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## Recent advances in light-triggered cancer immunotherapy

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Light-triggered phototherapies, such as photodynamic therapy (PDT) and photothermal therapy (PTT), have shown strong therapeutic efficacy with minimal invasiveness and systemic toxicity, offering opportunities for tumor-specific therapies. Phototherapies not only induce direct tumor cell killing, but also trigger anti-tumor immune responses by releasing various immune-stimulating factors. In recent years, conventional phototherapies have been combined with cancer immunotherapy as synergistic therapeutic modalities to eradicate cancer by exploiting the innate and adaptive immunity. These combined photoimmunotherapies have demonstrated excellent therapeutic efficacy in preventing tumor recurrence and metastasis compared to phototherapy alone. This review covers recent advancements in combined photoimmunotherapy, including photoimmunotherapy (PIT), PDT-combined immunotherapy, and PTT-combined immunotherapy, along with their underlying anti-tumor immune response mechanisms. In addition, the challenges and future research directions for light-triggered cancer immunotherapy are discussed.

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### 1. Introduction

Cancer is a leading cause of mortality and morbidity worldwide. Much progress and technical improvements against cancer have been made over the past few decades, contributing to a decline in cancer mortality rates. However, cancer remains a substantial burden, with more than 1.6 million newly diagnosed cases in 2017.<sup>1</sup> Several novel anti-tumor methods, such as chemotherapy, radiotherapy, gene therapy, photothermal therapy (PTT), and photodynamic therapy (PDT), have been developed to treat and prevent cancer.<sup>2</sup> Although these strategies have shown highly effective performances for cancer treatment, they only focus on regional therapy rather than fundamental therapy, hindering the prevention of metastasis and invasion.<sup>3</sup> However, the single treatment approach has become insufficient in eliminating the entire tumor owing to increased resistance to therapy. Therefore, the use of long-lasting therapeutics with multiple agents is a promising strategy for cancer treatment.

Immunotherapy, which exploits the innate immune system to eliminate malignant cells, has attracted considerable attention over the past few years.<sup>4–8</sup> Cancer cells have multiple

resistance mechanisms, such as local immune evasion, induced tolerance, and systemic disruption of T cell signaling, enabling them to escape immune recognition and subsequent destruction by the innate immune system.<sup>9</sup> Furthermore, through immune editing, tumors can grow unrestrained and become clinically apparent.<sup>10</sup> Since receiving the Nobel Prize in immunology in 2018, immunotherapy has prompted many researchers to use the immune system to protect humans against cancer. Immunotherapy against cancers include various approaches ranging from the use of IL-2 or IFN- $\alpha$  cytokine to stimulate the host's immune system,<sup>11</sup> employing cell-based therapies for T cell regulation,<sup>12–15</sup> to using antibodies against cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), programmed cell-death protein 1 (PD-1) and its ligand (PD-L1) for immune checkpoint blockade.<sup>6,16–19</sup> Immune systems activated through the abovementioned methods exhibited considerably improved anti-tumor effects and yielded increased memory effects, thereby successfully preventing tumor metastasis and recurrence. However, several recent studies have reported that immunotherapy is either ineffective or inconsistent in terms of therapeutic outcomes. Without cancer specificity, immunotherapy can induce several immune side effects, including the hyperimmunization of normal cells, autoimmune responses, organ toxicity, and increased drug resistance.<sup>20–22</sup> Therefore, one strategy to improve the efficacy of immunotherapeutic drugs and reduce their side effects is by combining immunotherapy with region-selective therapeutic methods.

In this regard, many studies that combine immunotherapy with external therapy have been reported.<sup>23–28</sup> Although chemotherapy,

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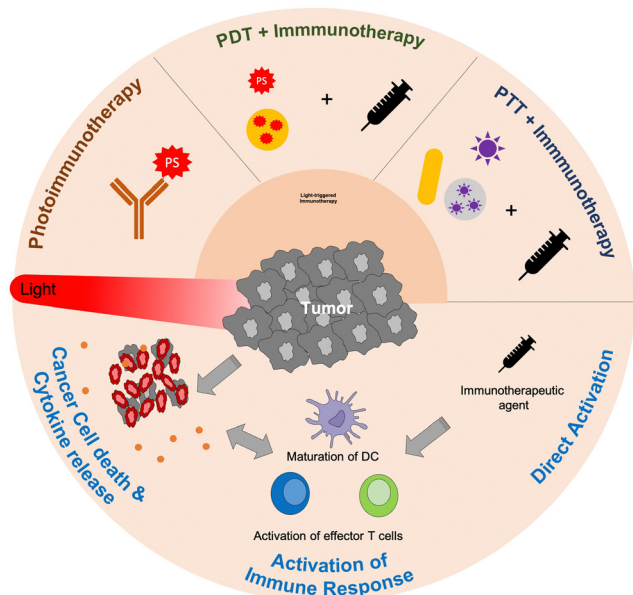


Fig. 1 Schematic representation of combinatorial treatment strategies of light-triggered immunotherapy.

radiotherapy, and surgery can be effective in tumor regression, these methods are generally not associated with the induction of systemic tumor immunity.<sup>29</sup> On the other hand, photoirradiation can trigger an immune-active response by itself in three major ways: (1) laser irradiation could induce tissue stress, disintegration, and ejection itself. (2) Photochemical reactions alter the chemical bonds on cell surfaces and generate reactive oxygen species (ROS) with the assistance of a photosensitizer, leading to both the death of irradiated tissues and a host immune response.

(3) Photothermal reactions that produce elevated heat from laser irradiation could effectively destroy the tumor vasculature owing to its high sensitivity to temperature.<sup>29–34</sup> Therefore, phototherapy could be a good candidate for synergistic therapeutic effects owing to its applicability as a tumor-focused treatment, which leads to tumor regression and prevents toxic effects in healthy tissues.<sup>35–37</sup>

In this review, we focus on dual-mode therapies that combine immunotherapy and light-based therapy (photoimmunotherapy). The types of phototherapies integrated with immunotherapy for cancer treatment are summarized in Fig. 1. First, we discuss the three latest developments in photoimmunotherapy: photoimmunotherapy (PIT), photodynamic therapy (PDT)-combined immunotherapy, and photothermal therapy (PTT)-combined immunotherapy. In addition, we present the immunological effects of each phototherapy, as well as those of the combined therapies. The last section summarizes the advantages and disadvantages of photoimmunotherapy and discusses its future aspects in detail.

## 2. Photoimmunotherapy

Photodynamic therapy (PDT) is a minimally invasive therapeutic method used in cancer treatment.<sup>38–40</sup> In PDT, a non-toxic

photosensitive dye (photosensitizer) absorbs incident light and subsequently produces cytotoxic ROS *via* photochemical reactions involving the transfer of excited electrons. PDT has advantages over conventional cancer therapies such as surgery, chemotherapy, and radiotherapy owing to its minimal systemic toxicity and repeatability in the tumor region. In addition, PDT can trigger an immunostimulatory effect on the immune system.<sup>29,31,41</sup> However, the use of PDT is limited owing to skin photosensitivity, phototoxicity, tumor selectivity, and the unfavourable distribution of photosensitizers leading to normal tissue damage.<sup>42,43</sup> A potential method to overcome these disadvantages is to provide tumor selectivity to photosensitizers through the conjugation of antibodies, *i.e.*, photoimmunotherapy (PIT).<sup>44</sup> Since the lifetime and radius of action of reactive oxygen species are  $<0.04 \mu\text{s}$  and  $<0.02 \mu\text{m}$ , respectively, dimensions smaller than the size of tumor cells ( $10 \mu\text{m}$ ), PIT can offer enhanced performance compared to the conventional PDT method.<sup>45</sup>

### 2.1 PIT

The PIT strategy, which has been studied for approximately 30 years, is based on photosensitizers conjugated to monoclonal antibodies (mAbs) that bind to particular tumor antigens in a specific manner. Therefore, its clinical effects depend on the antigen expressed on the tumor cell surface and not on normal tissues. Unfortunately, a considerable number of antigens identified in human tumors are not exclusive to tumor tissues but are also expressed in normal tissues. Despite this challenge, photoimmunotherapy (PIT) can still achieve adequate therapeutic efficacy in situations where mAbs exhibit limited accessibility to normal tissues or when the tissue remains unexposed to laser irradiation or normal daylight. Some target antigens and photosensitizer conjugates for PIT are provided in Table 1, along with their therapeutic responses.

As described in Table 1, various antigens have been used for tumor-specific targeting. In most studies, the photosensitizing and phototoxic performances of photoimmunoconjugates depend on the ratio of mAbs to the photosensitizer. In addition, the binding region of mAbs should not be interrupted or shielded by the conjugation reaction with the photosensitizer to ensure specific targeting. Therefore, the development of high-quality photoimmunoconjugates is very important.

Generally, the conjugation between mAbs and the photosensitizer depends on the reaction of functional groups such as  $-\text{NH}_2$ ,  $-\text{COOH}$ , and  $-\text{SH}$ . In addition, hydrophilic moieties have been introduced into photosensitizers, which are hydrophobic, to improve their water solubility.<sup>46</sup> Photoimmunoconjugates have been characterized to evaluate their therapeutic performances by altering the pharmacokinetics, biodistribution, specific and non-specific binding to antigens, and internalization.

Targeted PIT molecular therapeutic agents show anti-tumor effects by generating ROS that contribute to cancer cell destruction *via* direct cancer cell death, destruction of cell membranes or tumor vasculature, leading to tumor ischemia, and activation of the immune response.<sup>31,38,47</sup>



Table 1 Target antigens and therapeutic effects with PIT

Target antigen	Photosensitizer	Anti-tumor response	Ref.
CAMAL	Hematoporphyrin	Leukemia cells producing CAMAL were selectively killed.	49
Leu1	Chlorin e6	Human leukemia cells were selectively photodestructed.	50
CD4	Pyrene liposome	Human leukemia cells were selectively destructed.	51
CA 125	Chlorin e6	Immunoconjugate was accumulated in ovarian tumor, but showed dark toxicity.	52
ICAM	Tin chlorin e6	Melanoma cells were photolysed by a point rupture of the plasma membrane.	53
CA 125	Poly-L-lysine chlorin e6	Cationic photoimmunoconjugates showed enhanced cancer-cell uptake and phototoxicity.	54
CD44	<i>m</i> -Tetrahydroxyphenylchlorin	Tumor selectivity of conjugates increased compared to free photosensitizers.	55
CD44	Aluminium(III) phthalocyanine tetrasulfonate	Photoimmunoconjugates were more toxic to head and neck cancer cells than free photosensitizers.	56
CEA, ErbB2	Aluminium(III) phthalocyanine tetrasulfonate	Photo-induced toxicity of conjugates to ovarian carcinoma cells was increased by using internalized mAbs.	57
CEA, ErbB2, EpCAM	Porphyryn isothiocyanates	Photo-induced toxicity of conjugates to carcinoma cells was increased by using internalized mAbs.	58
CEA	IRDye800DX	Single PIT treatment caused rapid cell death and significantly inhibited tumor growth.	59
FAP	ZnF <sub>16</sub> Pc	Ferritin nanoparticle-based PIT (nano-PIT) selectively eliminated CAFs, enhanced T cell infiltration, and increased tumor suppression.	48

Recently, nanotechnology has been adopted in PIT for enhanced specific delivery of therapeutic agents to tumor regions through enhanced permeation and retention (EPR) effects, as well as active targeting *via* antibody–antigen conjugation. Zhen *et al.* reported a nanoparticle-based PIT utilizing ferritin as a photosensitizer carrier and an anti-fibroblast-activation protein (FAP) single-chain variable fragment (scFv) for targeting mAbs.<sup>48</sup> The nano-PIT system successfully eliminated carcinoma-associated fibroblasts (CAF) from tumors but caused slight damage to healthy tissues owing to its localized specific treatment. This approach prevents tumor growth *via* CAF elimination. Further studies have shown that the CAF elimination-induced destruction of the extracellular matrix (ECM) in the surrounding tumors sensitizes cancer cells to immunotherapeutic effects *via* the infiltration of CD8+ T cells into tumors. Although PIT efficiently triggers a specific anti-cancer PDT response, its application is limited to *in vitro* use, and only a few studies have shown *in vivo* therapeutic effects. There are several reasons for these inconsistent results. First, most conventional photosensitizers absorb visible light that cannot penetrate deep tissues, thus deteriorating the light-triggered therapeutic performance of PIT. Second, the hydrophobic nature of conventional photosensitizers disrupts their conjugation to mAbs and leads to poor water solubility of the immunoconjugates, thereby reducing the targeting capacity of the conjugate. Third, the reproducibility of photoimmunoconjugate production could be affected by conjugation procedures, potentially reducing their integrity and antigen-binding capacities.<sup>58</sup> Therefore, additional work is required to overcome these limitations to achieve successful *in vivo* therapeutic performance.

## 2.2 Near-infrared PIT (NIR-PIT)

Near-infrared (NIR, 700–1000 nm) light with deep tissue penetrability had already been broadly applied in imaging and therapy *in vivo*.<sup>60</sup> Several NIR light-sensitive photosensitizers have been discovered for *in vivo* PDT agents.<sup>61,62</sup> PIT has also been combined with NIR-sensitive photosensitizers to improve the anti-tumor response. Kobayashi *et al.* found that the NIR

phthalocyanine dye IR700, conjugated to mAbs (mAb-IR700), along with NIR light irradiation, enhanced the target-selective anti-tumor performance with immune activation.<sup>63–79</sup> As shown in Fig. 2(a) and (b), the tumor volume significantly reduced after NIR-PIT with mAb-IR700 treatment compared to the untreated control, while survival was prolonged in mice. In addition, dendritic cell (DC) maturation was confirmed using CD80 and CD86 markers after NIR-PIT-mediated cancer cell death (Fig. 2(c)). The molecular and cellular mechanisms underlying cell death induced by NIR-PIT have recently been elucidated.<sup>80</sup> IR700 molecules (a photoactivating chemical, APC) were conjugated with three hydrophilic sulfonic acid moieties that could be released from APC after NIR irradiation and subsequent hydrolysis under electron-donor-rich conditions. This light-triggered cleavage results in the formation of less soluble phthalocyanine molecules (Fig. 3(a)). The diminished water solubility contributed to the stacking and aggregation between IR700 conjugated to mAbs and mAbs bound to target antigens on the cell membrane, which damages the target membrane proteins, subsequently depleting the cell membrane integrity (Fig. 3(b)). Next, the targeted cancer cells rapidly swell and release cytoplasmic contents into the ECM through membrane rupture. In this cell death process, ROS generation by APCs plays a minor role, although conventional PDT could partially induce phototoxic cell death.<sup>23</sup> Danger signals released from targeted dying cells such as calreticulin, ATP, HMGB1, and heat shock proteins (HSPs), potently induce the phenotype maturation of DCs and their functional activation, characterized by an increase in CD80 and CD86 markers. In turn, the activated mature DCs cause cellular immune responses, leading to CD8+ T cell expansion, which contributes to cancer cell killing and confers a key feature of immune memory (Fig. 3(c)).

NIR-PIT has been applied to various types of cancers with overexpressed target membrane proteins and has shown successful *in vitro* and *in vivo* performances. Based on these early results, NIR-PIT was fast-tracked for approval by the US Food and Drug Administration for the treatment of malignant head and neck cancer in 2019.

Overall, NIR-PIT is a good platform for photo-triggered cancer immunotherapy with several advantages: (i) target



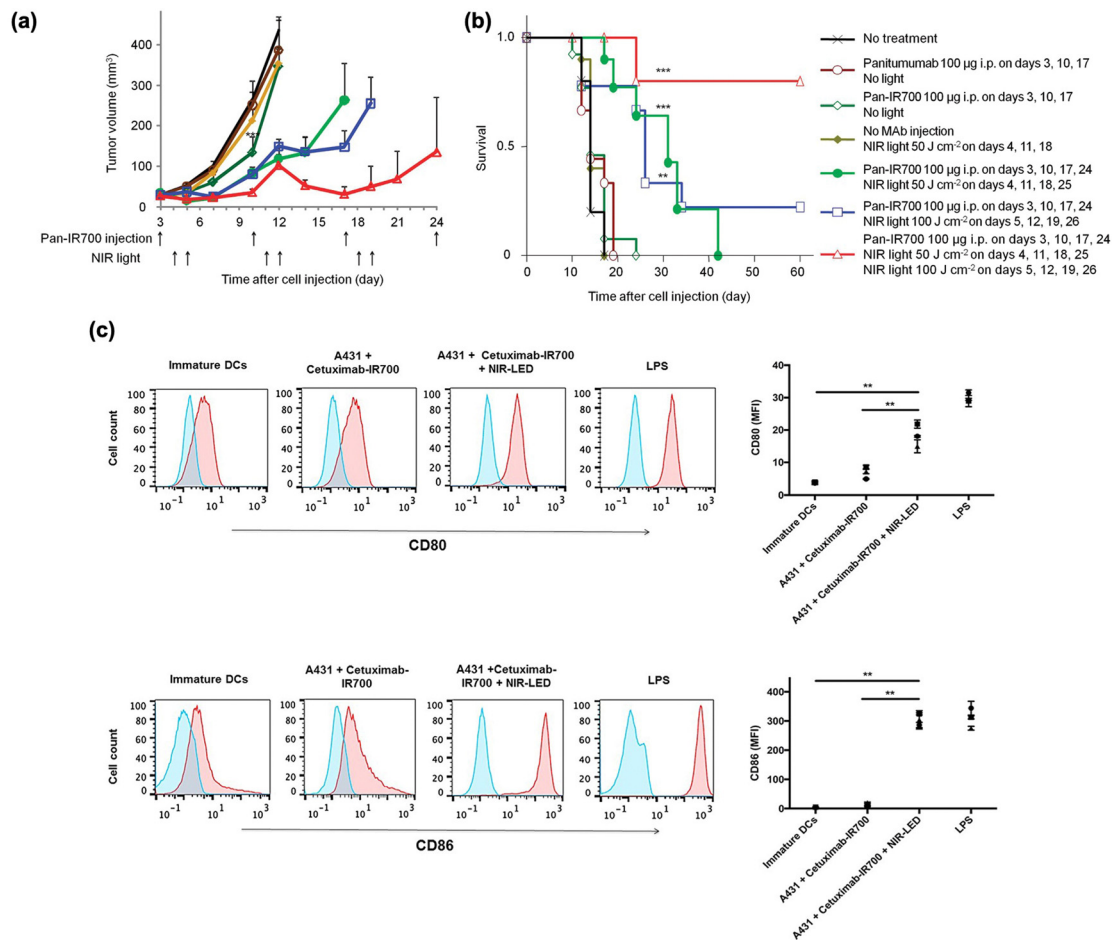


Fig. 2 Therapeutic performance of mAb-IR700 and immune activation. (a) Tumor volume reduction and (b) survival after NIR-PIT with repeated light irradiation. (c) DC maturation after NIR-PIT. Reproduced with permission from ref. 62 and 73. Copyright © 2024, American Chemical Society and Impact Journals, LLC.

specificity and selective phototoxicity, (ii) deep-tissue penetration of NIR light, (iii) high NIR-PIT repeatability, and (iv) flexibility of the platform that can be applied to various different biomarkers and antibodies. Thus, NIR-PIT is a promising novel approach for phototriggered cancer immunotherapy.

### 3. Photodynamic therapy-combined immunotherapy

#### 3.1 Immunogenic response of PDT

PDT directly causes both apoptotic and necrotic cell death by producing reactive singlet oxygen, whereas other cytotoxic agents usually induce apoptotic cell death.<sup>23</sup> During necrotic and/or apoptotic cell death, an acute inflammatory response can occur by attracting leukocytes such as DCs and neutrophils into the tumor site, thus increasing antigen presentation. HSP70 plays an important role in promoting tumor antigen delivery and stimulating anti-tumor immunity.<sup>81</sup> HSP70 is a protective protein that mitigates external stress, such as heat and inflammation, by interacting with the peptide segments of proteins. This engagement shields proteins from aggregation

or misfolding, ultimately averting consequential cellular death. Notably, both HSP70 expression and APC recruitment increased at the tumor site after PDT, which leads to the activation and maturation of DCs, resulting in the cross-presentation of the antigen peptide and the activation of CD8<sup>+</sup> effector T cells.<sup>82</sup> Furthermore, PDT-induced acute inflammation releases various types of cytokines. IL-6, a proinflammatory cytokine, was detected in increased levels after PDT.<sup>83</sup> IL-1 $\beta$ , IL-8, TNF- $\alpha$ , and IFN- $\gamma$  contents were also elevated after PDT.<sup>84,85</sup> These cytokines can trigger the immune response by directing the migration and activation of phagocytes and lymphocytes.

Tumor-specific and memory immunity following PDT has been demonstrated in several studies. Cubeddu *et al.* proved that MS-2 fibrosarcoma in mice were able to resist a rechallenge with tumor cells in a tumor-specific manner after PDT, whereas immunosuppressed models were unable to inhibit the rechallenge.<sup>86</sup> Several studies have reported that systemic anti-tumor immunity could be induced by PDT.<sup>87,88</sup>

On the other hand, the immunosuppressive properties of PDT have also been reported.<sup>89</sup> Bowen *et al.* reported that hematoporphyrin derivative-mediated PDT resulted in 50% suppression of contact hypersensitivity, which is a T cell-



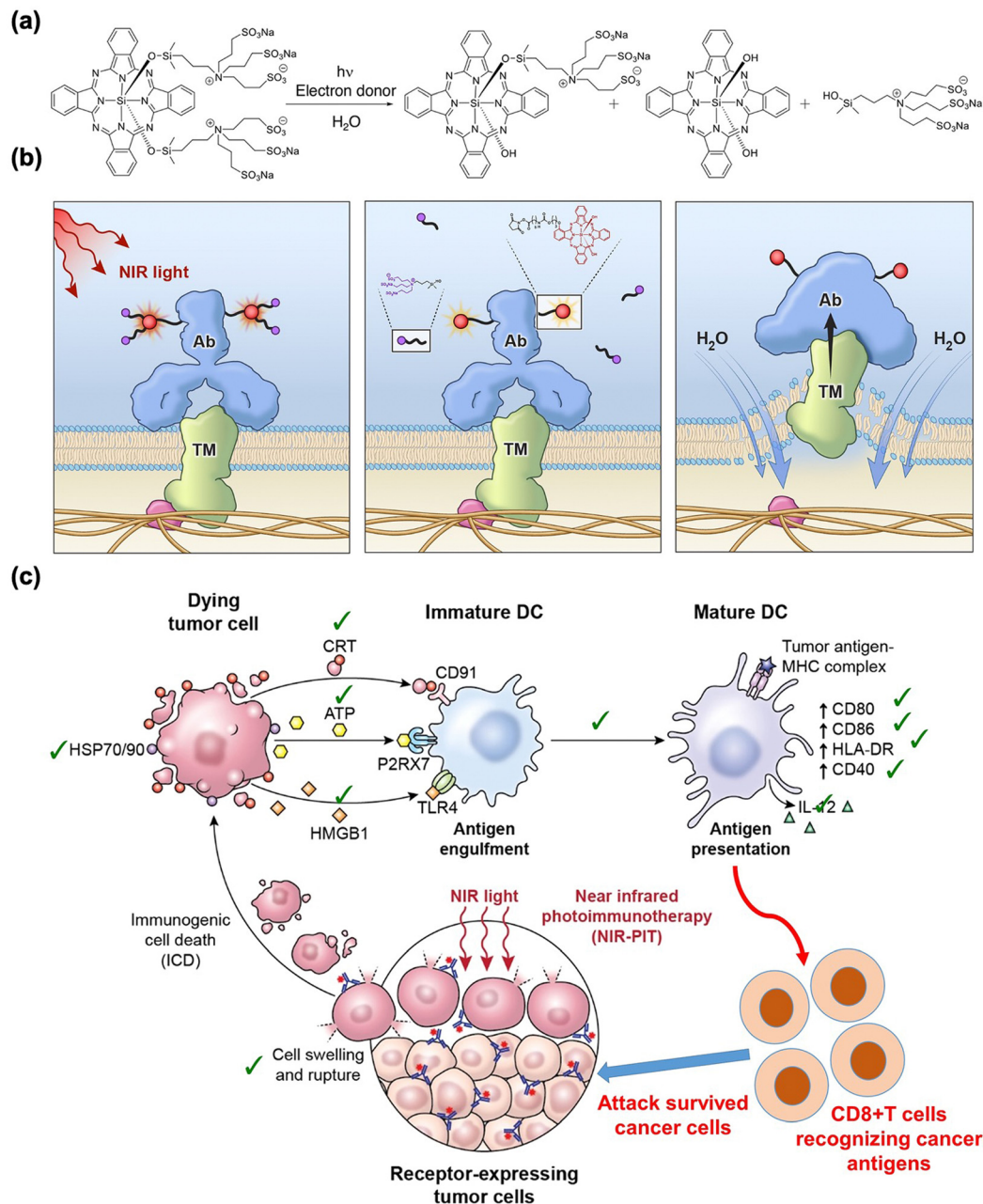


Fig. 3 Schematic illustration of the (a) chemistry, (b) physiological action of NIR-PIT, and (c) immunogenic cell death mechanism of NIR-PIT. Reproduced with permission from ref. 26. Copyright©2024, American Chemical Society.

mediated immune response.<sup>90</sup> Several subsequent studies have also shown the immunosuppressive effect of PDT using different photosensitizers.<sup>91–94</sup> Although the exact mechanism of the immunosuppressive effect of PDT has not been discovered yet, Hamblin *et al.* proposed that the local inflammation response following PDT may trigger a counteraction, leading to immune suppression through regulatory T cell (Treg) activation.<sup>89</sup> Elevated levels of the vascular endothelial growth factor (VEGF) after PDT might also inhibit DC maturation and increase immature phenotypes.<sup>95,96</sup>

These contradictory observations may be attributed to different photosensitizers, light treatments, fluency levels, and

evaluation time points. Therefore, to elicit a robust immunostimulatory effect with PDT, a combination of PDT and immunotherapy may be a good candidate for powerful cancer therapy with an intensified anti-cancer immune response.

### 3.2 PDT-combined immunotherapy

There have been several reports on the combination of PDT with various immunostimulatory agents, which can be divided into four broad classes.

**3.2.1 PDT combined with cytokines.** The administration of cytokines potentiates the effects of PDT. Cytokines are potent anti-tumor agents with a multitude of effects, including



neutrophil and T cell activation, the induction of anti-tumor toxicity, augmentation of natural killer (NK) cell activity, and the increase of cytokine release. PDT can stimulate immune responses by enhancing the release of proinflammatory cytokines as mentioned above, while ROS, including singlet oxygen, can function as signaling molecules to produce proinflammatory cytokines through redox biology.<sup>97</sup> Thus, combining PDT and cytokine administration can improve the therapeutic effect. Bellnier *et al.* combined photofrin II-mediated PDT with TNF- $\alpha$  and TNF- $\alpha$ -inducible drugs (5,6-dimethylxanthenone-4-acetic acid, DMXAA) in SMT-F, mouse mammary adenocarcinoma, and RIF-1, radiation-induced fibrosarcoma mouse models, respectively.<sup>98,99</sup> Both combinations improved the anti-tumor response with a low dose of the respective therapeutic agents. Localized tumor treatment with granulocyte-macrophage and granulocyte colony stimulating factors (GM-CSF and G-CSF, respectively), cytokines that regulate granulocyte biofunctions, combined with PDT agents significantly reduced tumor growth and increased survivability in mouse models.<sup>100,101</sup> CSF increases the number of granulocytes in the activation state, possibly extending the repertoire of antigen-presenting cells and facilitating the induction of a specific anti-tumor immune response.<sup>102</sup> Korbely *et al.* reported the combination of interferon- $\gamma$  (INF- $\gamma$ ) immunotherapy with PDT.<sup>103</sup> While INF- $\gamma$  treatment alone was not effective in boosting the PDT response, its combination with  $\gamma$ -inulin, a carbohydrate derived from the *Compositae* plant, resulted in a four-fold prolongation in the post-PDT recurrence time of B16BL6 mouse models. The author suggested that INF- $\gamma$  treatment can improve the activity of macrophage and dendritic cells in conjunction with  $\gamma$ -inulin.

**3.2.2 PDT combined with immuno-adjuvants.** Another class of PDT combination therapy involves the administration of immune adjuvants that enhance the immune response. Adjuvants are compounds that serve to improve or prolong immune responses with minimal toxicity.<sup>104</sup> Aluminum salt (Alum) is the most widely used adjuvant, but it is limited by its inefficient elicitation of Th1-dependent cellular immunity, which is an effective way of anti-cancer activity.<sup>105</sup> Recently, DC activation has attracted significant attention as the optimal target for adjuvants. In particular, stimulating pattern recognition receptors (PRRs) expressed from cells such as Toll-like receptors (TLRs) can trigger the production of proinflammatory cytokines and hasten DC maturation, resulting in an innate immune response as well as the subsequent development of adaptive immunity.<sup>106</sup> Thus, novel agonists which are able to activate PRR signaling, especially TLRs, have been investigated for their potential as an effective immunogenic cancer therapy method.<sup>107</sup> Wang *et al.* combined 5-aminolevulinic acid (ALA)-mediated PDT with imiquimod in a human model of premalignant Bowenoid papulosis.<sup>108</sup> Imiquimod is an FDA-approved TLR7 agonist, which stimulates an immune response *via* the induction of cytokines such as IFN- $\alpha$ , TNF- $\alpha$ , and various type of ILs, GM-CSF, and G-CSF.<sup>109</sup> ALA-mediated PDT combined with imiquimod therapy showed complete remission in 60% of the Bowenoid patients. Since then, many investigations have

evaluated the clinical effectiveness of imiquimod immunotherapy combined with PDT in Bowen's disease,<sup>110–112</sup> squamous cell carcinoma,<sup>113,114</sup> and basal cell carcinoma.<sup>114–116</sup> Although patients have shown promising therapeutic effects with imiquimod and PDT, further research is required before clinical use.

Another broadly used TLR-related immunostimulant is CpG-oligodeoxynucleotide (CpG-ODN), which triggers the host immune system by activating B cells, NK cells, monocytes, and macrophages after TLR9 recognition.<sup>117</sup> Hamblin *et al.* reported a combination of CpG-ODN as an adjuvant and liposomal benzoporphyrin derivative-based PDT for metastatic mouse breast cancer therapy.<sup>118,119</sup> The CpG adjuvant and PDT improved the therapeutic efficacy *via* DC maturation and activation. Ahn *et al.* treated cell lysates, which contain immunogenic HSP70, with CpG-ODN after PDT in a human metastatic lung cancer mouse model.<sup>120</sup> Interestingly, injecting PDT-generated cell lysates with CpG-ODN suppressed the tumor growth and improved the immune defense through IFN- $\gamma$  secretion and cytotoxic T lymphocyte (CTL) activation.

Chitosan is a biodegradable polymer of D-glucosamine and N-acetyl-D-glucosamine and is known to stimulate immune-activity by inducing cytokine release and T cell upregulation.<sup>121</sup> A chitosan derivative, glycated chitosan (GC), has been used as an immunostimulator with photofrin-mediated PDT for mammary and lung cancer therapies.<sup>114,122</sup> Notably, the combined GC and PDT treatment increased the survival rate by approximately 2-fold compared to PDT alone. Furthermore, another chitosan derivative, schizophyllan (SPG), increased the macrophage-1 antigen-positive innate immune cell content and prolonged the retention time of the photofrin photosensitizer, leading to an increased rate of tumor cell death and enhanced tumor cure rates in SCCVII-bearing mice.<sup>123</sup> GC and SPG exhibited significant immunostimulatory activities; however, further studies are needed to understand the therapeutic mechanisms and improve their practical efficacy.

Bacterial-derived anti-cancer vaccines have been used as non-specific adjuvants to develop immunity against cancer.<sup>124</sup> *Bacillus Calmette-Guérin* (BCG) administration not only improved the curative efficacy of PDT treatment toward tumors, but also boosted the incidence of immune memory T cells in tumor-draining lymph nodes.<sup>125,126</sup> Another bacterial-derived immuno-adjuvant, *Corynebacterium parvum* (CP), also exhibited a synergistic effect on PDT cancer treatment.<sup>127</sup> The therapeutic performance of CP-PDT depends on the amount and the sequence of CP. This method is promising, but further clinical studies are required. OK-432, which kills *Streptococcus pyogenes*, has been tested in combination with hematoporphyrin-mediated PDT in mice bearing NR-S1 mouse squamous cell carcinoma.<sup>128</sup> The survival time was prolonged when OK-432 was injected 3 h before light irradiation, where OK-432 activated macrophages and neutrophils, leading to tumoricidal effects. Although the exact immunostimulatory mechanism of the abovementioned bacterial-derived vaccines remains unknown, it may be attributed to multiple immunomodulatory effects that could enhance and sustain the inflammatory response, followed by anti-cancer immunity.



**3.2.3 PDT combined with adoptive cellular therapies.** The third group of PDT-immunotherapy includes adoptive cellular therapy (ACT), which can alter or augment immunity.<sup>14</sup> ACTs use a patient's own immune-related cells with anti-cancer properties, which is an effective way to treat metastatic cancer.

PDT-combined ACT involves the *ex vivo* manipulation of immune cells, including immature DCs, T cells, and NK cells, and their subsequent reinfusion into the PDT-treated tumor bearing host. Immature DCs efficiently acquire antigens from apoptotic and necrotic tumor cells after PDT treatment, which transforms immature DCs into effective antigen-presenting cells, mature DCs, that migrate to regional lymph nodes for the activation of T lymphocytes. In addition, mature DCs could induce activation and proliferation of NK cells, which in turn lead to the maturation of immature DCs. These combination approaches can potentiate PDT-induced immune response and increase therapeutic efficiency (Fig. 4). Golab *et al.* investigated the anti-tumor activity of PDT by administering immature DCs.<sup>129</sup> Immature DCs co-cultured with PDT-treated murine colon carcinoma cells (C-26) efficiently induced anti-tumor responses through the maturation and production of IL-12, and stimulation of cytotoxic T and NK cells. In addition, intratumoral DC administration combined with PDT resulted in *in vivo* tumor regression.<sup>130</sup> Most importantly, DC treatment with PDT led to the regression of contralateral untreated tumors, including multiple lung metastases. Korbelik *et al.* showed the therapeutic performance of mTHPC-mediated PDT combined with ACT using NK cells genetically modified to produce IL-2 (NK92MI cells) in human cervical squamous cell carcinoma and human colorectal adenocarcinoma mouse models.<sup>131</sup> They found that the transfer of NK92MI cells substantially improved the therapeutic outcome of PDT.

### 3.2.4 PDT combined with checkpoint blockade inhibitors.

One immune escape mechanism of tumor cells is the regression of tumor-infiltrating effector T cells through the expression of immunosuppressive immune checkpoint molecules such as CTLA-4, PD-1, and its ligand PD-L1. Recently, targeting these molecules to stimulate anti-tumor immunity has been the primary focus of cancer immunotherapy, and three immune checkpoint inhibitors have been approved by the FDA: ipilimumab, pembrolizumab, and nivolumab against CTLA-4 and PD-1.<sup>132,133</sup> Although checkpoint blockade inhibitors have yielded impressive results in preclinical and clinical studies,

there are still considerable variations in their individual therapeutic outcomes. Therefore, combination strategies have attempted to improve their effectiveness by combining immune checkpoint inhibitors with tumor therapeutic modalities, including PDT. Ossendorp *et al.* reported PDT combined with a CTLA-4 blockade in MC38 and CT26 double-tumor mouse models.<sup>134</sup> Local PDT treatment with the systemic administration of anti-CTLA-4 inhibited the growth of both local and distant tumors. The PD-L1 blockade has also shown an enhanced anti-tumor effect when combined with PDT.<sup>135</sup> The therapeutic efficacy of the combination of PDT and checkpoint blockade may be attributed to the depletion of tumor-infiltrating Treg, thereby favoring the subsequent increase in intratumoral effector T cells.

**3.2.5 Nanoparticle-based combined photodynamic-immunotherapy.** Nanomedicine has been intensively developed for applications in cancer therapy with several specific aspects: (1) targeted delivery to tumor tissues through either active or passive targeting utilizing the enhanced permeability and retention (EPR) effect. (2) Therapeutic capacity with one or multiple modalities. (3) Versatility and ease of surface functionalization. (4) Long circulation for highly efficient cargo delivery and cancer therapy. (5) Capability of stimuli-responsive therapy or release of therapeutic agents in a tumor-specific manner, and (6) biocompatibility and physicochemical stability. In addition to these features, nanomedicines can help modulate anti-tumor immunity by inducing cancer cell death to elicit immunogenic cell death or stimulate the tumor immune microenvironment. Therefore, a synergistic combination of nanomaterials and immunotherapy has recently been used to treat malignant cancers.

Various types of nanoparticles (NPs) have been developed to selectively deliver photosensitizers to cancer cells and activate anti-cancer immunity simultaneously. A summary of the different NPs used for PDT-combined immunotherapy is provided in Table 2. There are three major combinations of PDT with immunotherapeutic agents: (1) immunostimulators such as CpG-ODN and imiquimod (R837), (2) immune checkpoint inhibitors, and (3) indoleamine 2,3-dioxygenase (IDO) inhibitors. These approaches suppress primary and secondary tumors with memory effects induced by mature DC and activated T cells resulting from PDT-combined immunotherapy.

First, immunostimulators have been used in some studies in combination with PDT. Liu *et al.* used graphene quantum dots containing a Cy3 photosensitizer, Gd<sup>3+</sup> as the MR probe, and CpG-ODN (PC@GCpD(Gd)) for combined PDT immunotherapy.<sup>136</sup> The TLR-9 adjuvant, CpG-ODN, enhanced the anti-tumor immune response through the maturation of DCs and secretion of TNF- $\alpha$  and IL-6, thus stimulating CD4<sup>+</sup> and CD8<sup>+</sup> T cell infiltration to enhance the anti-tumor immunity. Other nanomaterials, including metal-organic frameworks (MOFs) and DNA nanostructures, have been reported as PDT and CpG-ODN therapeutic agents, efficiently suppressing primary and distant tumors *via* DC and T cell expansion, respectively.<sup>137,138</sup>

Neoantigens have recently emerged as next-generation immunotherapeutics.<sup>139–141</sup> Neoantigens are mutant peptide

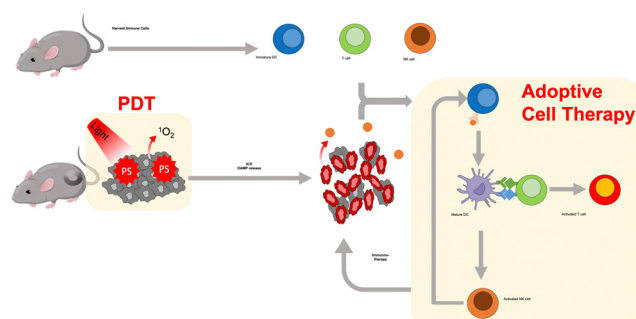


Fig. 4 Schematic illustration of PDT-combined adoptive cell transfer.



Table 2 Summary of recent research of NP-based PDT-combined immunotherapy

NPs	Immunotherapeutic agents	Advantages	Therapeutic effects	Ref.
PC@GCpD(Gd)	CpG ODN	Multimodal-NPs combining PDT and immunotherapy with MRI/FI dual imaging modalities	Complete suppression of EMT6 murine mammary cancer model by maturation of DCs and activation and infiltration of T cells	136
W-TBP/CpG (MOF)	CpG ODN	Efficient delivery of CpGs through charge interaction between cationic MOF and anionic CpGs	Strong anti-tumor immunity with 97% tumor regression and abscopal effect in a bilateral TUBO mouse model	137
bMSN(CpG/Ce6)-Neoantigen	CpG ODN, neoantigen	Biodegradable NPs, image-guided therapeutic NPs	Recruitment of DCs to tumor sites by PDT, followed by neoantigen-specific CD8 $\alpha$ <sup>+</sup> CTL response Strong synergistic effect on local and distant tumors in multiple tumor models	142
NCP@pyrolipid (polymer NPs)	PD-L1 inhibitor	Chemotherapy and PDT combined NPs	Regression of primary and distant tumors in CT26 and MC38 mouse models by inducing tumor-specific T cell response	143
ZnP@pyro	PD-L1 inhibitor	Biocompatible NPs	Eradication of the primary tumors, prevention of lung metastases in the 4T1 mTNBC murine model Complete inhibition of the non-irradiated distant tumors in 4T1 and TUBO mouse models	144
CAT@S/Ce6-CTPP/DPEG	PD-L1 inhibitor	pH responsive mitochondria-targeting NPs	Eradication of tumors with enhanced PDT effects Synergistic effect by anti-tumor immunities in 4T1 mouse model	145
Ce6/MLT@SAB (serum albumin-Aluminum hydroxide) UCNP	PD-1 inhibitor	DC-activatable peptide (melittin, MLT) containing NPs	Synergistic immunogenic PDT with MLT, increase in therapeutic effects by combination with anti-PD-1 immunotherapy in the 4T1 mouse model	146
Ce6-CAT/RPNPs/PEGDA	Imiquimod (R837), CTLA-4 inhibitor	NIR-active NPs, stimulating immune responses with two immunogenic agents	Direct DC maturation by UCNP-Ce6-R837 in the CT26 mouse model, elimination of primary and secondary tumors by the long-term immune memory effect	147
PpIX-1MT	Imiquimod (R837), CLTA-4 inhibitor	Long-term tumor retention and multiple rounds of therapy by <i>in situ</i> polymerization	Strong anti-tumor response, abscopal effect and long-term immune memory protection in the CT26 mouse model	148
Pegylated PPA-NLG919 liposome	1-Methyltryptophan (1MT), IDO-1 inhibitor	Caspase-responsive NPs	Activation of CD8 <sup>+</sup> T cells <i>via</i> IDO inhibition, eradication of both primary tumor and lung metastasis in the CT26 mouse model	149
pRNVs/HPPH/IND	Indoximod (IND), IDO inhibitor	MMP-2 responsive NPs, tumor-cell specific release of NLG919	Enhanced anti-tumor efficacy in both CT26 and 4T1 mouse models by reducing intratumoral Treg infiltration and improving intratumoral infiltration of IFN- $\gamma$ + T cells	150
		pH-responsive NPs	ICD by PDT and vehicle, significant inhibition of both primary and distant tumors by increasing CD8 <sup>+</sup> T cell infiltration in the B16F10 mouse model	151

fragments of major histocompatibility complexes (MHCs) on the surface of cancer cells that are absent in normal cells. Thus, the use of neoantigens could allow for tumor- and patient-specific T cell activation-mediated cancer immunotherapy. Recently, Moon *et al.* developed biodegradable mesoporous silica NPs (MSNs) as nanocarriers for PDT-combined immunotherapy. As MSNs can undergo hydrolysis under aqueous conditions through nucleophilic interaction between hydroxyl groups in water and unbridged oxide on the surface of MSNs, they have been widely used as a platform for drug delivery.<sup>152</sup> Moon *et al.* loaded CpG-ODN, neoantigen, photosensitizer chlorin e6, and <sup>64</sup>Cu into MSNs for positron emission tomography-guided PDT and cancer vaccination in MC38 and B16F10 tumor mouse models (Fig. 5).<sup>142</sup> PDT alone could reduce only primary tumors but not distant tumors, whereas the combined therapy showed a good abscopal effect through DC maturation and neoantigen-specific CD8 $\alpha$ <sup>+</sup> CTL responses (Fig. 5(b)–(d)).

Immune checkpoint inhibitors have also been used in several studies for PDT-combined immunotherapy. Lin *et al.*

combined polymeric core-shell NPs containing a photosensitizer pyropheophorbide-lipid (pyrolipid) conjugate and PD-L1 blockade to treat MC38 and CT26 bilateral tumor models.<sup>143</sup> PDT using pyrolipid NPs induced strong ICD in CT26 cells, which resulted in an effective vaccination against cancer cells. Although pyrolipid NPs alone effectively inhibited tumor growth under light irradiation, their combination with the PD-L1 blockade reduced tumor size by 2.9% compared to the control group. Furthermore, distant tumors that did not receive local light irradiation were almost eliminated. This abscopal effect can be attributed to the infiltration of CD8<sup>+</sup> T cells by the PD-L1 blockade. Lin *et al.* also demonstrated that pyrolipid-loaded Zn-pyrophosphate (ZnP) NPs can kill tumor cells with light irradiation directly by inducing apoptosis and necrosis, followed by disrupting the tumor vasculature and increasing tumor immunogenicity.<sup>144</sup> The anti-tumor therapeutic performance was enhanced after co-treatment with the PD-L1 blockade, as the primary and untreated distant tumors were eradicated by inducing tumor-specific cytotoxic T cell responses.<sup>153,154</sup> These studies proved that NP-mediated PDT can be synergized with





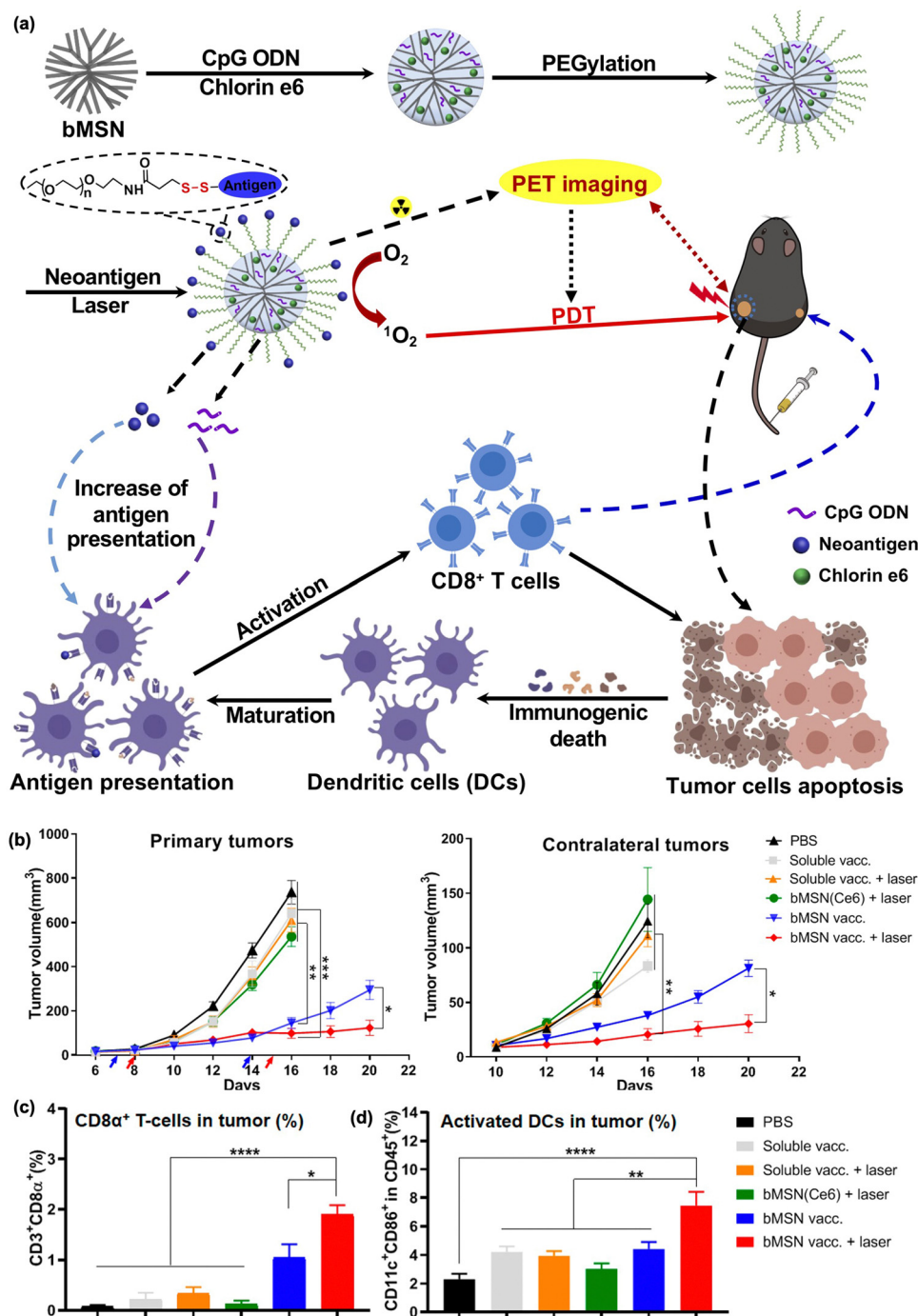


Fig. 5 (a) Schematic illustration for the synthesis of bMSN nanovaccines and the mechanism of therapeutic action. (b) Average primary and contralateral tumor growth in each group. Percentage of (c) CD3 + CD8 $\alpha$  + T cells and (d) CD11c + CD86 + DCs. Reproduced with permission from ref. 142. Copyright © 2024, American Chemical Society.

checkpoint blockade immunotherapy by activating tumor-specific innate and adoptive immune systems.

The oxygen-deficient (hypoxic) microenvironment of tumors can limit the effective use of PDT, which uses oxygen for cell death. Moreover, oxygen consumption induced by PDT further increases tumor hypoxia, which can lead to cancer recurrence and progression through the activation of various angiogenic factors, significantly impeding therapeutic outcomes. Therefore, it is of

great interest to develop PDT agents that can provide oxygen to hypoxic tumor microenvironments. Liu *et al.* reported hydrogel-based PDT agents that were gelled *in situ* through light irradiation.<sup>148</sup> Catalase (CAT)-conjugated chlorin e6 (Ce6), a photosensitizer capable of decomposing H<sub>2</sub>O<sub>2</sub> into oxygen and water, was combined with a polymeric matrix comprising poly(ethylene glycol) double acrylate (PEGDA) and imiquimod (R837)-loaded poly(lactic-co-glycolic acid) (PLGA). After injecting



this mixture into the tumor site, polymerization was initiated by light-triggered ROS generation by Ce6. The hydrogel produces oxygen by decomposing the tumor endogenous  $H_2O_2$  with the gel-retained CAT, which improved the PDT efficacy substantially. Furthermore, the immune response can be activated through DC maturation by recognizing R837, a TLR7 agonist. The *in situ* gelation of Ce6-CAT/PEGDA resulted in ICDs, inducing ICD infiltration into tumors, while the hypoxic conditions of the tumor created a reversed immunosuppressive tumor microenvironment, enhancing the therapeutic performance of PDT and immunotherapy. Combination therapy with the CTLA-4 blockade effectively inhibited primary and distant tumor growth, with a memory effect. Increased amounts of effector memory T cells in the spleen and  $IFN-\gamma$  generated an immune response. Additional details of the checkpoint blockade combination therapies are provided in Table 2.

Indoleamine 2,3-dioxygenase (IDO1 and IDO2) is an intracellular enzyme that catalyzes the conversion of tryptophan into kynurenine.<sup>155</sup> Kynurenine produced by IDO is a potent suppressor of CD8+ effector T and NK cells, while inducing the activation of Treg and myeloid-derived suppressor cells (MDSCs).<sup>156,157</sup> In addition, IDO is known to be resistant to immune checkpoint inhibitors.<sup>158</sup> Therefore, many IDO inhibitors have been developed for effective cancer immunotherapy and are currently undergoing preclinical and clinical trials. Recently, various IDO inhibitors have been used in combination with PDT. Zhang *et al.* reported primary and lung metastasis therapies based on PDT combined with the IDO1 inhibitor 1-methyltryptophan (1MT).<sup>149</sup> As shown in Fig. 6(a) and (b), the PpIX photosensitizer was linked to 1MT by bridging the caspase-3 substrate peptide (PpIX-1MT). After light irradiation, the photosensitizer produces reactive oxygen species that induce the apoptotic death of cancer cells, followed by an increase in caspase-3 activation and the production of tumor antigens. Subsequently, 1MT is released through caspase-3 substrate cleavage, thereby activating the CD8+ T cells and immune responses. Upon irradiation, PpIX-1MT yielded a higher ratio of CD3 + CD8+ effective T cells to CD3 + CD4+ Tregs than the other groups after IDO blockade (Fig. 6(c)). In addition, immune-activated factors such as  $TNF-\alpha$ ,  $IFN-\gamma$ , IL-17, CD-8, CD-86, and granzyme-B were upregulated with PpIX-1MT under light irradiation, while immune-suppressive IL-10 was downregulated in both primary and lung tumors (Fig. 6(d)). Other studies have shown the therapeutic potential in different tumor models or the abscopal effect of PDT combined with IDO inhibition, highlighting the potential of IDO inhibitors for highly efficient photodynamic immunotherapy.<sup>150,151,159</sup>

As described above, the combination of PDT with immunotherapy has been actively studied to achieve enhanced therapeutic outcomes. It is noteworthy that PDT, presented as an alternative for advanced cancer patients, has demonstrated biosafety, as evidenced by the FDA approval of the photosensitizer photofrin. Consequently, the combination of clinically pertinent photosensitizer-based PDT with immunotherapy holds the potential to yield clinical advantages, leading to efficacious therapeutic outcomes. One limitation of PDT-related

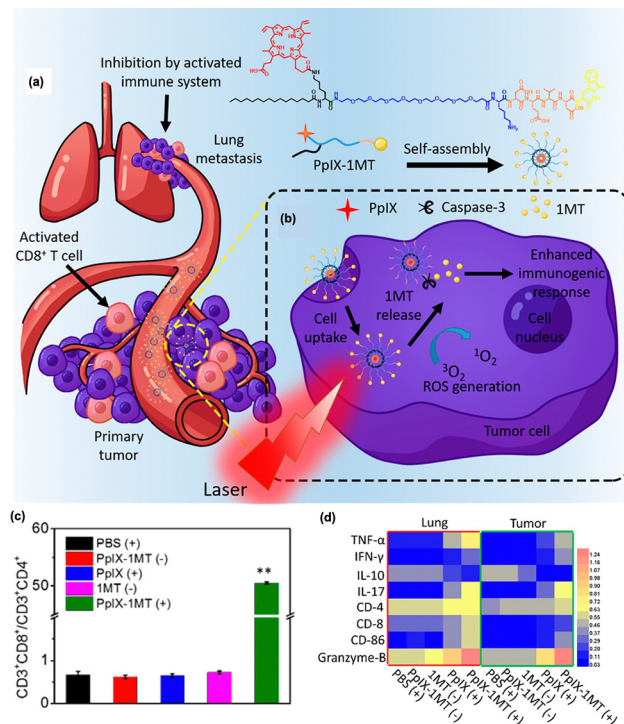


Fig. 6 (a) Structure of PpIX-1MT and (b) therapeutic mechanism involving a series of cascade activations to inhibit primary tumor and lung metastasis. (c) Ratio of CD3 + CD8+ T cells to CD3 + CD4+ T cells after treatment, showing higher CD8+ effector T cell contents and lower CD4+ regulatory T cell contents due to the IDO inhibitor. (d) Relative values of the immune-associated factors determined by western blot within the tumor and lung. "(+)" and "(−)" represent the sample with and without light irradiation. Reproduced with permission from ref. 149. Copyright © 2024, American Chemical Society.

therapy is that light cannot pass through deep tissue, which restricts the use of PDT in tumors under the skin or on the lining of internal organs or cavities.<sup>160</sup> Moreover, PDT is less effective in treating large tumors, as light sources cannot cover the all parts of the tumors.<sup>160,161</sup> For these reasons, PDT is usually considered as a local treatment. To overcome these limitations, it is important to combine PDT with immunotherapy to stimulate the immune system and improve the natural ability of the immune system to enhance the effectiveness of PDT.

## 4. Photothermal therapy-combined immunotherapy

### 4.1 Immunogenic response of photothermal therapy

Photothermal therapy (PTT) is another photo-triggered therapeutic method that uses materials that can absorb and convert NIR light energy to thermal energy, leading to cancer cell death with hyperthermia. Conventional PTT, necessitating hyperthermia exceeding 50 °C, frequently results in undesired damage to the surrounding healthy tissues or recruited immune cells. In contrast, the emerging mild-temperature PTT, with a temperature range from 38 °C to 43 °C, shows great potential with much lower phototoxicity to neighboring normal tissues.<sup>162</sup>



Heat ablation mainly changes the cell membrane integrity, resulting in the dysfunction of the cell cytoskeleton, thereby leading to cell necrosis and apoptosis and the release of tumor-associated antigens in the central zone where the laser is directly irradiated.<sup>163</sup> In the surrounding peripheral zone, the induction of heat shock proteins (HSPs) plays a major role in stimulating the anti-tumor immune response.<sup>164,165</sup> The HSP content, especially HSP70 and HSP72, on the cancer cell surface increases in response to stress, *i.e.*, the unfolded protein response (UPR), after heat treatment, followed by recruitment of NK cells as well as DC maturation and activation of tumor cell-killing cytotoxic CD8+ T cells. In addition, HSP is also released from cancer cells as HSP alone, HSP-tumor antigen complexes, and HSP exosomes, all of which can induce activation and attract DCs and subsequently prime cytotoxic T cells by interacting with various surface receptors of APCs, including TLR, CD40, and CD91.<sup>166</sup> In essence, the localized photothermal effect can induce an anti-tumor immune response through the expression and release of HSP, NK cell recognition, and activation of DCs and cytotoxic T cells.

#### 4.2 Photothermal therapy-combined immunotherapy

Since its development by Chen *et al.* in 1997, PTT-combined immunotherapy has been known as a laser immunotherapy method, which involves using a laser dye for photothermal destruction and an immunoadjuvant for immune stimulation.<sup>167</sup> In this initial report, indocyanine green (ICG) dye was used as a PTT agent in combination with glycated chitosan (GC) as an

immunoadjuvant. ICG-GC co-administration successfully eradicated tumors and increased resistance to tumor rechallenge by enhancing the immune response, which resulted from PTT-induced tumor antigen exposure, subsequent recognition of the exposed tumor antigen by GC, and an enhanced immunological effect on the treated and metastatic tumors.<sup>30,167,168</sup> They also combined ICG-mediated PTT with imiquimod as an immunoadjuvant for clinical studies of metastatic melanoma treatment, and showed ICG-imiquimod therapy to be a safe and useful method with promising therapeutic outcomes.<sup>169</sup> This pioneering work demonstrated that PTT-combined immunotherapy is potentially a fruitful arena for effective cancer therapy.

Recently, several studies combining PTT with immunotherapy studies have been conducted using nanotechnology (Table 3). The combination of immunoadjuvants, such as GC, GM-CSF, CpG-ODN, ovalbumin (OVA) and PTT have shown enhanced anti-cancer activities.<sup>170–174</sup> Xing *et al.* combined GC immunostimulators and carbon nanotube (CNT)-mediated PTT to treat murine mammary carcinoma.<sup>170</sup> CNTs are NIR-absorbing carbon materials that exhibit dimensional advantages across cell membranes without any cytotoxicity.<sup>175</sup> After the application of this combined method, GC delivered into tumor cells serves as both pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), resulting in an increase in the immunogenicity of tumors and APC activation. Both primary and secondary tumors were thoroughly eradicated.

Meaningful results were reported by Lu *et al.*, in which hollow CuS NPs and CpG-ODNs were used as photothermal

Table 3 Summary of recent research of NP-based PTT-combined immunotherapy

NPs	Immunotherapeutic agents	Advantages	Therapeutic effects	Ref.
Carbon nanotubes	GC	Ease of cell internalization and protein delivery, NIR active optical property	Eradication of both primary and secondary tumors in EMT6 mouse models with higher DAMPs and PAMPs and enhanced immunogenicity	170
Cationic graphene oxide	CpG-ODN	Ease of negative CpG ODN delivery	Synergistic therapeutic effects in the CT26 mouse model	171
Chitosan-coated hollow CuS NPs	CpG-ODN	Biodegradable and NIR absorbing NPs	Suppression of primary and distant tumors in the EMT6 mouse model by DC maturation and CD8+ T cell activation, and subsequent immune response	172
Au NPs-CpG-ODN hydrogel	CpG-ODN	Facile loading and release of CpG-ODN	Suppression of primary tumor in the EG7-OVA mouse model <i>via</i> enhanced HSP70 expression and immune response	173
OVA-ICG mixture nanovaccine	OVA	Simple fabrication, high antigen loading efficiency	Suppression of primary tumor and rechallenging in the B16 mouse model	174
PLGA-ICG-R837	Imiquimod, CTLA-4 inhibitor	Biodegradable polymer	Suppression of secondary tumor and rechallenging in 4T1 and CT26 mice models by DC maturation and blocking action of Treg cells	176
Au nanorods-R837	Imiquimod, PD-1 inhibitor	Biocompatible NPs	Suppression of primary tumor and rechallenging by enhanced CD8+ T cell infiltration	177
Fe <sub>3</sub> O <sub>4</sub> -R837	Imiquimod, PD-L1 inhibitor	Magnetic targeting and MR imaging active NPs	Suppression of primary and secondary tumors through DC maturation Inhibition of metastasis by NK cells, B cells, CD8+ T cells, and CD4+ T cells in distant tumors	178
NGL919/IR780 micelles	IDO inhibitor (NGL919)	—	Suppression of primary and secondary tumors Upregulation of CD8+ T cells and downregulation of T <sub>reg</sub> cells	179
rGO	IDO inhibitor (epacadostat), PD-L1 inhibitor	High loading efficiency of the IDO inhibitor	Inhibition of primary and secondary tumor growth in the CT26 mouse model Increase in CD45+ leukocytes, CD4+ T cells, CD8+ T cells and decrease in Treg cell infiltration in distant tumors	180



and immunostimulating agents, respectively.<sup>172</sup> Hollow CuS NPs broke down after laser irradiation, before reassembling and transforming into polymeric structures, leading to enhanced tumor retention and therapeutic efficacy. PTT induced tumor cell death and the release of tumor antigens, whereas the CpG immunoadjuvant triggered the activation of NK cells and DCs. Furthermore, IFN- $\gamma$  production in the tumor and spleen was significantly increased, resulting in successful anti-tumor immunity. Hollow CuS- and CpG-ODN-mediated photothermal immunotherapy elicits effective systemic anti-tumor immune responses that can eradicate primary and secondary tumors.

Although various types of nanomaterial-based vaccines have been developed for combined immunotherapy, their clinical use is limited owing to their low antigen loading efficiency, low yield, complex fabrication steps, and high cytotoxicity. Sun *et al.* reported a simple synthetic method to prepare natural carriers loaded with ICG in high loading and high yields.<sup>174</sup> OVA, which is immunogenic, was used as a model antigen. OVA-ICG NPs were prepared by simply mixing these two components, resulting in a high antigen-loading efficiency of over 80%, high yield, high reproducibility, and excellent biocompatibility. OVA-ICG induced high levels of MHC-II, CD80, and

CD83 expression, indicating the maturation of DCs. Subsequently, matured DCs activate CD8+ cytotoxic T cells and initiate an immune response to eliminate primary tumors and prevent tumor rechallenge. These results demonstrated that PTT-based long-term anti-cancer efficacy could be supported by a combination of immunostimulating agents or adjuvants.

More rigorous and promising therapeutic studies have been conducted by combining PTT with certain types of immune checkpoint blockades.<sup>176</sup> Liu *et al.* reported that PLGA-coated NPs containing ICG and imiquimod (R837) (PLGA-ICG-R837) achieved vaccine-like anti-tumor immunity and showed synergistic effects when PLGA-ICG-R837 was combined with a CTLA-4 blockade (Fig. 7(a)). After administration of NPs and tumor ablation, DC maturation was significantly increased by tumor destruction post-PTT, as well as by the immune-stimulating effect of the adjuvant imiquimod (Fig. 7(b)). In addition, immune activation was observed in secondary tumors *via* a substantial increase in CD8+ CTL and CD4+ effector T cells by the CTLA-4 blockade, which reduced the number of Tregs (Fig. 7(c)). Based on these mechanisms, this strategy successfully inhibited secondary tumor growth and rechallenge after the primary tumor treatment (Fig. 7(d)).

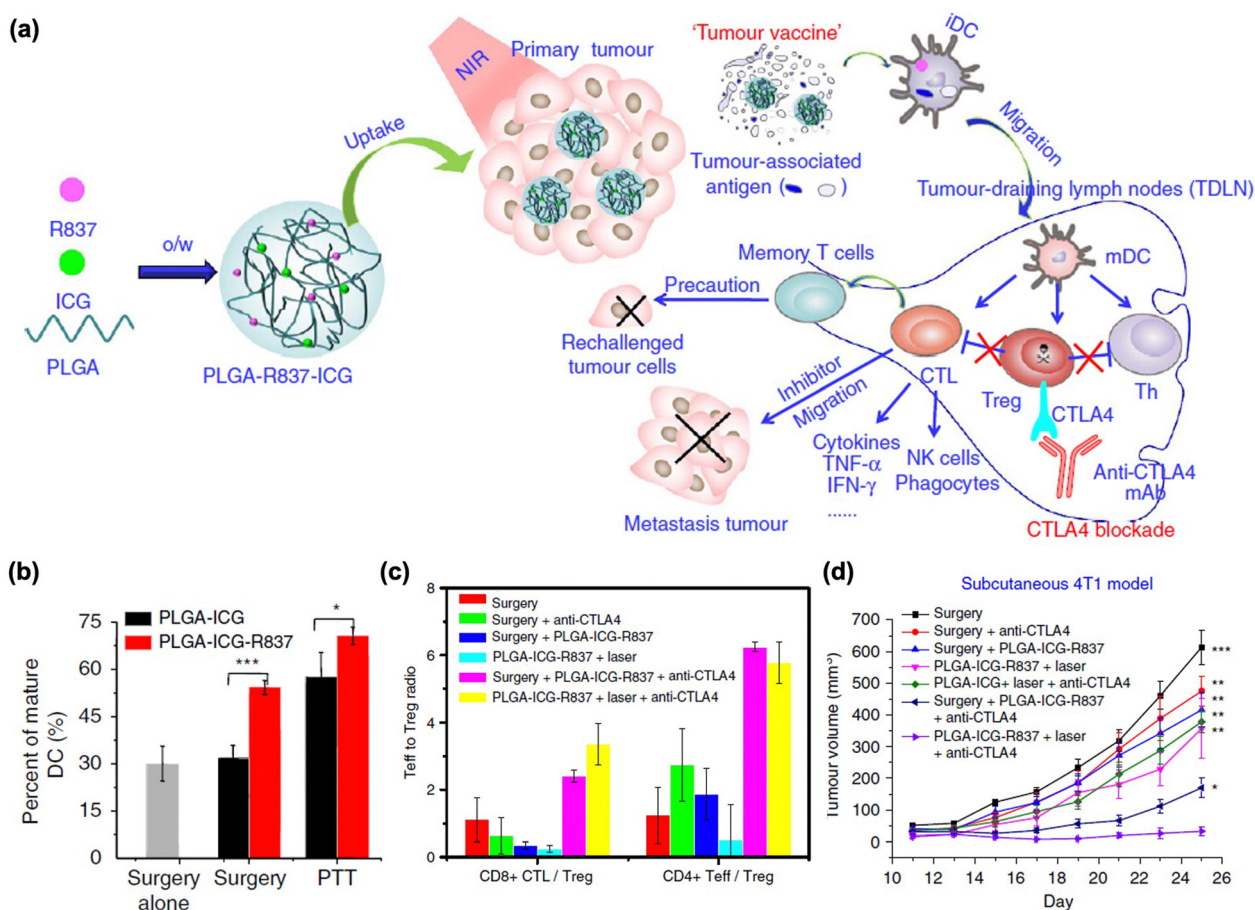


Fig. 7 (a) The mechanism of anti-tumour immune responses induced by PLGA-ICG-R837-based PTT in combination with the checkpoint-blockade. (b) DC maturation after PLGA-ICG-R837 treatment. (c) CD8+ CTL: Treg ratios and CD4+ effector T cells: Treg ratios in the secondary tumours upon various treatments to remove the first tumours. (d) Tumour growth curves of different groups of mice. Reproduced with permission from ref. 176. Copyright © 2024, Nature Publishing Group.



Recently, the regulation of T cell metabolism has attracted considerable attention as a target of immunotherapy.<sup>181</sup> As mild heating resulting from local light irradiation could upregulate tumor cell protective and T cell suppressive proteins related to T cell metabolism after PTT, inhibiting these T cell metabolite proteins could enhance the immune response to PTT. In this regard, IDO inhibition has been utilized to further improve the therapeutic effect in combination with PTT.<sup>179,182–185</sup> Ma *et al.*

used an IDO inhibitor (epacadostat)-loaded PEG-coated rGO in combination with a PD-L1 blockade inhibitor (Fig. 8(a)).<sup>180</sup> Here, they observed that tumor ablation led to the maturation of the DCs. Furthermore, with the aid of an IDO inhibitor, this method showed higher IFN- $\gamma$ , CD4<sup>+</sup> T cell and CD8<sup>+</sup> T cell contents, while inhibiting the Treg function. In addition, these immune responses were significantly augmented in distant tumors in combination with PD-L1 blockade therapy (Fig. 8(b)–(f)).

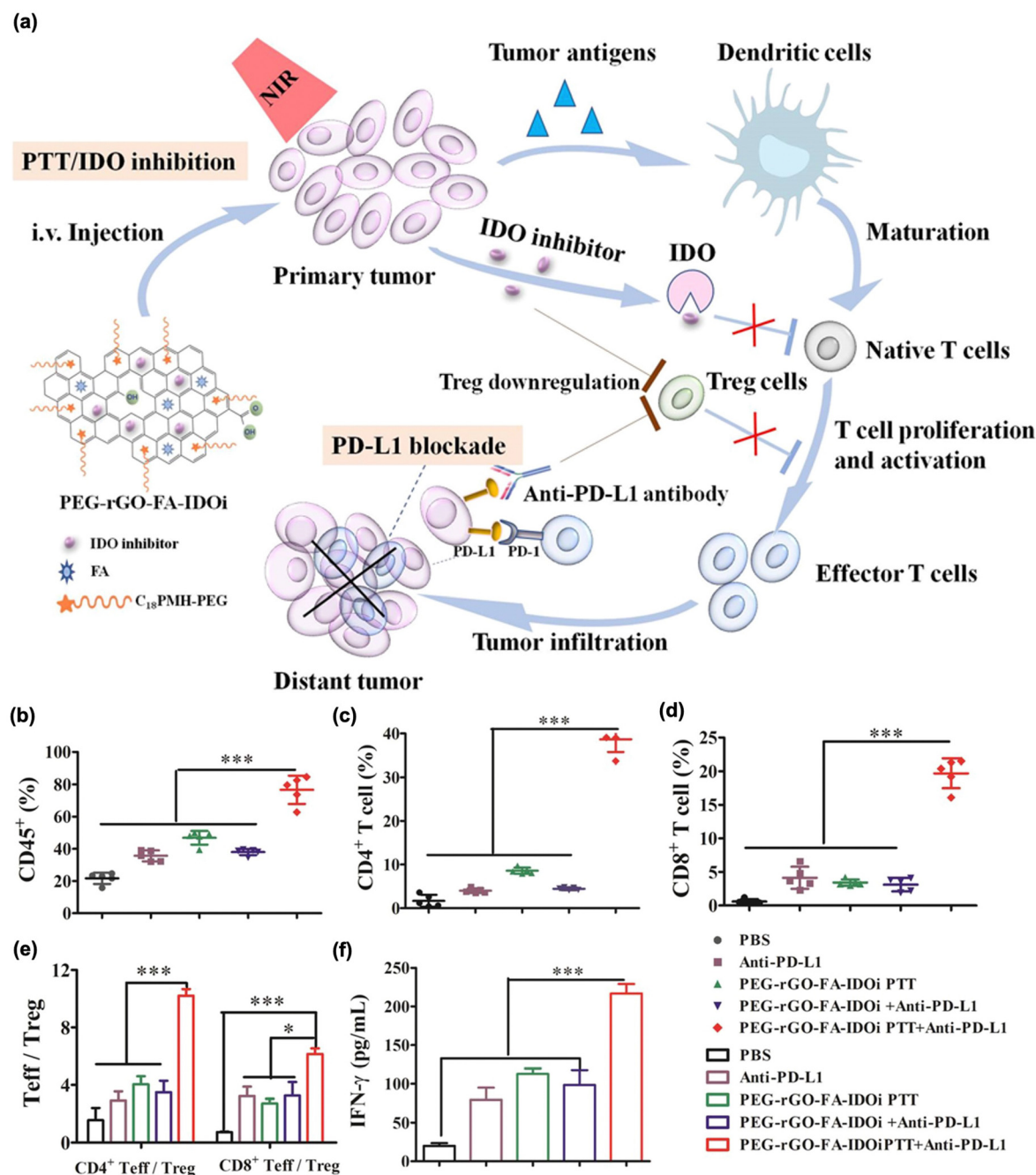


Fig. 8 (a) The mechanism of anti-tumour immune responses induced by PEG-rGO-FA-IDOi-based PTT in combination with checkpoint-blockades. Percentage of (b) CD45<sup>+</sup> leukocytes, (c) CD3 + CD4<sup>+</sup> T cells, (d) CD3 + CD8<sup>+</sup> T cells, (e) CD3 + CD4<sup>+</sup> Foxp<sup>+</sup> effector T cells (CD4<sup>+</sup> Teff): CD3 + CD4<sup>+</sup> Foxp<sup>-</sup> regulator T cells (Treg) ratios and CD3 + CD8<sup>+</sup> T cells: Treg ratios in distant tumors. (f) Production of IFN- $\gamma$  in serum was determined through ELISA. Reproduced with permission from ref. 180. Copyright © 2024, American Chemical Society.



Overall, PTT provides anti-tumor immunity by killing primary tumor cells and releasing tumor antigens. However, long-term therapy has been limited to inducing memory effects to prevent tumor metastasis. In particular, mild local heating of

tumors owing to the narrow or shallow penetration depth of NIR lasers results in not only a temporary immune response but also an increase in some proteins that can block T cell expansion. Therefore, combining PTT with immunotherapeutic

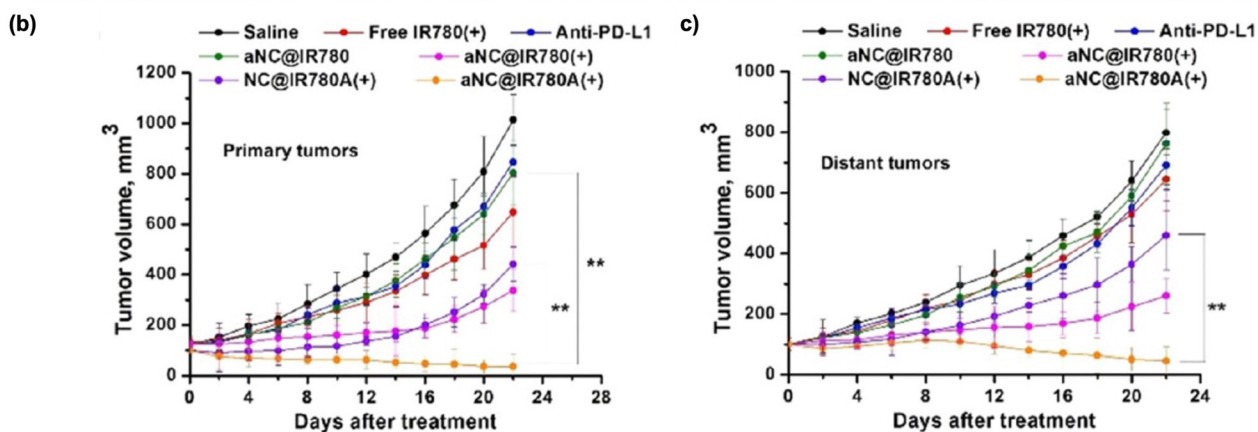
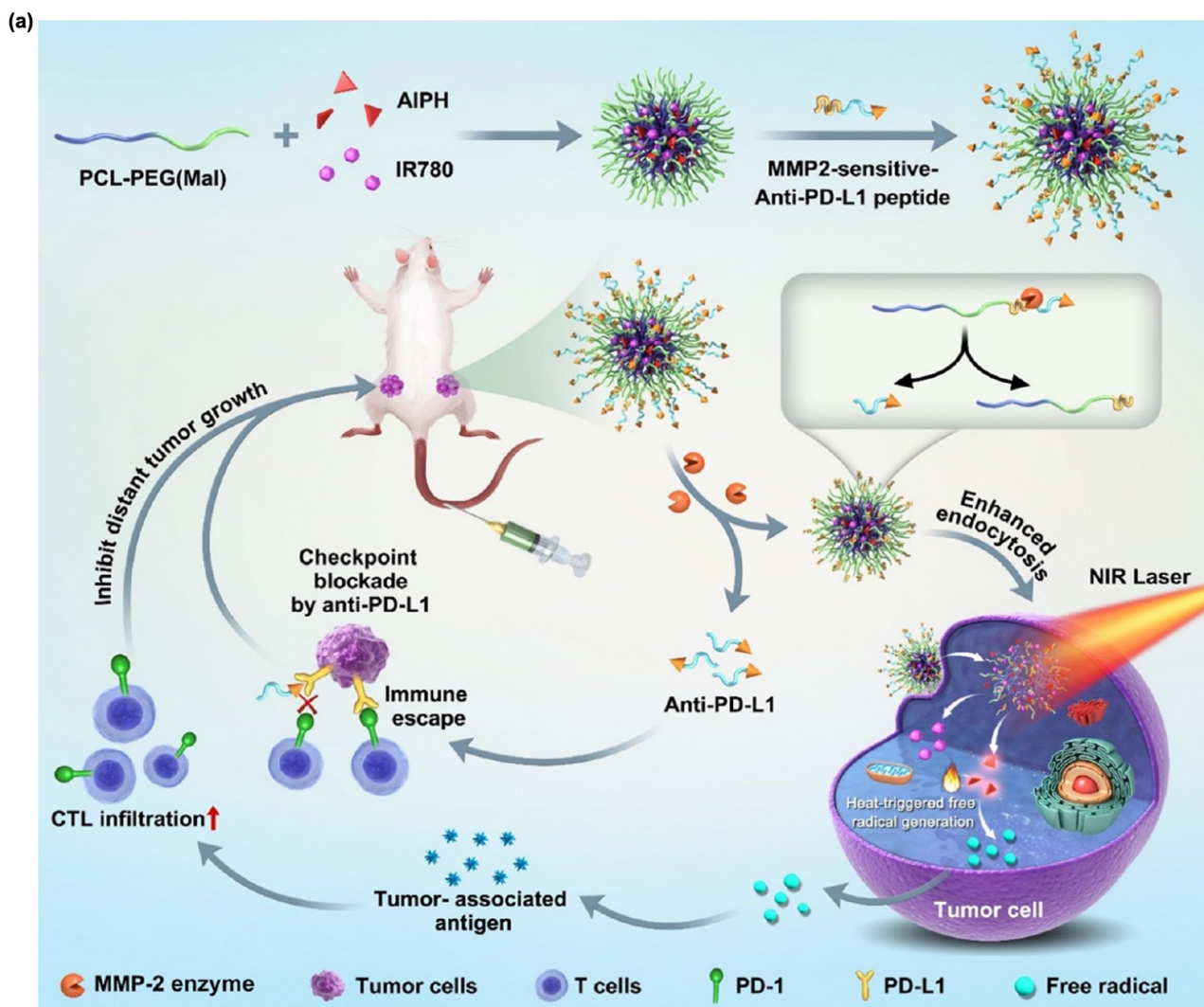


Fig. 9 (a) Schematic illustration of IR780/AIPH nanocomposites encapsulated within an anti-PD-L1 peptide selectively cleaved by MMP for the treatment of primary hypoxic tumors and distant tumors. Average primary (b) and distant (c) tumor growth of each group. Reproduced with permission from ref. 195. Copyright©2024, American Chemical Society.



agents such as checkpoint blockers, immunostimulators, and adjuvants is a promising strategy for effective anti-cancer therapy with long-term memory effects to prevent tumor rechallenge and metastasis.

## 5. PDT–PTT-combined immunotherapy

Integrated trimodal therapy, encompassing PDT, PTT, and immunotherapy, has been reported to enhance the anti-cancer efficacy. Given the previously discussed ability of PDT and PTT to stimulate immune responses *via* ICD, the synergistic combination of PTT and PDT is likely to activate long-term immunity more effectively compared to either PTT or PDT alone.<sup>186–188</sup> Meng *et al.* proposed nanoparticles consisting of highly conjugated and donor–acceptor alternating molecular photosensitizer BTAC6.<sup>186</sup> These BTAC6 NPs exhibited superb NIR absorption with enhanced intersystem crossing (ISC), resulting in efficient energy conversion into cytotoxic ROS and heat. From these properties, BTAC6 NPs activated immune cells, promoting therapeutic efficacy even in hypoxic tumors, and restricting tumor recurrence. Another recent article showed PDT/PTT/immunotherapy designed by Cui's group.<sup>189</sup> The authors prepared a trimodal therapeutic nanoplatform by loading CaCO<sub>3</sub>-coated gold nanostars with the Ce6 photosensitizer (GNS@CaCO<sub>3</sub>/Ce6) into human NK cells. The three parts, namely, GNS for PTT, Ce6 for PDT, and NK cells for adoptive cellular immunotherapy, significantly inhibited primary tumor growth *via* increased secretion of cytokines including TNF- $\alpha$ , IFN- $\gamma$ , IL-18, granzyme A, perforin, FASL, and TRAIL. Shi *et al.* reported Cu<sub>9</sub>S<sub>5</sub>-based nanoparticles decorated with CpG (CSPM@CpG).<sup>190</sup> This strategy can enhance CpG uptake by NP penetration and subsequent production of immune cytokines, leading to the stimulation of the immune response. Liu *et al.* developed low molecular weight hyaluronic acid (HA) modified black phosphorous (BP) nanoparticles (HA-BP) as trimodal therapeutics.<sup>191</sup> In tumor microenvironments, there are two types of tumor associated macrophages (TAMs), anti-tumor M1 macrophages and pro-tumor M2 macrophages.<sup>192</sup> It has been proven that low molecular HA can induce the conversion of TAMs to M1 macrophages as well as reverse TAMs from M2 into M1 macrophages to promote anti-tumor therapeutic efficacy.<sup>193,194</sup> HA-BP showed good photothermal and photodynamic activity, and great potential in inducing M1 phenotype from TAMs rather than M2. Li's group constructed core–shell nanoparticles that contained IR780 and 2,2'-azobis-[2-(2-imidazolin-2-yl)propane]-dihydrochloride (AIPH) encapsulated within a matrix metalloproteinase (MMP)-sensitive PLGVRGC-anti-PD-L1 peptide shell (Fig. 9(a)).<sup>195</sup> IR780, AIPH, and peptide serve as the PTT agent, oxygen-independent PDT agent, and anti-PD-L1 precursor for immune checkpoint blockades, respectively. IR780/AIPH with MMP-sensitive PLGVRGC-anti-PD-L1 peptide significantly inhibited primary and distant tumors compared with the control groups (Fig. 9(b) and (c)). This nanohybrid showed effective PTT-induced oxygen-independent

free radical generation even under hypoxic primary tumor conditions, which led to ICD. Moreover, the anti-PD-L1 peptide, released subsequent to MMP-selective cleavage, functions as an antagonist against PD-L1 on cancer cells. This action further augments immune activation by facilitating the infiltration and activation of CD8<sup>+</sup> T cells. Notably, the outcomes from the 4T1 xenograft mouse model showcase significant inhibition of both primary and distant tumors. These findings suggest that the proposed combination strategy holds substantial promise as an approach for PTT/PDT/immunotherapy in hypoxic tumors.

Beyond the combination of PTT/PDT/immunotherapy, various studies are underway to explore the integration of PTT and PDT with other therapeutic modalities to achieve more effective cancer treatment and immunotherapy.<sup>23,196–198</sup>

## 6. Conclusions and perspectives

Phototherapy, PDT, and PTT can trigger anti-tumor immune responses during direct ROS- or heat-mediated cancer cell death. However, additional treatment modalities are required to achieve long-term therapeutic efficacy. Cancer immunotherapy activates the host immune system to kill primary tumors, thereby preventing tumor recurrence and metastasis through a long-term memory immune response. Hence, the combination of phototherapy and immunotherapy may be a good candidate for effective cancer treatment. Particularly, photoimmunotherapy (PIT) and NIR-PIT can provide disease-specific therapeutic outcomes by activating the immune system. With the aid of immunostimulators and checkpoint inhibitors, enhanced anti-tumor immunity and therapeutic efficacy against primary and distant tumors have been achieved using PDT and PTT in various tumor models. In particular, NP-based combination therapy provides advantages in specific functions, such as tumor-specific delivery, multimodality, high loading of therapeutics, and stimuli-responsive release of cargo.

Although light-triggered cancer immunotherapy has shown high treatment efficacy and synergistic effects, several issues and challenges remain to be addressed. First, most of the current studies use multiple components or treatments for each therapy. The therapeutic components do not have the same formula when combined with an immune checkpoint inhibitor. Several steps are required to achieve therapeutic outcomes and incorrect delivery or unwanted side reactions remain issues to be addressed. Therefore, it is necessary to design a photoimmunotherapeutic platform in which each therapeutic element is well arranged and released under tumor-specific stimuli, leading to an appropriate cascade immune-stimulating reaction. Second, the challenge of deep tissue penetration should be addressed because phototherapies are limited to treating only superficial cancers owing to the low penetration depth of the light source. There have been a few recent studies on enhancing the phototherapy efficiency with improved tissue penetration.<sup>199</sup> Gao *et al.* reported the treatment of orthotopic malignant glioblastoma using NIR (NIR-II) photoactive nanomaterials.<sup>200</sup> NIR-II light ranging from 1000 to



1700 nm is superior to NIR-I light for deep tissue phototherapy. Meng *et al.* demonstrated the direct transarterial administration of MoS<sub>2</sub>-based PTT agents to efficiently eliminate orthotopic liver tumors.<sup>201</sup> However, further studies on deep-tissue phototherapy are necessary. Third, the mechanism of immune activation through combination therapy is yet to be fully understood. Multiple complex immune responses against cancer must be established in future clinical studies. Finally, phototherapeutic elements and nanoplatforms for combination therapy should be optimized for therapeutic efficacy, stability, biodistribution, and safety in further clinical applications.

## Author contributions

Writing – original draft preparation, writing, and preparing the final version of the manuscript, J. K. Yang; literature analysis and collection of references, H. Kwon; writing – review and editing and supervision, S. H. Kim. All the authors have read and agreed to the published version of the manuscript.

## Conflicts of interest

There are no conflicts to declare.

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