently, a few emerging options could provide leverage to offset this constrain. Although largely impermeable, it is known that a small percentage of NIR light could still infiltrate into deeper tissues.^[87] Logically, this led to many groups improvising ways to increase the thermal efficiency and stability of existing PTT agents.^[88] For example, Moon et al designed an extra coating of polydopamine over gold nanoparticles, endowing it with sustained absorption capacity.^[88a] Their work also opened new avenues to the possibility of triggering immunotherapeutic effects by combination with sub-therapeutic doses of anticancer drugs. More research into this domain will certainly expand the current library of phototherapy agents, potentially identifying a “blockbuster” agent. Modification to deliver the light source could also be improvised, and this was shown by Kobayashi et al. where they combined external and interstitial NIR light exposure. This successfully improved its efficacy as compared to a sole external NIR light source.^[89] Alternatively, we and others have investigated the possibility of improving penetration depth through photothermal agents absorbing NIR-II window (1000–1700 nm).^[90] It is also worth mentioning that although heavy metal nanoparticles are typically better agents, caution should be exercised if selecting them as they are known to accumulate long term in tissues and questions still remain on their

safety and toxicity profiles.^[91] Increasing the accumulation in tumor tissue or residual tumor cells by improving the targeting ability of phototherapy agents through the use of antibody drug conjugates should be considered as well. Lastly, in PDT, oxygen availability is another key challenge to generate sufficient ROS for ablating tumor cells. An innovative method to overcome hypoxia by Lin et al made use of a metal-organic framework that is able to catalyze a series of reactions whereby intracellular H_2O_2 is eventually converted to ROS.^[73b, 78] Recently, we have also developed hybrid core-shell semiconducting nanoparticles featuring MnO_2 sheets that promotes O_2 evolution in hypoxic solid tumors.^[92]

Combining immune checkpoint immunotherapy with the assistance of phototherapy could greatly improve the outcome of cancer prognosis as described in this review. What remains to be seen is the discovery of immune checkpoints offering better control and efficacy to immunosuppression. One potential immune checkpoint is the targeting of CD47, an integrin-associated receptor protein expressed at high levels on the surface of various cancer cells. Binding of CD47 with anti-CD47 disrupts the interaction with SIRP-alpha on macrophages and dendritic cells, reversing the “don’t eat me” signal and thus increasing antitumor activity.^[93] An interesting alternative is the utilization of non-immunotherapeutic methods to trigger an immunotherapeutic response as reported by Moon and coworkers.^[88a] Aside from exploiting immunotherapeutic tools, it will be interesting to note the incorporation of other phototherapeutic strategies^[47h, 94] that could work in conjunction with immunotherapy, offering the possibility of a complete recovery.

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The table of contents

Phototherapy is a promising modality that offers an elegant solution to eradicate tumors through the simple application of light irradiation and has emerged as an excellent tool to complement immunotherapies for a more efficacious, synergized cancer treatment regime. This review focuses on the recent development of advanced approaches combining phototherapy with immunoadjuvants and immune checkpoint blockade for cancer therapy.

Keyword: Phototherapy; photothermal; photodynamic; cancer immunotherapy; immune checkpoint blockade; immunoadjuvant

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Recent Progresses in Phototherapy-synergized Cancer Immunotherapy

ToC figure

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