Review

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Phototherapy meets immunotherapy: a win–win strategy to fight against cancer

<https://doi.org/10.1515/nanoph-2021-0209>

Received May 1, 2021; accepted June 30, 2021;

published online July 13, 2021

Abstract: Phototherapy usually includes photodynamic therapy (PDT) and photothermal therapy (PTT) to induce cell death. PDT utilizes the sensitization of the photosensitizers to generate reactive oxygen species by the intersystem crossing while PTT undergoes nonradiative decay to generate heat. Cancer immunotherapy has evolved as a new therapeutic modality to eradicate tumor cells by activating antigen-presenting cells, and thus, inducing innate or adaptive immune responses. Phototherapy is able to stimulate the immune system, usually by inducing immunogenic cell death (ICD). Photoimmunotherapy (PIT) is an oncological treatment that combines the phototherapy of the tumor with immunotherapy treatment. Combining phototherapy with immunotherapy enhances the immunostimulating response and has synergistic effects for metastatic cancer treatment. PIT is able to enhance the antitumor immune response by ICD and prevent tumor metastases and recurrence. In this review article, we would like to summarize the recent advances in the development of phototherapy (such as PDT, PTT, and synergistic PDT/PTT) triggered immunotherapy for cancer treatment. In

addition, immunotherapy triggered by phototherapy and other therapeutic modalities will be discussed. PIT may be a win-win strategy to fight against cancer.

Keywords: immunotherapy; photodynamic therapy; photosensitizer; photothermal therapy.

1 Introduction

Cancer remains one of the most common diseases and is responsible for the increasing cases of death every year [1]. The development of nanomedicine has proven to be an effective strategy for cancer therapy, holding great promise for enhancing the therapeutic efficacy as well as reducing side effects [2–4]. Phototherapy, including photodynamic therapy (PDT) and photothermal therapy (PTT), usually utilizes phototherapeutic agents to selectively kill cancer cells under appropriate light irradiation [5–8]. It is considered as a noninvasive therapeutic technique with good selectivity and nondrug resistance, compared with the traditional therapies, such as chemotherapy and radiation therapy [9–13]. Photosensitizers play a fundamental role in the photosensitization process. During PDT, photosensitizers will undergo intersystem crossing (ISC) to generate reactive oxygen species (ROS). There are two general ways for the photosensitization process, one is the electron/hydrogen transfer (type I process), during which superoxide and hydroxyl radicals will be generated. The other one is the energy transfer process (type II process) for the direct sensitization of nontoxic triplet oxygen ($^3\text{O}_2$) to cytotoxic singlet oxygen ($^1\text{O}_2$) (Figure 1(a)) [14]. PTT usually utilizes the photogeneration of heat by the nonradiative transition to kill cancer cells. Photosensitizers, including inorganic nanomaterials [15–17], such as black phosphorus (BP) [18–21], metal-organic frameworks [22], gold nanoparticles (NPs) [23], and organic compounds [24–27], such as near-infrared (NIR) small molecules [28–30], semiconducting polymers [24, 31], can be used for efficient ROS or heat generation (Figure 1(b)). However, phototherapy still remains an unsatisfactory method because it may result in adverse effects on normal tissues. These

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nontargeted photosensitizers can also be uptaken by normal tissues. In addition, there is a high probability of cancer metastasis and recurrence, which is partly driven by tumor-driven immunosuppression (Table 1).

Nanotechnology provides researchers with the opportunity for real-time studying and manipulating macromolecules and cancer progression in the earliest stages [32]. NPs are one hundred to ten thousand times smaller than human cells. Therefore, they are able to interact with biomolecules readily on both the surface and inside cells [32]. They have a great potential to detect disease and deliver treatment by gaining access to a variety of areas in the body [33–35]. Nanotechnology can provide rapid and sensitive detection of cancer-related molecules, enabling scientists to detect molecular changes even when they occur only in a small percentage of cells [36]. Uniquely, the use of NPs for cancer, comes down to its ability to be functionalized readily and tuned easily. They are capable of delivering or acting as the therapeutic/diagnostic agents, or both [37–40]. Besides, they are able to passively accumulate at the tumor site by the enhanced permeability

and retention (EPR) effect, or actively target cancer cells by modification, or be delivered across traditional biological barriers in the body [41–43].

Immunotherapy, a revolutionary cancer treatment, relies on the activation of the immune system to eliminate cancer and has attracted increasing attention because of its clinical efficacy [32, 33, 44–46]. The activation or boosting of the inherent immunological systems will be beneficial to recognize and further kill cancer cells [47]. Developing agents that are effective in patients with various types of cancer is one of the most challenging works for researchers. Immuno-checkpoint blockade (ICB), adoptive T cell therapy, and cancer vaccine are usually three different kinds of immunotherapy [48]. ICB therapy usually takes advantage of blocking the inhibitory pathways, such as programmed cell death protein 1 pathway (PD-1/PD-L1) and cytotoxic T-lymphocyte-associated antigen 4 with an antagonist [45]. It proves to be one of the most effective approaches to treating different types of cancers in the clinic. Chimeric antigen receptor T (CAR-T) therapy, a kind of cellular therapy, takes advantage of a patient's own immune system cells to rally an attack on cancer. They have been made by the removal of a specific set of cells from the blood, modification in a lab to intensify the immune response to cancer, and finally re-injecting them into the patient [46]. For example, recently, Gu et al. developed a biodegradable hydrogel reservoir that can encapsulate CAR-T cells to target the human chondroitin sulfate proteoglycan 4 for implantation into the tumor-resection cavity [34]. The post-surgery local delivery of combination immunotherapy could represent a translational route for preventing the recurrence of cancers. CAR-T cells, engineered with antigen-targeting regions fused with signaling chains of the T cell receptor and costimulatory molecules genetically, have achieved outstanding progress in the clinic, especially for the treatment of hematologic malignancies.

Cancer vaccines are able to activate the tumor-specific immunological response against cancer cells because they often contain tumor-associated antigens (TAAs) and immune adjuvants [47]. For example, Liang et al. developed a proton-driven nanotransformer-based vaccine (NTV) comprised a polymer-peptide-based nanotransformer and a loaded antigenic peptide [35]. The NTV induces a robust immune response without substantial systemic toxicity, offering a safe and robust strategy for cancer immunotherapy (CIT). In our previous work, we have reported several cancer nanovaccines, such as albumin binding vaccines which can self-assemble *in vivo* for efficient vaccine delivery and potent CIT [37], size-transformable antigen-presenting cell-mimicking nanovesicles for antigen-specific CD8⁺ T cell

Figure 1: (a) Illustration of the mechanism of phototherapy. (b) Classification of nanomaterials for photoimmunotherapy and the relationship between photodynamic therapy, photothermal therapy, and immunotherapy.

Table 1: Full names and corresponding abbreviations in the text.

Full name	Abbreviation	Full name	Abbreviation
Photodynamic therapy	PDT	Epidermal growth factor receptor	EGFR
Photothermal therapy	PTT	Dendritic cells	DCs
Intersystem crossing	ISC	Tumor-associated antigens	TAAAs
Activating antigen-presenting cells	APCs	Chimeric cross-linked polymersomes	CCPS
Immunogenic cell death	ICD	Doxorubicin hydrochloride	DOX
Photoimmunotherapy	PIT	2-(1-Hexyloxyethyl)-2-devinyl pyropheophorbide-a	HPPH
Reactive oxygen species	ROS	Indoximod	IND
Black phosphorus	BP	Endoplasmic reticulum	ER
Metal organic frameworks	MOFs	Phosphatidylserine	PS
Nanoparticles	NPs	Transforminggrowth factor-β	TGF-β
Enhanced permeability and retention	EPR	Interleukin-10	IL-10
Immuno-checkpoint blockade	ICB	Diselenide-bridged hollow mesoporous organosilica nanocapsules	HMSeN
Chimeric antigen receptor T cells	CAR-T cells	Annexin A5	ANX5
Nanotransformer-based vaccine	NTV	High mobility group protein B1	HMGB1
Damage-associated molecular patterns	DMAPs	Indoleamine 2,3-dioxygenase	IDO
Tumor microenvironment	TME	Interferon γ	IFN-γ
Extracellular matrix	ECM	Indocyanine green	ICG
Chemodynamic therapy	CDT	Toll-like receptor type 7 and 8	TLR7/8
Calreticulin	CRT	Tumor necrosis factor	TNF
Natural killer cells	NK cells	Hyaluronic acid	HA
Organic semiconducting pro-nanostimulant	OSPS	Docetaxel	DTX
Annti-programmed death-ligand 1	anti-PD-L1	Photoacoustic	PA

preactivation [38], and DNA-RNA nanocapsules loaded with tumor neoantigens [39]. In addition, genetically engineered cell-membrane-coated magnetic NPs [40], hybrid cellular membrane nanovesicles [41], and bi-adjuvant neoantigen nanovaccines [42] have also been investigated by our group. These methods may not only enhance the therapeutic efficacy but also provide long-term immune memory effects to inhibit cancer recurrence [18]. There is a great hope that with immunotherapy, cancers may be curable diseases in the years to come.

2 Photoimmunotherapy

Phototherapy, including PDT and PTT, employs photosensitization to generate ROS by ISC or heat by nonradiative decay to induce cell death. Combining phototherapy with immunotherapy may enhance specificity and further the therapeutic efficacy to prevent tumor metastasis and recurrence [47]. In most cases, cancer immunotherapeutic targets lack cancer specificity because some of them may be expressed in normal tissues. The nontargeted release of the immunotherapeutic agents into these normal tissues can sometimes result in severe side effects [32], including fever, hypotension, and skin reactions as well as lab abnormalities [44]. Phototherapy only works with laser irradiation to the tumor site and the photosensitizers are nontoxic in dark

conditions, therefore the normal tissues will suffer from diminished side effects [3, 14]. Therefore, improving cancer specificity is of tremendous significance. Photoimmunotherapy (PIT) is an oncological treatment that combines PDT of the tumor with immunotherapy treatment. PIT selectively destroys cancer cells, leading to immunogenic cell death (ICD) that initiates local immune reactions to release cancer antigens from dying cancer cells [43]. Combination phototherapy with immunotherapy may, to some extent, improve the efficacy and reduce the side effects.

ICD is an anticancer strategy during PIT [43]. Necrotic tumor cells will attract antigen-presenting cells which present the TAAAs to naive T cells. This will lead to the activation of cytotoxic T cells recruited to the tumor tissues. The immune response will be triggered by inducing damage-associated molecular patterns (DAMPs) released from dying tumor cells [43]. Tumor mass consists of not only a heterogeneous population of cancer cells but also a variety of resident and infiltrating host cells, secreted factors, and extracellular matrix (ECM) proteins, collectively known as the tumor microenvironment (TME) [49]. Although ICD can enhance the immunotherapeutic efficacy of cancer, the elevated ROS in the TME will severely weaken the ICD and tumor-infiltrating T lymphocytes. Tumor progression is influenced by interactions of cancer cells with their environment that ultimately determine

whether the primary tumor is eradicated, metastasizes, or establishes dormant micrometastases [49]. The TME can also shape therapeutic responses and resistance, justifying the recent impetus to target components of the TME. Since the high ROS level in the TME will lead to an immunosuppressive condition, the ROS level should be suppressed [43]. In the PDT process, the ROS level should be high enough to induce ICD. Modulation of the level of the TME is a wise approach to enhance the therapeutic efficacy, for example, scavenging of extracellular ROS for the reversal of immunosuppressive environment is of tremendous importance to solve the problem. To solve this problem, we designed a pH-sensitive covalently cross-linked polyethylene glycol to act as a tumor ECM targeting ROS nanoscavenger [43]. Such nanoscavenger is capable of sweeping away the ROS from TME and relieving the immunosuppressive ICD elicited by specific chemotherapy and prolongs the survival of T cells for personalized CIT. In a breast cancer model, elimination of the ROS in the TME elicited antitumor immunity and increased infiltration of T lymphocytes, resulting in a highly potent antitumor effect.

In this review, we will briefly summarize the recent progress of photoimmunotherapy (PIT) based on nanomaterials. It will be divided into four parts, PDT [49–56] or PTT triggered immunotherapy [57–62], PDT/PTT synergistically triggered immunotherapy [63–74], and immunotherapy induced by combinational phototherapy with other therapeutic modalities [75–83], such as chemotherapy, chemodynamic therapy (CDT), and gas therapy. We hope that this review could help the researchers make a comprehensive understanding of the latest advances and prospects of combinational phototherapy with immunotherapy, leading to new paradigms in cancer treatment.

2.1 Photodynamic therapy triggered immunotherapy

PDT is able to elicit immunogenicity by inducing ICD through the release of calreticulin exposure and dying tumor cell debris, resulting in the enhanced antigen presentation and activation of T cells to kill the residual tumor [51, 54, 55]. Photosensitizers can generate ROS to induce ICD with laser irradiation. CpG oligodeoxynucleotides (or CpG ODN) are short single-stranded synthetic DNA molecules that contain a cytosine triphosphate deoxynucleotide (“C”) followed by a guanine triphosphate deoxynucleotide (“G”) [84]. CpG can induce production from dendritic cells (DCs) of cytokines to activate natural killer (NK) cells [85]. Then the activated

NK cells mediate enhanced cellular cytotoxicity or direct killing of NK-sensitive cancers. For example, Yang et al. synthesized black phosphorous quantum dot vesicles, a kind of inorganic photosensitizer, for synergistic PDT with immunotherapy by the simultaneous release of small BP quantum dots with deep tumor penetration and CpG with enhanced immunotherapy [21]. In addition, the photosensitizers can be combined with the antibodies for checkpoint blockade to achieve efficient tumor suppression. Epidermal growth factor receptor (EGFR) overexpression in ovarian cancer is closely associated with poor prognosis and proves to correlate with poor survival outcomes in women with ovarian cancers. To inhibit the EGFR, Hasan et al. encapsulated the liposome benzo-porphyrin and cetuximab antibody for EGFR into a stable preformed plain liposome by passive physical adsorption [26]. The inhibition of EGFR signaling has enhanced PDT-mediated ovarian cancer cell death.

Recent studies have shown that DCs are able to provoke T cells, thus the DC vaccine is effective for CIT [55]. For traditional DC vaccines, DCs will be extracted from the patient's blood and conditioned with antigens and adjuvants before being reinfused into the host. We designed a versatile polymersomal nanoformulation that enables the generation of TAAs through PDT-initiated ICD, and enhances immune responses to the TAA through the use of an immune adjuvant chimeric cross-linked polymerosomes (CCPS) (Figure 2) [55]. CCPS was prepared by self-assembly of a triblock copolymer, polyethylene glycol-poly(methyl methacrylate-co-2-amino ethyl methacrylate (thiol/amine))-poly 2-(dimethylamino)ethyl methacrylate (PEG-P(MMA-co-AEMA (SH/NH₂))-PDMA). CCPS are capable of encapsulating doxorubicin hydrochloride (DOX) and 2-(1-hexyloxyethyl)-2-devinyl pyropheophorbide-a (HPPH), a photosensitizer to facilitate PDT for ROS generation to induce ICD. Such a combination is able to enhance the recruitment of DC and the population of TAAs, thus eliciting an immune response cascade. In addition, CCPS, with primary and tertiary amines as an adjuvant, can stimulate DCs recruited to form an *in situ* DC vaccine after combination with TAAs for MC38 colorectal cancer treatment. ROS generation initiated by PDT was able to induce immunogenic cell death (Figure 2).

TME features hypoxia, acidosis, high interstitial fluid pressure, and increased ECM stiffness [49]. pH-sensitive NPs are able to be triggered by the TME for drug delivery or enhanced therapeutics [10, 54]. To extend the previous work, a pH-responsive nanovesicle formula was developed to act as a nanocarrier because an intelligent cancer vaccine should avoid the blood product handling to the

Figure 2: *In situ* DC vaccine exploiting chimeric cross-linked polymersomes (CCPS) as adjuvant with tumor-associated antigens (TAAs) for MC38 colorectal cancer immunotherapy.

(a) Self-assembly of the versatile copolymer for polymersomal nanoformulation encapsulating HPPH and DOX. (b) Immune response cascade after injection of CCPS/HPPH/DOX with laser irradiation for *in situ* DC vaccine formation, CD8⁺ T cell activation, and tumor cell death. Reprinted with permission from reference [55]. Copyright 2019 American Chemical Society.

largest degree but offer enhanced immune response and antitumor efficacy (Figure 3) [54]. After co-encapsulation of HPPH, a commercial photosensitizer, and an indoleamine 2,3-dioxygenase (IDO) inhibitor, indoximod (IND), such NPs showed significant antitumor efficacy at a single low dose injection in a B16F10 melanoma tumor model after light irradiation. Such enhanced efficacy can be attributed to the following three points: the first is the efficient singlet oxygen generation by HPPH with irradiation but the NPs alone cannot induce ¹O₂ generation; the second is the

immune response provocation caused by increased DC recruitment after ICD, and the third is the TME modulation by IND for CD8⁺ T cell development by enhancing P-S6K phosphorylation.

Although ICD elicited PDT is mediated through the generation of ROS to induce endoplasmic reticulum (ER) stress, it is worth noting that the ROS will, in turn, trigger ER stress to stimulate the downstream DAMPs/danger signaling pathways [51]. Therefore, modulation of ROS-induced ER stress tends to improve the therapeutic

Figure 3: (a) Illustration of synthesis of pH-responsive nanovesicles by co-assembly of HPPH, IND, and pH-responsive polypeptide. (b) Single low-dose injection of NPs to promote host immunity and induce tumor cell death. Reprinted with permission from reference [54]. Copyright 2020 American Chemical Society.

efficacy of PDT-mediated ICD. However, the short half-life of $^1\text{O}_2$ (10–320 ns) and limited diffusion (10–55 nm) hinder the accumulation in the ER and limit ER stress induction. It is desirable to design and prepare ER-targeting photosensitizers to enhance the therapeutic efficacy of PDT-induced ICD. To solve the problem, we synthesized an efficient ER-targeting photosensitizer TCPP-TER(4,4',4'',4'''-(porphyrin-5,10,15,20-tetrayl)tetrakis(N-(2-((4-methylphenyl)sulfonamido) ethyl) benzamide), which was encapsulated by reduction-responsive PEG (PEG-s-s-1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino-(polyethylene glycol)]₂₀₀₀) NPs (Figure 4) [51]. The as-obtained Ds-sP/TCPP-TER NPs could selectively accumulate in the ER and locally generate ROS with laser irradiation. The ER stress was induced, ICD was amplified, and the immune cells were activated, leading to a superior immunotherapeutic efficacy.

Apoptosis and necrosis are two mechanisms involved in cell death. Apoptosis is considered as a naturally occurring physiological process while necrosis is a pathological one caused by external agents, such as toxins and infections [86, 87]. Apoptosis is a highly timely, regulated process whereas necrosis is a random and unregulated one [86, 87]. We postulate that blocking phosphatidylserine (PS) exposure on dying tumor cells *in vivo* as a way to 'convert' apoptosis to secondary necrosis could be an effective way to create *in situ* autologous tumor-cell vaccines. The exposure of PS on the outer leaflet of the plasma membrane of the apoptotic cells is a major 'eat-me' signal for phagocytes, such as macrophages [88]. PS recognition by macrophages triggers the release of immunosuppressive cytokines (transforming growth factor- β (TGF- β) and interleukin-10 (IL-10)), which will prevent the maturation

Figure 4: Illustration of the synthesis of ER-targeting Ds-sP/TCPP-TER NPs for specific accumulation in the ER and *in situ* ROS production upon laser irradiation to induce ER stress and amplify ICD. Reprinted with permission from reference [51]. Copyright 2020 American Chemical Society.

of antigen-presenting DCs and quench inflammation. Thus, macrophage clearance of apoptotic cells will be capable of enhancing the immunosuppression of the TME and thus prevent the stimulation of the host immune system. Under the secondary necrosis condition, DCs have more chances to take up tumour-associated antigen epitopes (TAEs) and receive further costimulation from DAMPs, which can stimulate the immune system and trigger antitumor immune responses.

To solve this problem, we proposed a straightforward *in situ* therapeutic vaccination approach to initiate anti-tumor immunity (Figure 5) [53]. Diselenide-bridged hollow mesoporous organosilica nanocapsules (HMSeN) were designed to immobilize Annexin A5 (ANX5) to achieve photosensitizer blockade. By oxidation- or reduction-responsive diselenide-bond cleavage, HMSeN undergo degradation in either bio-oxidative or bioreductive conditions, thus leading to the on-demand burst release of ANX5 in oxidative TME or reductive intracellular environment. The released ANX5 will then bind to PS, as a result, the recognition of apoptotic cells by macrophages

is inhibited and phagocytic clearance is delayed or blocked. We coated hyaluronate-modified bacterial outer membrane vesicles (HOMVs) onto the HMSeN (HMSeN@HOMV). HMSeN-ANX5@HOMV plus laser-induced a substantial tumoral release of high mobility group protein B1, nearly twice as much as that induced by HMSeN@HOMV plus laser treatment. Furthermore, secondary necrosis of the apoptotic cells releases TAEs and DAMPs to stimulate the immune system for reinvigorating antitumor immune responses.

2.2 Photothermal therapy-induced immunotherapy

PTT is able to induce cell death by generating heat through nonradiative transition [3]. However, many aspects restricted the therapeutic efficacy of PTT, such as nonspecificity of the photothermal agents toward cancer cells, lack of deeper heating of tumor tissues, and thermo-tolerance after initial treatment [3]. Nanotechnology can

Figure 5: Schematic showing the development of *in situ* therapeutic cancer vaccines. Diselenide-bridged HMSeN immobilized with ANX5 protein was coated with HOMVs. Following intravenous administration, HMSeN-ANX5@HOMV efficiently accumulated in the tumor tissue, degraded, and released a burst of ANX5 protein in either oxidative TME (ROS) or bioreductive intracellular (GSH) environment. The released ANX5 protein blocked PS exposure on dying tumor cells. It inhibited phagocytic clearance by macrophages, thereby converting immunosuppressive apoptosis into immunostimulatory secondary necrosis, which simultaneously rendered the primary tumor immunogenic and inflamed the TME. Then, DCs presented the TAEs to T cells and provoked antitumor immune responses. ROS, reactive oxygen species; HA, hyaluronate; CT, chemotherapy; RT, radiotherapy; M ϕ , macrophage; Treg, T regulatory cell. Reprinted with permission from reference [53]. Copyright 2020 Nature Publishing Group.

detect cancer-related molecules rapidly and sensitively, enabling scientists to detect molecular changes even when they occur only in a small percentage of cells [36]. Combining PTT with NPs may achieve better therapeutic efficacy. For PTT-induced immunotherapy, the high expression of IDO following interferon γ stimulation results in overconsumption of L-tryptophan and kynurenine accumulation in the TME [57, 61]. The depletion of tryptophan can prohibit mechanistic target of rapamycin (mTOR) pathway interference with PS6K phosphorylation, induce regulatory T cells, and inhibit CD8⁺ T cell activation [61]. NPs based on NIR dyes, such as indocyanine green (ICG), and IDO inhibitors, are usually used for immunosuppression. Such NPs usually can be combined with PD-L1 checkpoint blockade for efficient PIT [30, 57, 61]. For example, Ma et al. integrated PEGylated IDO inhibitor (Epacadostat) and ICG into a core-shell nanostructure *via* intermolecular interactions, which can

transform into small dual-drug complexes (<40 nm) at TME (Figure 6) [30]. Such NPs could undergo enhanced cellular uptake to trigger an antitumor immune response *in situ* and modulate IDO-mediated immunosuppression. More significantly, the prodrug nanoplateform combined with PD-L1 checkpoint blockade synergistically promotes the antitumor immunity and efficiently inhibits the growth of both primary and abscopal tumors.

Common photothermal agents are triggered by the NIR I (NIR-I, <1000 nm) light, while for *in vivo* study, the therapeutic efficacy is restricted by the wavelength [89]. The second NIR light (NIR-II, 1000–1700 nm) is superior to NIR-I light because it possesses better biological transparency with a lower maximum permissible exposure limit [89]. NIR-II light may result in a more homogeneous release and distribution of DAMPs in the deeper parts of the tumors, especially for *in vivo* studies. To address this issue, Pu et al. developed a photothermal-activatable polymer

Figure 6: Schematic illustration of the TME-responsive prodrug nanoplatform with deep tumor penetration for efficient synergistic PIT. (a) PEGylated IDOi and ICG can be co-assembled into a core-shell nanostructure via molecular interactions. The core-shell nanostructure can be disassembled into the small dual-drug complexes (IDOi/ICG NPs, <40 nm) due to the MMP-induced PEG detaching TME. (b) The prepared prodrug nanoplatform with prolonged blood circulation, deeper penetration, and enhanced tumor accumulation to achieve effective antitumor photo-immunotherapy, IDO inhibition, and PD-L1 blockade. Reprinted with permission from reference [30]. Copyright 2020 Elsevier publishing group.

nanoagonist (APNA) for NIR-II light-regulated photo-thermal CIT (Figure 7) [31]. Such NPs consist of a semi-conducting polymer backbone with NIR-II light-absorbing ability as a photothermal agent. It was conjugated with a potent toll-like receptor type 7 and 8 (TLR7/8) agonist (Resiquimod: R848) as the immunostimulant by a thermal

cleavable linker. Upon NIR-II light irradiation, the NPs mediate the photothermal effect to directly eradicate the tumor and elicit the ICD of cancer cells to promote anti-tumor immunity. *In situ* cleavage of the thermolabile linker was triggered at the tumor site to activate the TLR7/8 agonist to further potentiate antitumor immune response.

Figure 7: Scheme of APNA-mediated NIR-II photothermal immunotherapy.

(a) Chemical structure of pBODO-PEG-VR and preparation of APNA. (b) Mechanism of antitumor immune response by APNA-mediated NIR-II photothermal immunotherapy. TAAs, tumor-associated antigens; DAMPs, damage-associated molecular patterns; iDC, immature DC; mDC, mature DC; HMGB1, high-mobility group box 1 protein. Reprinted with permission from reference [31]. Copyright 2021 Nature publishing group.

Such an activating antigen-presenting cell-mediated spatiotemporal potentiation of CIT enables complete inhibition of the primary tumor and efficient inhibition of either distal tumor or lung metastasis.

2.3 Photodynamic and photothermal therapy triggered immunotherapy

PDT relies on the photogeneration of ROS but the efficacy is limited by the oxygen level in the TME [14]. Lack of deeper heating of tumor tissues, and thermo-tolerance after initial treatment may limit the therapeutic efficacy of PTT [3]. Therefore, photodynamic and photothermal synergistic therapy may be a better choice to compensate for each other to achieve better therapeutic efficacy. There exist two different macrophages in the tumor. M1 macrophages release the proinflammatory cytokines (such as interleukin-6 [IL-6], IL-12, IL-23, and tumor necrosis

factor), reactive nitrogen, and oxygen species (such as NO, H₂O₂, NOS, and superoxide), resulting in an anti-tumor effect [90]. In contrast, M2 macrophages release pro-tumor cytokines (IL-4, IL-10, IL-13, growth factors, and matrix metalloproteinase-9 [MMP-9] in TME to promote tumor progression and metastasis. Coinstantaneous re-regulating macrophage subtype (from pro-tumor M2 to antitumor M1) can be an efficient strategy for tumor immunotherapy. As a polysaccharide, hyaluronic acid (HA) has been widely used for advanced gene and drug delivery, owing to its biodegradability, biocompatibility, nonimmunogenicity, and tumor targeting specificity (CD44 receptor) [71]. For example, Liu et al. developed low molecule weight HA modified BP NPs to improve the stability and targeting specificity of BP and remodel the phenotype of tumor-associated macrophages (TAMs) (Figure 8) [71]. HA-BP down-regulated the expression of CD206 (M2 macrophage marker) by 42.3% and up-regulated the ratio of CD86 (M1 macrophage marker) by

Figure 8: The synthetic scheme of HA-BP nanoparticles and the function of HA-BP nanoparticles *in vivo*. Reprinted with permission from reference [71]. Copyright 2019 Elsevier publishing group.

59.6%, indicating that HA-BP NPs have great potential in remodeling TAMs from the M2 phenotype toward the M1 phenotype to significantly improve tumor immunotherapeutic efficacy.

During the phototherapy process, ROS-responsive drug release is an intelligent way to minimize the side effects and improve the therapeutic efficacy. Pu et al. reported a ROS responsive organic semiconducting pro-nanostimulant (OSPS) for PTT and PDT (Figure 9) [66]. OSPS is made up of a semiconducting polymer NP core which is conjugated with an immunostimulant through a $^1\text{O}_2$ cleavable linker. OSPS generates both heat and $^1\text{O}_2$ to exert combinational phototherapy not only to ablate tumors but also to produce TAAs. With NIR laser irradiation, the semiconducting polymers nanoparticles (SPN) core within OSPS generates both heat and $^1\text{O}_2$, leading to the release of TAAs. Meanwhile, the $^1\text{O}_2$ cleavable linker is destroyed to trigger the release and activation of caged NLG919 to promote both activation and proliferation of

T cells but suppression of Treg cells. The released TAAs in conjunction with activated immunostimulants induce a synergistic antitumor immune response after OSPS-mediated phototherapy, resulting in the inhibited growth of both primary/distant tumors and lung metastasis in a mouse xenograft model. As a result, OSPS integrates phototherapy with remote-controlled immune checkpoint blockade therapy to achieve an amplified therapeutic efficacy in inhibiting primary/distant tumor growth and lung metastasis.

3 Immunotherapy induced by phototherapy and other therapeutic modalities

Other therapeutic modalities, such as CDT [76, 77], gas therapy [78], and chemotherapy [80, 81] have been

Figure 9: Illustration of OSPS-mediated photoactivatable cancer immunotherapy. (a) Photoactivation of OSPS for synergistic therapeutic action including phototherapy and checkpoint blockade immunotherapy. (b) Structure and NIR photoactivation mechanism of OSPS. Reprinted with permission from reference [66]. Copyright 2019 John Wiley and Sons.

combined with phototherapy to induce immune response, thus enhancing the therapeutic efficacy. Chemotherapy utilizes chemo-drug, such as docetaxel (DTX), doxorubicin, to induce cell apoptosis. For example, Qian et al. developed a NIR dye IR820 as the carrier to generate the supramolecular assembly of DTX to form NPs with high drug encapsulation [81]. In addition, a pre-designed peptide with 27 amino acid units (named CF27) was introduced to induce self-cross-linking of the high drug-loading NPs in tumors. Such NPs show excellent PDT/chemotherapy-enhanced immunotherapy. Kim et al. reported BP nanosheet loaded with DOX, cancer growth inhibitor (programmed death-ligand 1 and small interfering RNA), and targeting agent (chitosan–polyethylene glycol) for PDT/chemo-immunotherapy of colorectal cancer [80].

Compared with PDT, CDT is another efficient way to produce ROS by the Fenton or Fenton-like reactions. Cu(II) and Fe(II)/Fe(III) derivatives, such as oxide, sulfide, have been widely used as CDT agents [77]. Such therapy avoids the limitation of the penetration depth faced by phototherapy. For example, Pang et al. developed a simple copper doped covalent organic polymerized-p-phenylenediamine-5,10,15,20-tetra-(4-aminophenyl)porphyrin NPs (abbreviated as Cu-PPT NPs) for cancer therapy [77].

Encapsulated Cu(I)/Cu(II) ions permitted Cu-PPT with glutathione peroxidase-mimicking, Fenton-like, and catalase-mimicking activity to regulate TME. Further combining with anti-programmed death-ligand 1 (anti-PD-L1) checkpoint blockade therapy successfully suppressed the distant tumor growth and cancer metastasis.

Gas therapy is another attractive therapeutic modality by generating toxic gas, such as CO and NO, to induce cell apoptosis. Wu et al. developed a hydrophilic and viscous hydrogel of poly(vinyl alcohol) with conjugation of chitosan and polydopamine [78]. NO donor was formed on a red phosphorus nanofilm deposited on a titanium implant. Under the irradiation of NIR light, peroxynitrite ($\bullet\text{ONOO}^-$) was formed by the reaction between the released NO and superoxide produced and finally the antibacterial mechanism of the $\bullet\text{ONOO}^-$ against the methicillin-resistant *Staphylococcus aureus* (MRSA) biofilm. The excellent osteogenesis and biofilm eradication by released NO under NIR irradiation indicated the noninvasive tissue reconstruction of MRSA-infected tissues through phototherapy and immunotherapy.

Live tumor-targeting microorganisms, such as anaerobic bacteria and even oncolytic viruses, have emerged as therapeutic agents by themselves. In particular, *Salmonella typhimurium* can selectively colonize in tumor tissues

Figure 10: Bacterial colonization in CT26 tumor-bearing mice and healthy mice after intravenous injection.

(A) Schematic illustration of bacteria-triggered tumor thrombosis and the subsequent photothermal tumor ablation. The enhanced NIR absorbance of the tumor is visualized by *in vivo* PA imaging. (B and D) Representative photographs of solid Luria-Bertani (LB) agar plates (B) and quantification (D) of bacterial colonization in various organs harvested from CT26 tumor mice at different time points after injection of bacteria. (C and E) Representative photographs of solid LB agar plates (C) and quantification (E) of bacterial colonization in various organs of healthy mice in a month. Reprinted with permission from reference [75]. Copyright 2020 American Association for the Advancement of Science.

because of the immunosuppressive, hypoxic, and biochemically unique microenvironment within solid tumors. Based on these observations, Liu et al. developed a bacteria-based PIT using intact microbes without any chemical modification or loading of additional payloads (Figure 10) [75]. The bacterial proliferation within tumors would activate innate immunocytes to release proinflammatory factors and disrupt the tumor vasculature, resulting in an/the influx of blood cells into extravascular spaces. Such bacteria-induced tumor-specific thrombosis would darken tumor color with strong NIR absorbance. Because of the increased tumor-specific NIR absorbance, effective photothermal ablation of tumors could be achieved in five different types of tumor models.

3.1 Outlook

In this review, we have summarized the nanomaterials for PIT with enhanced therapeutic efficacy, including immunotherapy triggered by PDT, PTT, PDT/PTT synergistic therapy, CDT, and gas therapy. Such nanomaterials-assisted combinational PIT to trigger systemic antitumor immune responses may be able to enhance tumor-specific immune responses by laser irradiation and this would significantly improve the therapeutic outcome and avoid the side effects, to some extent. However, PIT still has a long way to go before clinical translation.

First, the biocompatibility and the long-term systemic toxicity of these nanomaterials for PIT should always be considered and monitored. These materials should also be able to be produced in mass, allowing for easy clinical translation at an affordable cost. It would be a wise strategy to choose Food and Drug Administration-approved biomaterials. In addition, it is extremely important to choose appropriate animal models to estimate the therapeutic results. Most animal models reported in the literature are created by subcutaneous injection of cell lines, which is easy to perform but far from satisfactory for clinical trials. Humanized mice with transgenic expression of human genes or engrafted with hematopoietic cells are recommended for the study prior to clinical trial because the immune system in these mice is more similar to that of the human, compared with that of immunocompetent mice.

The relationship between nanomaterials and immune responses should be understood in depth for the further design of novel cancer PIT strategies. Nanomaterials with better control of immunological responses should always be considered because this will reduce side effects. For instance, designing ER targeting phototoxic nanomaterials for effective ICD could activate strong tumor-specific

immune responses. Moreover, immunosuppressive TME is another huge challenge for cancer PIT. Developing nanosystems to modulate TME should be recognized as a smart strategy to fight against cancer. For example, mild hyperthermia induced by PTT could significantly improve the infiltration and activation of CAR-T cells in solid tumors and enhance the therapeutic efficacy of CAR-T cells. Nanomaterials-based PIT may bring about excellent therapeutic benefits to cancer patients in the foreseeable future.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Research funding: The authors acknowledge the financial support from the National University of Singapore Start-up Grant (NUHSRO/2020/133/Startup/08) and NUS School of Medicine Nanomedicine Translational Research Program (NUHSRO/2021/034/TRP/09/Nanomedicine).

Conflict of interest statement: The authors declare no conflicts of interest regarding this article.

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