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Combining Photothermal-Photodynamic Therapy Mediated by Nanomaterials with Immune Checkpoint Blockade for Metastatic Cancer Treatment and Creation of Immune Memory

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The pursuit of effective treatments for metastatic cancer is still one of the most intensive areas of research in the biomedical field. In a not-so-distant past, the scientific community has witnessed the rise of immunotherapy based on immune checkpoint inhibitors (ICIs). This therapeutic modality intends to abolish immunosuppressive interactions, re-establishing T cell responses against metastasized cancer cells. Despite the initial enthusiasm, the ICIs were later found to be associated with low clinical therapeutic outcomes and immune-related side effects. To address these limitations, researchers are exploring the combination of ICIs with nanomaterial-mediated phototherapies. These nanomaterials can accumulate within the tumor and produce, upon interaction with light, a temperature increase (photothermal therapy) and/or reactive oxygen species (photodynamic therapy), causing damage to cancer cells. Importantly, these photothermal-photodynamic effects can pave the way for an enhanced ICI-based immunotherapy by inducing the release of tumor-associated antigens and danger-associated molecular patterns, as well as by relieving tumor hypoxia and triggering a pro-inflammatory response. This progress report analyses the potential of nanomaterial-mediated photothermal-photodynamic therapy in combination with ICIs, focusing on their ability to modulate T cell populations leading to an anti-metastatic abscopal effect and on their capacity to generate immune memory that prevents tumor recurrence.

1. Introduction

Metastases account for over 90% of the cancer-related deaths.[1] This scenario is aggravated by the inability of the gold-standard treatments to efficiently act on metastases. Notwithstanding, both radio- and chemo-therapies can induce/support metastases' development.[2] On the other hand, immunotherapy can potentially exert an effect on metastases and prevent tumor recurrence.[3]

Currently, the available cancer immunotherapies include cytokine therapy[4] adoptive T-cell immunotherapy,[5] tumor vaccines,[6] and immune checkpoint inhibitors (ICIs).[5,7] Particularly, cancer immunotherapy based on the use of ICIs has been showing promising results, being some of them already approved by the FDA/EMA.[8,9] The ICIs can target immune regulatory receptors expressed by cancer cells (programmed death-ligand 1 (PD-L1)) and T cells (programmed death 1 (PD-1), cytotoxic T-lymphocyte antigen 4 (CTLA-4)) that block the development of antitumoral immunological responses.[9,10] Furthermore, ICIs targeting metabolic checkpoints, such as indoleamine-2,3 dioxygenase (IDO1), are also under investigation.[11] As a matter of fact, 27 out of the 32 FDA-approved ICI-based treatments are used for treating metastatic cancer.[9] Despite the potential of ICIs for treating metastatic cancer, this type of therapy is expensive, and it has been associated to low clinical responses and immune-related side effects.[12,13] In fact, some patients display an innate resistance to ICIs while others acquire it in the course of the treatment.[14] The scarce formation of antitumoral T cells (e.g., due to the unavailability of tumor-associated antigens (TAA)) or their impaired function (related to the cancer cells or to the immunosuppressive tumor microenvironment) are some factors responsible for the lack of efficacy of ICIs—reviewed in detail in refs. [14,15]. For instance, the hypoxic tumor microenvironment contributes to T cell exhaustion.[16] In turn, the side effects associated to ICIs (known as immune-related adverse events) can affect distinct organs, presenting a varying severity

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that may even lead to life-threatening complications (e.g., pneumonitis, myocarditis).[13,17]

The combination of ICIs with phototherapies mediated by nanomaterials can potentially overcome the limitations of the former. These nanomaterials can display a high tumor-homing capacity and produce, upon interaction with light, a temperature increase (photothermal therapy (PTT))[18,19] and/or reactive oxygen species (photodynamic therapy (PDT)), causing damage to cancer cells.[20,21] As importantly, this effect can also i) induce the release of TAA,[22] ii) prompt the release of danger-associated molecular patterns (DAMPs),[23,24] iii) relieve tumor hypoxia,[25] and iv) induce a pro-inflammatory response.[26,27] In this way, these photo-triggered effects enable several events that improve the therapeutic outcome of ICI-based immunotherapies. Furthermore, the nanomaterials may also encapsulate the ICIs, mediating their delivery to the tumor microenvironment, which can contribute to improve the ICIs' specificity.[28,29] The nanomaterials can also mediate the delivery of immunostimulating agents (toll-like receptor (TLR) agonists such as CpG oligodeoxynucleotides (ODNs) or R837) that will aid in the establishment of an antitumoral immunological response.[30,31]

In this progress report, the potential of nanomaterials' mediated PTT/PDT in combination with ICI-based immunotherapies for treating metastatic cancer is analyzed. Firstly, the main PTT/PDT triggered events that can enhance the efficacy of ICI-based immunotherapies are overviewed (Section 2). Afterward, the effect of nanomaterials' mediated PTT/PDT combined with ICIs/immunostimulants' action in improving dendritic cells' (DCs) maturation (Section 3.1), in modulating the T cell populations leading to an anti-metastatic effect (Section 3.2), and in generating immune memory that prevents tumor recurrence (Section 3.3) are reviewed. Finally, an outlook regarding the state-of-the-art and future directions is provided (Section 4). For the sake of brevity, the combination of nanomaterials mediated immuno-PTT/PDT with other therapeutic modalities (e.g., chemotherapy, radiotherapy) and immuno-photothermal/photodynamic approaches that employ other delivery systems than nanomaterials were not reviewed.

2. PTT/PDT Triggered Events That Enhance ICI-Based Immunotherapies

Nanomaterials mediated PTT/PDT can trigger several events that can enhance the efficacy of ICI-based immunotherapies (**Figure 1**). Generally, the immuno-phototherapies mediated by nanomaterials begin with the intravenous administration of these nanostructures.[32,33] Once in circulation, the nanomaterials must bypass rapid clearance mechanisms (e.g., renal filtration, opsonization) and avoid accumulation in detoxifying off-target organs (liver and spleen).[34] Eventually, the nanomaterials will reach the tumor zone, extravasating from the tumor leaky vasculature (presents fenestrae with 200–1200 nm) to the tumor interstitial space.[35] Furthermore, nanostructures' retention in the tumor is also promoted by the impaired lymphatic drainage present at this site.[36] This phenomenon is known as the enhanced permeability and retention (EPR) effect.[35,37] The ability of nanostructures to achieve tumor accumulation by taking advantage of the EPR effect is dependent on their physicochemical properties (size, surface charge, corona composition, and decoration with targeting ligands), and it has been extensively reviewed by our and other research groups elsewhere.[32,34,38] Furthermore, the dynamic and short-lived bursts occurring in the tumor vasculature can also be exploited by nanomaterials in order to achieve tumor accumulation.[39] Alternatively, the nanomaterials can also be administered directly into the primary tumor (intratumoral injection).[40,41] Such may facilitate the investigation of the nanomaterials' role in the attainment of an abscopal effect after the immuno-PTT/PDT.

Afterward, the tumor zone is exposed to laser light. Depending on the optical properties of the nanomaterials, these can produce a temperature increase (PTT) and/or reactive oxygen species (PDT) upon interaction with light.[42] In this regard, near infrared (NIR; 750–1000 nm) light is generally used due to its minimal/negligible interactions with biological components (e.g., water, collagen, melanin, and hemoglobin).[19,20,43] In this way, the NIR light displays a high tissue penetration depth, reaching the tumor-homed nanomaterials with minimal off-target interactions.[32]

The nanomaterials' mediated photothermal-photodynamic effect can per se induce toxicity toward cancer cells.[43,44] In multiple cases, the amplitude of this effect enables the eradication of the irradiated tumor.[29,30,33,45] As importantly, the nanomaterials' photothermal-photodynamic effect can also trigger the i) TAA release, ii) DAMPs release, iii) tumor hypoxia relief, and iv) pro-inflammatory response (discussed in Sections 2.1–2.4; Figure 1). Such events pave the way for a PTT/PDT enhanced ICI-based immunotherapy against metastasized cancer cells/smaller distant tumors (discussed in Section 3; Figure 1).

2.1. TAA Release Induced by Nanomaterials' Mediated PTT/PDT

Nanomaterials' mediated PTT and PDT can induce cellular damage, causing the release of TAA (derived from the cancer cells' debris/residues post-PTT/PDT) to the tumor microenvironment.[22,30,46] Immature DCs can then capture the released TAA, processing and expressing them as TAA-major histocompatibility complex complexes.[47] These events lead to DCs' maturation, which will then migrate to the lymph nodes to later prime naïve T cells to be specific for the TAA.[47,48] In this regard, Yan et al. verified that, after the PTT mediated by folic acid- and poly(ethylene glycol) (PEG)-functionalized reduced graphene oxide (rGO) nanoparticles, the amount of mature DCs (mDCs) found on the lymph nodes near the tumor site increased from 16% to 23% (**Figure 2**).[45]

In another study, the percentage of mDCs on the tumor-draining lymph nodes also increased from 13% to 22% after the PDT mediated by PEGylated upconversion nanoparticles (UCNPs) loaded with chlorin e6 (Ce6).[30]

2.2. DAMPs Release Induced by Nanomaterials' Mediated PTT/PDT

Nanomaterials' mediated PTT/PDT originates stressed and/or necrotic cancer cells that release and expose a selection

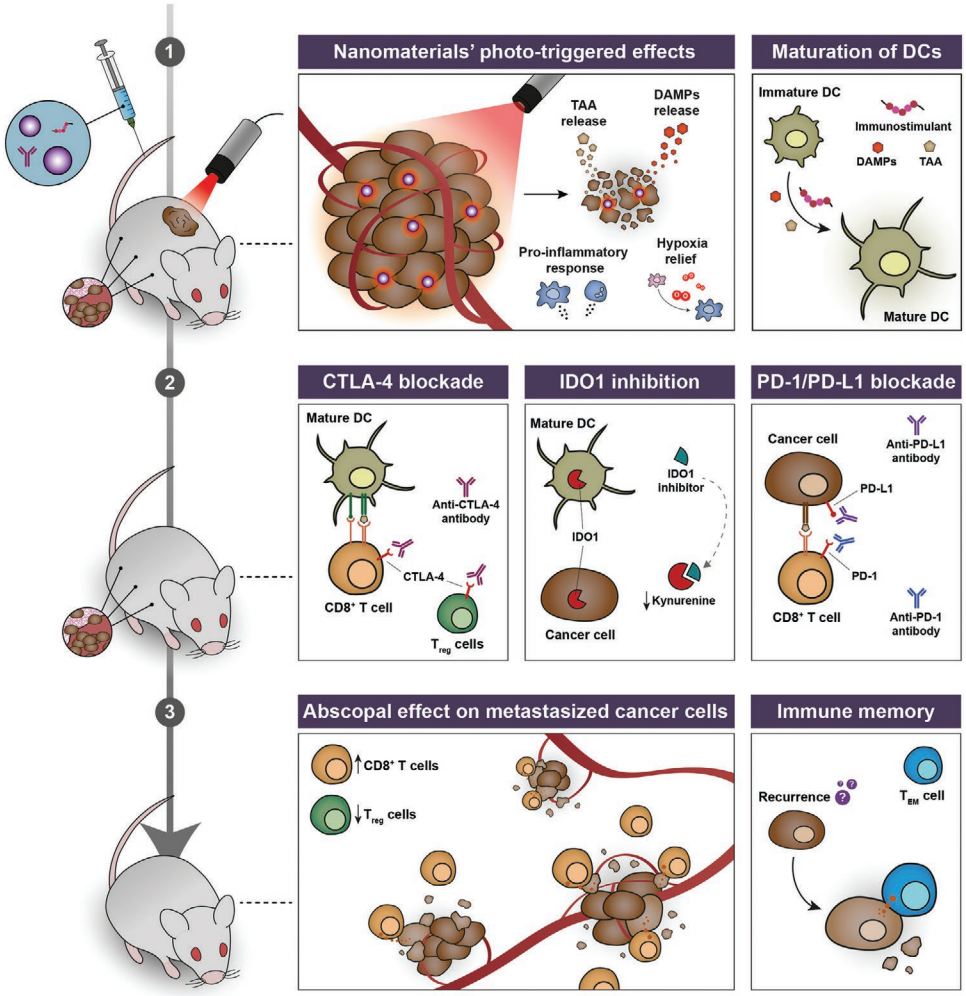

Figure 1. Representation of the different events occurring during nanomaterials' mediated PTT/PDT in combination with ICIs/immunostimulants that can lead to an abscopal effect on metastasized cancer cells and prevent tumor recurrence. 1) Initially, the nanomaterials, ICIs, and immunostimulants are administered, and the primary tumor is exposed to laser light. The tumor-homed nanomaterials produce a photothermal-photodynamic effect that can eradicate the primary tumor. This photothermal-photodynamic effect can also induce the release of TAA and DAMPs, relieve tumor hypoxia, and trigger a pro-inflammatory response. These photo-triggered events will pave the way for a PTT/PDT enhanced ICI-based immunotherapy. Then, the immunostimulants and the released TAA and DAMPs can induce the maturation of DCs. 2) Subsequently, the immunosuppressive actions of CTLA-4, IDO1, and PD-1/PD-L1 are abrogated by the ICIs. 3) The immune checkpoint blockade leads to an increase in the levels of $CD8^+$ T cells in the metastatic sites as well as to a decrease in the levels of T regulatory (T_{reg}) cells. Such events enable $CD8^+$ T cells' action against metastasized cancer cells, leading to the elimination of distant tumors. At later stages, effector memory T (T_{EM}) cells can rapidly generate an antitumoral response after reencountering the TAA, hence preventing tumor recurrence.

of molecules. These DAMPs are used as signals to trigger a response from the immune system.[49,50] This mechanism of immunogenic cell death is associated with i) the exposure of chaperones (e.g., calreticulin (CRT)) in the cancer cells' surface,[49,51] ii) the secretion of ATP and nucleotides/nucleic acids,[23,51] and iii) the release of TLR agonists (e.g., high-motility group box 1 (HMGB1)).[23,52] These molecules will bind to specific receptors on antigen-presenting cells, inducing their maturation, or can act as "eat me" signals, helping in the phagocytic process.[53]

In this regard, Wang and co-workers demonstrated that the PDT mediated by Ce6-loaded PEGylated $Cu_{2-x}Se$-based nanoparticles induced CRT exposure and triggered the release of ATP and HMGB1.[54] In another work, the PTT mediated by PEGylated gold nanocages also increased CRT exposure on cancer cells' membrane and induced a high level of ATP and HMGB1 release from cancer cells.[55] Such events induced DCs' maturation and activation.[55]

2.3. Tumor Hypoxia Relief Induced by Nanomaterials' Mediated PTT

The photothermal effect mediated by nanomaterials can improve the blood flow to the tumor site, improving tumor oxygenation.[56,57] Tumor hypoxia relief can also be achieved by engineering nanomaterials that can decompose H_2O_2 into

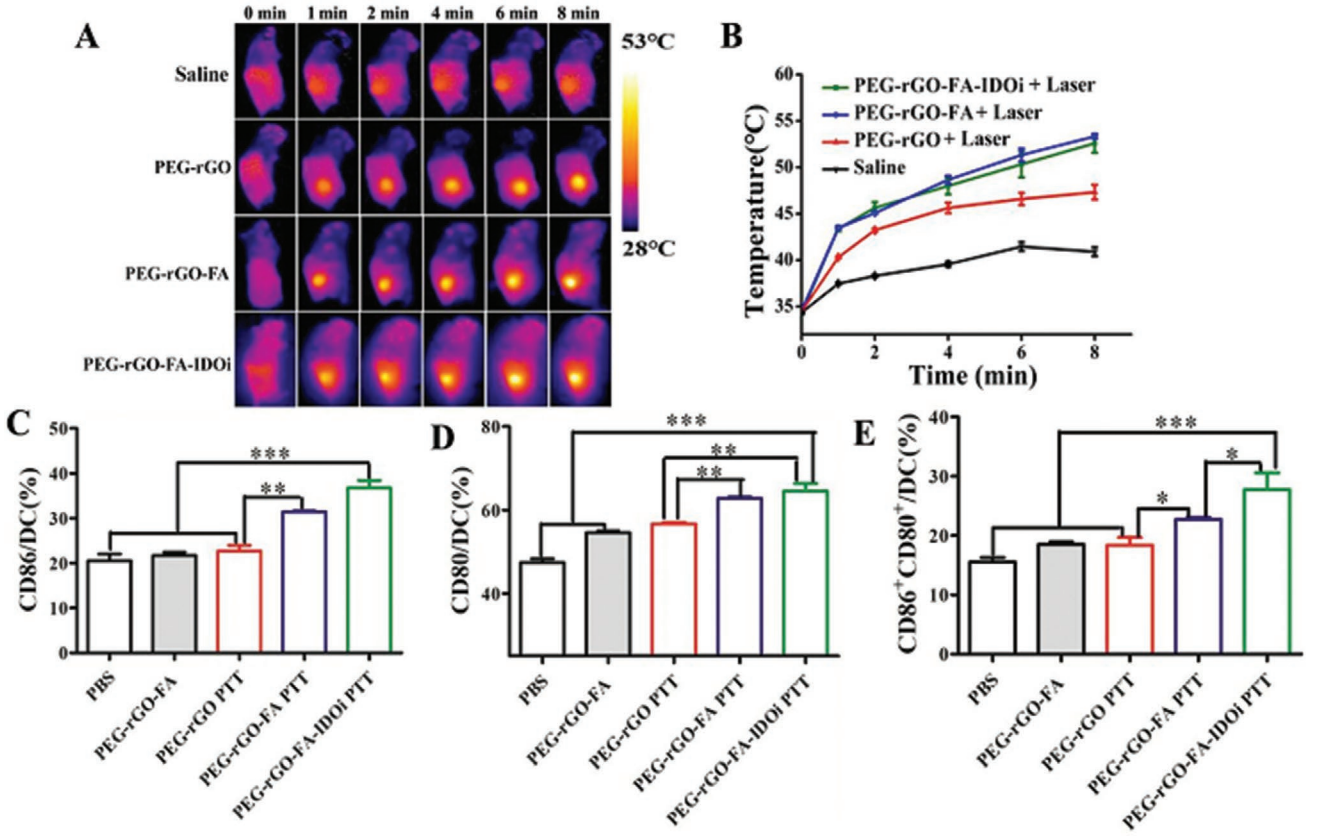

Figure 2. The effect of the PTT mediated by FA- and PEG-functionalized rGO nanoparticles in DCs' maturation. A) Infrared thermographic maps of mice after intravenous injection with the different formulations and exposure to laser irradiation. B) Temperature variation curves of the tumor area after the different treatments. Percentage of C) $CD86^+$, D) $CD80^+$, and E) $CD86^+CD80^+$ DCs in the lymph nodes after the different treatments. Reproduced with permission.[45] Copyright 2019, American Chemical Society. PEG-rGO: PEGylated rGO nanoparticles; PEG-rGO-FA: FA- and PEG-functionalized rGO nanoparticles; PEG-rGO-FA-IDOi: Epacadostat loaded FA- and PEG-functionalized rGO nanoparticles; PTT/Laser: NIR light irradiation.

O_2 (e.g., MnO_2 nanoparticles[58]) or by incorporating oxygen carriers in the nanoformulations (e.g., perfluorocarbons[59]). For instance, Huang and co-workers verified that mice treated with cancer cell membrane coated Fe_3O_4 nanoparticles incorporating indocyanine green (ICG) plus NIR light display decreased levels of hypoxia-inducible factor 1-α in the tumor, indicating that the nanomaterials' mediated PTT could relieve tumor hypoxia.[57]

Tumor hypoxia has an important role in the establishment of an immunosuppressive environment, contributing to macrophages' polarization into a pro-tumoral state.[60] Relieving tumor hypoxia can contribute to switch macrophages' polarization from the type 2 anti-inflammatory (pro-tumoral) state to the type 1 pro-inflammatory (antitumoral) state,[61] hence playing an important role in the induction of antitumor immunity. In this regard, Li et al. demonstrated that, after the PTT mediated by albumin-coated gold nanorods, the expression levels of macrophages' type 2 markers at the tumor site significantly decreased.[62] A similar capacity to prompt macrophages' polarization to the antitumoral state was also demonstrated in the PTT mediated by PEGylated GO.[63]

2.4. Pro-Inflammatory Response Induced by Nanomaterials Mediated PTT/PDT

The PTT/PDT mediated by nanomaterials can trigger a local acute inflammatory response.[64] In this process, inflammatory cells (e.g., neutrophils, mast cells, and monocytes/macrophages) are recruited to the tumor zone in an effort to eliminate cells' debris and to neutralize the DAMPs' source.[64,65] Furthermore, inflammatory cytokines and chemokines are released by the different cells of the tumor microenvironment, having an important role in antitumor immunological responses.[65] Moreover, such release is also of paramount importance since several cytokines (e.g., IL-2, IL-12, and IL-15) have been reported to improve the outcome of the therapies based on CTLA-4 and PD-1/PD-L1 blockade.[66]

For instance, Wang et al. verified that the PTT mediated by PEGylated conjugated polymer nanoparticles could induce an up-regulation of the expression levels of IL-1β, IL-6, IL-12, and TNF-α (pro-inflammatory cytokines) on macrophages.[26] PEGylated hollow gold nanoshells exposed to NIR light also increased the serum levels of several pro-inflammatory cytokines (IL-1β, IL-6, IL-12, and TNF-α) as well as of the

chemokines CXCL-1, CCL-2, and CCL-4, that are responsible for the recruitment of neutrophils, monocytes, macrophages, and DCs.[27,67]

3. Nanomaterials' Mediated PTT/PDT in Combination with Immunostimulants and/or ICIs

The nanomaterials' photothermal/photodynamic effects (analyzed in Section 2) in combination with immunostimulants and/or ICIs can improve DCs' maturation (discussed in Section 3.1), modulate T cell populations leading to an abscopal effect (Section 3.2) and generate immune memory (Section 3.3). These events can culminate in the elimination of the primary and distant tumors as well as in the prevention of tumor recurrence.

3.1. Nanomaterials' Mediated PTT/PDT in Combination with Immunostimulants for an Improved DCs' Maturation

The sole delivery of immunostimulating agents by nanomaterials can potentially induce a higher maturation of DCs.[30,68,69] For this purpose, R837 (Imiquimod; TLR-7 agonist) and R848 (Resiquimod; TLR-7/8 agonist) have been encapsulated in the nanomaterials due to their hydrophobic character.[30,31] In contrast, the hydrophilic CpG ODNs (TLR-9 agonist) can be co-administered with the nanoparticles or incorporated on the nanomaterials' hydrophilic shell.[22,70,71] In this context, Chen et al. verified that the delivery of R837 by FA-polydopamine nanoparticles induced about 1.5-times higher DCs' maturation than free R837.[68]

Furthermore, the immunostimulating agents' action can synergize with the photothermal/photodynamic effects mediated by the nanomaterials, leading to an enhanced maturation of DCs.[30,70,72] For instance, Guo et al. demonstrated that the levels of mDCs in the tumor, after treatment with chitosan and CpG ODNs-coated CuS nanoparticles plus NIR light (nanomaterials' mediated PTT and CpG ODNs delivery) were 5.8- and 14.2-fold higher than those induced by CpG ODNs-coated CuS based nanoparticles (nanomaterials' mediated CpG ODNs delivery) and CuS based nanoparticles plus NIR light (nanomaterials' mediated PTT), respectively.[40] In another work, the PDT mediated by PEGylated UCNPs incorporating Ce6 and R837 also induced a 1.5- and 1.4-fold higher DCs' maturation than the nanomaterials' mediated PDT and the nanomaterials' mediated R837 delivery, respectively (**Figure 3**).[30]

The combined effect of nanomaterials' mediated PTT/PDT and immunostimulants' delivery on DCs' maturation is summarized in **Table 1**.

3.2. Nanomaterials' Mediated PTT/PDT in Combination with ICIs/Immunostimulants for Modulating the T Cell Populations Leading to an Abscopal Effect

The nanomaterials' mediated PTT/PDT and the immunostimulating agents' action can greatly improve DCs' maturation (reviewed in Section 3.1). This synergy can pave the way for prompting high levels of activated $CD8^+$ T cells into the primary tumor as well as into the secondary tumor/metastases.[33,40,73] In this case, the ablative effects on the primary tumor are mainly catalyzed by the nanomaterials' PTT/PDT, while strong abscopal effects on the secondary tumor/metastases mediated by the $CD8^+$ T cells are often abrogated by CTLA-4, IDO1, and PD-1/PD-L1 immunosuppressive actions.[29,30,70,72,74,75] In order to revert this immunosuppressive environment, ICIs have been combined with nanomaterials' mediated PTT/PDT (**Table 2**).

In this regard, combinatorial therapies based on nanomaterials' mediated PTT/PDT, immunostimulants' action and CTLA-4 blockade have been explored due to the capacity of the latter to restore T cell activation and inhibit the immunosuppressive activity of T regulatory (T_{reg}) cells (Table 2).[76] In this case, the Anti-CTLA-4 antibodies are generally intravenously administered after the nanomaterials' mediated PTT/PDT.[29,30]

Chen et al. demonstrated that the levels of T_{reg} cells in the secondary tumors were strongly decreased after treatment with R837 and ICG loaded poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles plus Anti-CTLA-4 antibody (Ab) (with and without NIR laser irradiation).[29] Furthermore, the highest infiltration of $CD8^+$ T cells in secondary tumors was observed for mice treated with R837 and ICG loaded PLGA nanoparticles combined with NIR light plus Anti-CTLA-4 Ab administration.[29] Hence, this treatment group displayed a high $CD8^+$ T/T_{reg} cells ratio.[29] Owing to these effects, the nanomaterials' mediated PTT and R837 delivery in combination with CTLA-4 blockade could eradicate the primary and secondary tumors as well as suppress the establishment of lung metastases.[29] Similar observations were reported by Xu et al. when investigating the effects on T_{reg} and $CD8^+$ T cells induced by the PDT mediated by R837 and Ce6 loaded PEGylated UCNPs in combination with Anti-CTLA-4 Ab administration (**Figure 4**).[30] In this case, this combinatorial immuno-photodynamic treatment led to the eradication of the primary tumor and strongly delayed the growth of the secondary tumor.[30]

IDO1 is mainly involved in the suppression of $CD8^+$ T cells and stimulation of T_{reg} cells through the effects of kynurenine.[77] Hence, several IDO1 inhibitors have been explored in combination with nanomaterials' mediated PTT/PDT (Table 2). For this purpose, these IDO1 inhibitors (e.g., NLG919, NLG8189, Epacadostat) have been incorporated on the nanomaterials' hydrophobic reservoirs, due to their poor solubility.[78,79] In this regard, Peng et al. verified that the PTT mediated by PEG-poly(ε-caprolactone) micelles co-encapsulating IR780 and NLG919 generated the highest infiltration of $CD8^+$ T cells in the secondary tumors as well as the greatest reduction in the T_{reg} cells in the spleen.[78] Such immuno-photothermal effect led to the eradication of the primary tumor as well as to a reduction in the growth of the secondary tumor and lung metastases.[78] In another work, the PDT generated by PEG-pyropheophorbide-a based liposomes (with and without NLG8189 encapsulated) induced the highest levels of $CD8^+$ T cells in the tumor.[79] However, a slight reduction on the levels of T_{reg} cells was only prompted by the NLG8189 loaded PEG-Pyropheophorbide-a based liposomes combined with NIR light.[79] This immuno-photodynamic effect induced the regression of the primary tumor, reduced the growth of the secondary tumor, and diminished the establishment of lung metastases.[79]

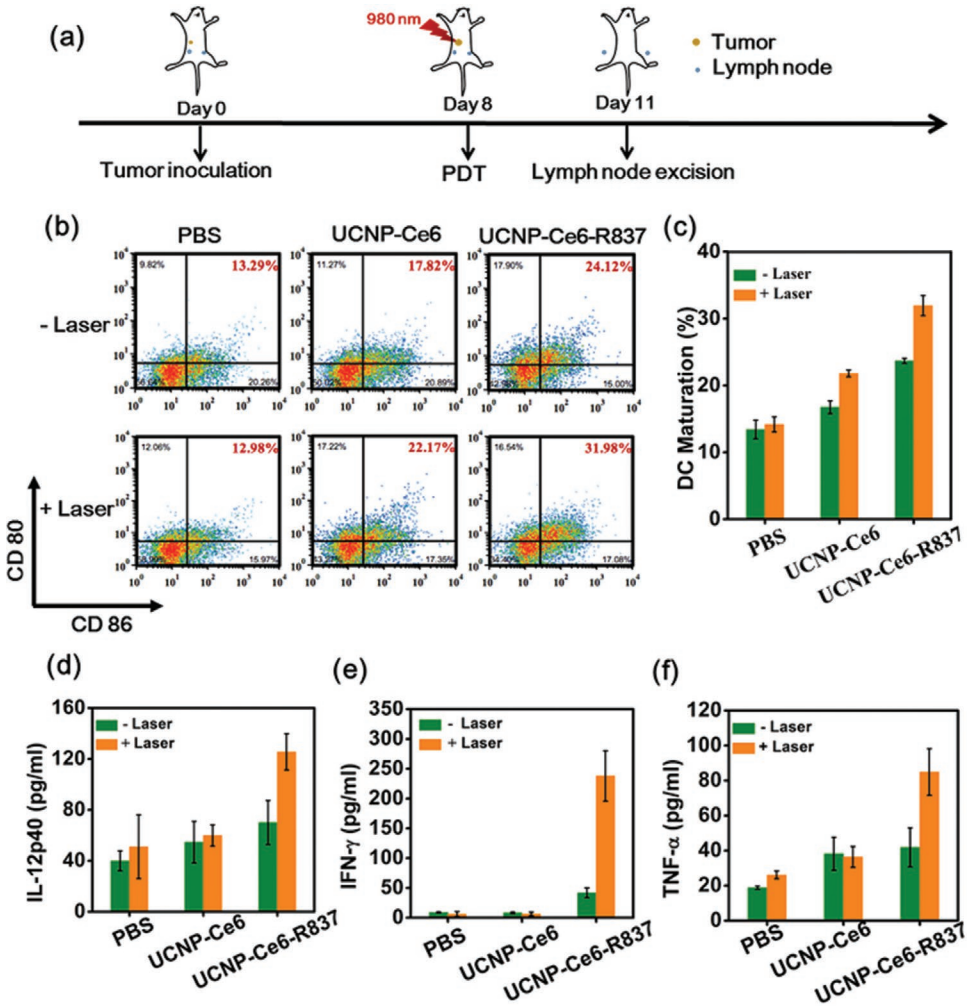

Figure 3. The effect of the PDT mediated by R837 and Ce6 loaded PEGylated UCNPs in DCs' maturation and pro-inflammatory cytokines' expression. a) Representation of the experimental approach. b,c) Levels of mDCs in the tumor-draining lymph nodes after the different treatments. Levels of d) IL-12p40, e) IFN-γ, and f) TNF-α in the sera of mice after the different treatments. Reproduced with permission.[30] Copyright 2017, American Chemical Society. UCNP-Ce6: Ce6 loaded PEGylated UCNPs; UCNP-Ce6-R837: R837 and Ce6 loaded PEGylated UCNPs; –Laser: non-irradiated; +Laser: NIR light irradiation.

Blockade of the PD-1 (expressed on T cells' surface)—PD-L1 (expressed on cancer cells) interaction is crucial to abolish its immunosuppressive effects on effector T cells[80]—Table 2. Similar to the Anti-CTLA-4 Ab, the Anti-PD-1 or Anti-PD-L1 Ab are generally administrated intravenously after nanomaterials' injection and laser irradiation.[74,81] In this regard, Yan et al. demonstrated that the PTT/PDT mediated by Ce6 loaded PEGylated UCNP/polydopamine nanoparticles, followed by Anti-PD-1 Ab administration, promoted the highest levels of $CD8^+$ T cells in the spleen.[81] Owing to this capacity, the nanomaterials' mediated PTT/PDT combined with PD-1 blockade could eradicate the primary tumor and almost prevented the establishment of metastases in all treated mice.[81] In turn, the sole use of nanomaterials' mediated PTT/PDT only eradicated the primary tumor, enabling an uncontrolled metastases' growth.[81]

Furthermore, PD-1/PD-L1 blockade in combination with nanomaterials' mediated PTT/PDT and other agents has also yielded promising results (Table 2). Lu and co-workers verified that the PTT mediated by R848 loaded PEGylated polydopamine nanoparticles followed by Anti-PD-L1 Ab administration induces higher levels of $CD8^+$ T cells in the primary and secondary tumor when compared to the nanomaterials' mediated R848 delivery plus PD-L1 blockade and to the nanomaterials mediated' PTT and R848 delivery.[74] Therefore, the nanomaterials mediated' PTT and R848 delivery combined with PD-L1 blockade promoted the best therapeutic outcome by inducing the strongest reduction in the primary and secondary tumors' growth.[74]

Yan and co-workers investigated the potential of the PTT mediated by FA-functionalized PEGylated rGO loaded with Epacadostat in conjugation with Anti-PD-L1 Ab administration.[40] This combinatorial treatment induced the highest levels of $CD8^+$ T cells in the secondary tumors as well as the strongest increase in the ratio of $CD8^+$ T/T_{reg} cells.[45] Such immune-photothermal effects enabled the eradication of the primary tumor and a stark decrease in the growth of the secondary tumor.[45]

Table 1. The effect of PTT/PDT mediated by nanomaterials in combination with ICIs/immunostimulants on DCs' maturation.

Nanoformulation	Immuno-agent	Photo-agent	Observations	Ref.
Chitosan and CpG ODNs-coated hollow CuS nanoparticles	CpG ODNs	CuS nanoparticles	CpG/CuS NPs combined with NIR light induced 5.8- and 14.2-fold higher levels of mDCs in the tumor than CpG/CuS NPs and CuS NPs plus NIR light, respectively. CpG/CuS NPs combined with NIR light induced 2.9- and 6.7-fold higher levels of mDCs in the tumor-draining lymph nodes than CpG/CuS NPs and CuS NPs plus NIR light, respectively.	[40]
R837 and ICG loaded PLGA nanoparticles	R837	ICG	R837/ICG/PLGA NPs combined with NIR light induced 1.3 and 1.2-times higher levels of mDCs in the tumor-draining lymph nodes than R837/ICG/PLGA and ICG/PLGA plus NIR light, respectively.	[29]
R837 and Ce6 loaded PEGylated UCNPs	R837	Ce6	R837/Ce6/UCNPs combined with NIR light induced 1.4- and 1.5-times higher levels of mDCs in the tumor-draining lymph nodes than R837/Ce6/UCNPs and Ce6/UCNPs plus NIR light, respectively.	[30]
R837 and Fe_3O_4 loaded PEG-PLGA nanoparticles	R837	Fe_3O_4 nanoparticles	R837/ Fe_3O_4 /PEG-PLGA NPs combined with NIR light induced 1.4- and 1.8-fold higher levels of mDCs in the tumor-draining lymph nodes than R837/Fe_3O_4/PEG-PLGA NPs and Fe_3O_4/PEG-PLGA NPs plus NIR light, respectively.	[72]
CpG loaded PEG-Chitosan -Ce6-based MSNs[a)]	CpG ODNs	Ce6	CpG/Ce6-MSNs combined with laser radiation induced 1.3-fold higher levels of mDCs in the tumor than Ce6-MSNs plus laser radiation.	[22]
AUNP12 and HAuNS[b)] loaded PLGA based nanoparticles	AUNP12; CpG ODNs (non-loaded)	HAuNS	AUNP12/HAuNS/PLGA NPs combined with NIR light plus CpG ODNs administration led to the highest levels of mDCs in the secondary tumor and spleen.	[70]
Polydopamine-Al_2O_3 nanoparticles	Al_2O_3; CpG ODNs (non-loaded)	Polydopamine	Polydopamine-Al_2O_3 NPs combined with NIR light plus CpG ODNs administration induced high levels of mDCs in the tumor-draining lymph nodes.	[90]
IR780 and Imatinib loaded PEGylated GITR[c)]-functionalized PLGA-based nanoparticles	Imatinib	IR780	IR780/Imatinib/GITR-PLGA NPs combined with NIR light induced high levels of mDCs in the tumor.	[91]
Epacadostat loaded PEG/FA/rGO	Epacadostat	rGO	Epacadostat/PEG/FA/rGO combined with NIR light induced 1.2-fold higher levels of mDCs in the lymph node near the tumor site than PEG/FA/rGO plus NIR light.	[45]
ACF[d)]-loaded HA[e)] and CpG-coated H_2TCPP[f)] based MOFs[g)]	CpG ODNs; ACF	H_2TCPP	CpG/ACF/H_2TCPP MOFs combined with laser radiation induced 1.2-fold higher levels of mDCs in tumor-draining lymph nodes than CpG/ACF/H_2TCPP MOFs and ACF/H_2TCPP MOFs plus laser radiation. This treatment also induced 1.4-fold higher levels of mDCs than ACF/H_2TCPP MOFs.	[92]
NLG919 incorporating PEG-Ppa[h)] based vesicles	NLG919	PPa	NLG919/PPa vesicles combined with laser radiation led to 1.5-fold higher levels of mDCs in the tumor-draining lymph nodes than NLG919/PPa vesicles.	[93]
CpG coated polydopamine/Ce6-GQD[i)] based nanoparticles	CpG ODNs	Ce6; polydopamine; GQD	CpG/polydopamine/Ce6-GQD NPs combined with laser radiation induced 1.2- and 1.4-fold higher levels of mDCs in the lymph nodes than CpG/polydopamine/Ce6-GQD NPs and polydopamine/Ce6-GQD NPs plus laser radiation, respectively.	[94]
1MT[j)]-IR820 nanoparticles	1MT; Anti-PD-L1 Ab (non-loaded)	IR820	1MT-IR820 NPs combined with laser radiation plus Anti-PD-L1 Ab administration induced the highest levels of mDCs in the tumor-draining lymph nodes.	[83]
CpG-Pd nanosheets	CpG ODNs	Pd nanosheets	CpG-Pd nanosheets combined with NIR light induced high levels of mDCs in the tumor, spleen, and blood.	[73]
Ce6 and aluminum hydroxide loaded BSA[k)] nanoparticles	Aluminum hydroxide; CpG ODNs (non-loaded)	Ce6	Ce6/aluminum hydroxide/BSA nanoparticles combined with laser radiation plus CpG ODNs administration led to 1.6-fold higher levels of mDCs in the tumor-draining lymph node than Ce6/aluminum hydroxide/BSA nanoparticles plus laser radiation.	[33]

[a)]Mesoporous silica nanoparticles; [b)]Hollow gold nanospheres; [c)]Glucocorticoid-induced TNFR-related (GITR) Protein; [d)]Acriflavine; [e)]Hyaluronic acid; [f)]Meso-tetra(4-carboxyphenyl)porphyrin; [g)]Metal-organic frameworks; [h)]Pyropheophorbide-a; [i)]Graphene quantum dots; [j)]1-methyl-tryptophan; [k)]Bovine serum albumin.

3.3. Nanomaterials' Mediated PTT/PDT in Combination with ICIs/Immunostimulants Can Generate Immune Memory That Prevents Tumor Recurrence

The photothermal/photodynamic effects mediated by nanomaterials in combination with ICIs/immunostimulating agents can facilitate the establishment of immune memory that protects against tumor recurrence (**Table 3**). In this case, the effector memory T (T_{EM}) cells play a crucial role in rapidly generating an antitumoral response after reencountering the TAA.[82]

In this context, Xu et al. verified that the levels of T_{EM} cells in the spleen after the PDT mediated by R837 and Ce6 loaded PEGylated UCNPs in combination with CTLA-4 blockade were strongly increased by up to 3.9-fold (compared to control).[30] Due to this effect, mice that received this treatment regimen resisted tumor re-inoculation.[30] Interestingly, the nanomaterials' mediated R837 delivery in conjugation with

Table 2. The effect of PTT/PDT mediated by nanomaterials in combination with ICIs/immunostimulants on the T cell populations and therapeutic outcome.

Nanoformulation	Immuno-agent	Photo-agent	Observations	Ref.
Chitosan and CpG ODNs-based hollow CuS nanoparticles	CpG ODNs	Hollow CuS nanoparticles	CpG/CuS NPs combined with NIR light induced 1.8- and 3.5-fold higher levels of IFN-γ-secreting $CD8^+$ T cells in the tumor than CpG/CuS NPs and CuS NPs plus NIR light, respectively.	[40]
			CpG/CuS NPs combined with NIR light induced 2.8- and 5.1-fold higher levels of IFN-γ-secreting $CD8^+$ T cells in the tumor-draining lymph nodes than CpG/CuS NPs and CuS NPs plus NIR light, respectively.	
			CpG/CuS NPs combined with NIR light induced 1.9- and 2.3-fold higher levels of IFN-γ-secreting $CD8^+$ T cells in the secondary tumor than CpG/CuS NPs and CuS NPs plus NIR light, respectively.	
			CpG/CuS NPs combined with NIR light induced the strongest decrease in the growth of the primary and secondary tumors.	
CpG loaded PEI[a]-PEG-GO	CpG ODNs	GO	CpG/PEI-PEG-GO combined with NIR light prompted the most potent effect by inducing tumor's regression	[95]
PEGylated SWCNTs[b]	Anti-CTLA-4 Ab (non-loaded)	SWCNTs	PEG/SWCNTs combined with NIR light plus Anti-CTLA-4 Ab administration induced 1.4- and 1.5-fold higher levels of $CD8^+$ T cells in the secondary tumor than Anti-CTLA-4 Ab administration and PEG/SWCNT plus NIR light, respectively.	[96]
			PEG/SWCNTs combined with NIR light plus Anti-CTLA-4 Ab administration induced 2.7- and 9.4-fold lower levels of T_{reg} cells in the secondary tumor than Anti-CTLA-4 Ab administration and PEG/SWCNT plus NIR light, respectively.	
			PEG/SWCNTs combined with NIR light plus Anti-CTLA-4 Ab administration led to the highest ratio of $CD8^+$ T/T_{reg} cells in the secondary tumor.	
			PEG/SWCNTs plus NIR, with or without Anti-CTLA-4 Ab administration, induced primary tumor eradication and induced the strongest decrease in the growth of the secondary tumor.	
			PEG/SWCNTs combined with NIR light plus Anti-CTLA-4 Ab administration can reduce the number of lung metastases.	
PEGylated and pyrolipid incorporating ZnP[c] based nanoparticles	Anti-PD-L1 Ab (non-loaded)	Pyrolipid	Pyrolipid/ZnP NPs combined with laser radiation followed by Anti-PD-L1 Ab administration led to primary tumor eradication and decreased the growth of the secondary tumor.	[97]
			Pyrolipid/ZnP NPs combined with laser radiation plus Anti-PD-L1 Ab administration almost abolished the occurrence of lung metastases.	
R837 and ICG loaded PLGA nanoparticles	R837; Anti-CTLA-4 Ab (non-loaded)	ICG	R837/ICG/PLGA NPs combined with NIR light plus Anti-CTLA-4 Ab administration induced 1.6- and 2.1-fold higher levels of $CD8^+$ T cells in the secondary tumor than R837/ICG/PLGA NPs plus Anti-CTLA-4 Ab and R837/ICG/PLGA NPs plus NIR light, respectively.	[29]
			R837/ICG/PLGA NPs plus Anti-CTLA-4 Ab administration, with or without NIR exposure, led to low levels of T_{reg} cells in the secondary tumor.	
			R837/ICG/PLGA NPs combined with NIR light plus Anti-CTLA-4 Ab administration led to the highest ratio of $CD8^+$ T/T_{reg} cells ratio in the secondary tumor.	
			R837/ICG/PLGA NPs combined with NIR light, with or without Anti-CTLA-4 Ab administration led to the primary tumor eradication and could eradicate the secondary tumors.	
			R837/ICG/PLGA NPs combined with NIR light plus Anti-CTLA-4 Ab administration almost totally suppressed the occurrence of lung metastases.	
R837 and Ce6 loaded PEGylated UCNPs	R837; Anti-CTLA-4 Ab (non-loaded)	Ce6	R837/Ce6/UCNPs combined with NIR light plus Anti-CTLA-4 Ab administration induced 2.1- and 2.3-fold higher levels of $CD8^+$ T cells in the secondary tumor than R837/Ce6/UCNPs plus NIR light and R837/Ce6/UCNPs plus Anti-CTLA-4 Ab, respectively.	[30]
			R837/Ce6/UCNPs plus Anti-CTLA-4 Ab administration, with or without NIR exposure, strongly decreased the levels of T_{reg} cells in the secondary tumor.	
			R837/Ce6/UCNPs combined with NIR light plus Anti-CTLA-4 Ab administration induced 4.0- and 2.0-fold higher levels of $CD8^+$ T/T_{reg} cells ratio in the secondary tumor than R837/Ce6/UCNPs plus NIR light and R837/Ce6/UCNPs plus Anti-CTLA-4 Ab, respectively.	

Table 2. Continued.

Nanoformulation	Immuno-agent	Photo-agent	Observations	Ref.
			R837/Ce6/UCNPs combined with NIR light plus Anti-CTLA-4 Ab administration led to the primary tumor eradication and strongly delayed the growth of the secondary tumor	
NLG919 and IR780 loaded PEG-PCL[d] micelles	NLG919	IR780	NLG919/IR780/PEG-PCL micelles combined with NIR light induced 2.3- and 3.9-fold higher levels of $CD8^+$ T cells in the secondary tumor than NLG919/IR780/PEG-PCL micelles and IR780/PEG-PCL micelles plus NIR light, respectively.	[78]
			NLG919/IR780/PEG-PCL micelles combined with NIR light induced 1.2- and 1.6-fold higher levels of $CD8^+$ T cells in the spleen than NLG919/IR780/PEG-PCL micelles and IR780/PEG-PCL micelles plus NIR light, respectively.	
			NLG919/IR780/PEG-PCL micelles combined with NIR light induced the lowest levels of T_{reg} cells in the spleen.	
			NLG919/IR780/PEG-PCL micelles plus NIR light could induce the ablation of the primary tumor as well as reduce the growth of the secondary tumor.	
			NLG919/IR780/PEG-PCL micelles plus NIR light reduced the establishment of lung metastases.	
R837 and Fe_3O_4 loaded PEG-PLGA nanoparticles	R837; Anti-PD-L1 (non-loaded)	Fe_3O_4 Nanoparticles	R837/Fe_3O_4/PEG-PLGA NPs combined with NIR light plus Anti-PD-L1 Ab administration induced the highest levels of $CD8^+$ T cells in the secondary tumor.	[72]
			Fe_3O_4/PEG-PLGA NPs combined with NIR light, with and without R837/Anti-PD-L1 Ab, led to primary tumor eradication.	
			R837/Fe_3O_4/PEG-PLGA nanoparticles combined with NIR light plus Anti-PD-L1 Ab administration induced the strongest reduction in the growth of the secondary tumors.	
			R837/Fe_3O_4/PEG-PLGA NPs combined with NIR light plus Anti-PD-L1 Ab administration suppressed the establishment of lung and liver metastases.	
CpG loaded PEG-Chitosan-Ce6-based MSNs	CpG ODNs	Ce6	CpG/Ce6-MSNs combined with laser irradiation induced the strongest reduction in the tumor's growth.	[22]
AUNP12 and HAuNS loaded PLGA based nanoparticles	AUNP12; CpG ODNs (non-loaded)	HAuNS	AUNP12/HAuNS/PLGA NPs combined with NIR light plus CpG ODNs administration led to the highest levels of $CD8^+$ T cells in the secondary tumor and spleen.	[70]
			AUNP12/HAuNS/PLGA NPs combined with NIR light plus CpG ODNs administration led to the lowest levels of T_{reg} in the secondary tumor and spleen.	
			HAuNS/PLGA NPs combined with NIR light, with or without AUNP12/CpG ODNs, induced the regression of the primary tumor.	
			AUNP12/HAuNS/PLGA nanoparticles combined with NIR light plus CpG ODNs administration induced the strongest decrease in the growth of the secondary tumor.	
PpIX[e]-1MT based nanoparticles	1MT	PpIX	PpIX-1MT NPs combined with laser radiation induced tumor regression.	[98]
			PpIX-1MT NPs combined with laser radiation could suppress the establishment of lung metastases.	
Polydopamine-Al_2O_3 nanoparticles	Al_2O_3; CpG ODNs (non-loaded)	Polydopamine	Polydopamine-Al_2O_3 NPs combined with NIR light, plus CpG ODNs administration, led to high levels of $CD8^+$ T cells in the tumor-draining lymph nodes.	[90]
			Polydopamine-Al_2O_3 NPs combined with NIR light plus CpG ODNs administration led to a strong tumor regression.	
IR780 and Imatinib loaded PEGylated GITR-functionalized PLGA-based nanoparticles	Imatinib	IR780	IR780/Imatinib/GITR-PLGA NPs combined with NIR light increased the activity of the tumor-homed $CD8^+$ T cells.	[91]
			IR780/Imatinib/GITR-PLGA NPs combined with NIR light led to low levels of T_{reg} cells in the tumor.	
			IR780/Imatinib/GITR-PLGA NPs combined with NIR light induced tumor eradication.	
IR820 loaded PEGylated TPP[f]- and CpG-functionalized GO[g]	DSPE[h]-PEG-CpG	GO; IR820	IR820/TPP/CpG/GO nanoparticles combined with NIR light induced the strongest reduction in the growth of the tumor.	[99]
Epacadostat loaded PEG/FA/rGO	Epacadostat; Anti-PD-L1 Ab (non-loaded)	rGO	Epacadostat/PEG/FA/rGO combined with NIR light plus Anti-PD-L1 Ab administration led to 6.2-fold higher levels of $CD8^+$ T cells in the secondary tumor than Epacadostat/PEG/FA/rGO plus NIR light and Epacadostat/PEG/FA/rGO plus Anti-PD-L1 Ab.	[45]

Table 2. Continued.

Nanoformulation	Immuno-agent	Photo-agent	Observations	Ref.
			Epacadostat/FA/PEG/rGO combined with NIR light plus Anti-PD-L1 Ab administration led to 3.3- and 2.7-fold higher ratio of $CD8^+$ T/T_{reg} cells in the secondary tumors than Epacadostat/PEG/FA/rGO plus Anti-PD-L1 Ab and Epacadostat/PEG/FA/rGO plus NIR light, respectively.	
			FA/PEG/rGO plus NIR light, with and without Epacadostat/Anti-PD-L1 Ab, induced primary tumor eradication.	
			Epacadostat/FA/PEG/rGO combined with NIR light plus Anti-PD-L1 Ab administration led to the greatest reduction in the growth of the secondary tumor.	
ACF and CpG loaded HA-coated H_2TCPP based MOFs	CpG ODNs; ACF	H_2TCPP	CpG/ACF/H_2TCPP MOFs combined with laser radiation induced tumor regression.	[92]
NLG919 incorporating PEG-PPa based vesicles	NLG919	PPa	PEG-PPa vesicles combined with laser radiation, with or without NLG919, induced the highest levels of $CD8^+$ T cells in the tumor.	[93]
			NLG919/PPa vesicles combined with laser radiation led to 2.9- and 2.4-fold lower levels of T_{reg} cells in the tumor than PEG-PPa vesicles plus laser radiation and NLG919/PEG-PPa vesicles, respectively.	
			NLG919/PPa vesicles combined with laser radiation led to 2.8 and 2.7-fold higher ratio of $CD8^+$ T/T_{reg} cells in the tumor than PEG-PPa vesicles plus laser radiation and NLG919/PEG-PPa vesicles, respectively.	
			NLG919/PPa vesicles combined with laser radiation induced the most potent antitumoral effect.	
CpG and Fe_3O_4 loaded PEG-PLGA-PLL[i)] nanoparticles	CpG ODNs	Fe_3O_4	CpG/Fe_3O_4/PEG-PLGA-PLL NPs combined with laser radiation could induce the eradication of the primary and secondary tumors.	[71]
NLG8189 loaded PEG-PPa-based liposomes	NLG8189	PPa	PEG-PPa-based liposomes combined with laser radiation, with or without NLG8189, led to the highest levels of $CD8^+$ T cells in the tumor.	[79]
			NLG8189/PEG-PPa-based liposomes combined with NIR light decreased the levels of T_{reg} cells in the blood.	
			NLG8189/PEG-PPa-based liposomes combined with laser radiation induced the regression of the primary tumor and the strongest reduction in the growth of the secondary tumor	
			NLG8189/PEG-PPa-based liposomes combined with laser radiation almost completely suppressed the establishment of lung metastases.	
R848 loaded PEGylated polydopamine nanoparticles	R848; Anti-PD-L1 Ab (non-loaded)	Polydopamine	R848/polydopamine NPs combined with NIR light, followed by Anti-PD-L1 Ab administration, led to the highest levels of T $CD8^+$ cells in the primary and secondary tumors.	[74]
			R848/polydopamine NPs combined with NIR light, followed by Anti-PD-L1 Ab administration, induced the strongest reduction in the growth of the primary and secondary tumors.	
CpG coated polydopamine/Ce6-GQD based nanoparticles	CpG ODNs	Ce6; polydopamine; GQD	CpG/polydopamine/Ce6-GQD based nanoparticles combined with laser radiation induced the strongest reduction in the growth of the tumor.	[94]
Ce6 loaded PEGylated UCNP/polydopamine nanoparticles	Anti-PD-1 Ab (non-loaded)	Ce6, polydopamine	Ce6/PEG/UCNP/polydopamine NPs combined with NIR light plus Anti-PD-1 Ab administration led to 1.2- and 1.7-fold higher levels of $CD8^+$ T cells in the spleen than Ce6/PEG/UCNP/polydopamine nanoparticles plus NIR light and Ce6/PEG/UCNP/polydopamine nanoparticles plus Anti-PD-1 Ab, respectively.	[81]
			Ce6/PEG/UCNP/polydopamine NPs plus NIR light, with or without Anti-PD-1 Ab administration could induce the eradication of the primary tumor.	
			Ce6/PEG/UCNP/polydopamine NPs combined with NIR plus Anti-PD-1 Ab administration almost completely suppressed the establishment of lung metastases.	
LyP-1 peptide functionalized PEGylated ^{D}PPA-1 peptide-Au-Pt nanohybrids	^{D}PPA-1 (encapsulated and non-encapsulated)	Au-Pt hybrids	LyP-1 functionalized PEG-^{D}PPA-1-Au-Pt nanohybrids combined with NIR light, followed by additional ^{D}PPA-1 administration, induced the eradication of the primary tumor and could eliminate some of the secondary tumors.	[85]
			LyP-1 functionalized PEG-^{D}PPA-1-Au-Pt nanohybrids combined with NIR light, followed by additional ^{D}PPA-1 administration, reduced the occurrence of lung metastases.	
1MT-IR820 nanoparticles	1MT; Anti-PD-L1 Ab (non-loaded)	IR820	1MT-IR820 NPs combined with laser radiation plus Anti-PD-L1 Ab administration led to the highest levels of $CD8^+$ T cells in the primary and secondary tumor.	[83]
			1MT-IR820 NPs combined with laser radiation plus Anti-PD-L1 Ab administration induced the highest ratio of $CD8^+T/T_{reg}$ cells in the primary tumor.	

Table 2. Continued.

Nanoformulation	Immuno-agent	Photo-agent	Observations	Ref.
			1MT-IR820 NPs combined with laser radiation plus Anti-PD-L1 Ab administration induced the strongest reduction in the growth of the primary and secondary tumors.	
R848 loaded chitosan-polyaniline nanoparticles	R848	Polyaniline	R848/chitosan-polyaniline NPs combined with NIR light could induce tumor eradication in some mice.	[31]
CpG-Pd nanosheets	CpG ODNs	Pd nanosheets	CpG-Pd nanosheets combined with NIR light induced 1.7- and 1.3-fold higher levels of $CD8^+$ T cells in the tumor and spleen, respectively, than Pd nanosheets plus NIR light.	[73]
			CpG-Pd nanosheets combined with NIR light induced tumor regression.	
ICG loaded PEG-Epacadostat conjugate nanoparticles	Epacadostat, Anti-PD-L1 Ab (non-loaded)	ICG	ICG/PEG-Epacadostat nanoparticles combined with NIR light plus Anti-PD-L1 Ab administration induced 2.3- and 1.6-fold higher levels of $CD8^+$ T cells in the secondary tumor than ICG/PEG-Epacadostat NPs plus Anti-PD-L1 Ab and ICG/PEG-Epacadostat NPs plus NIR light, respectively.	[100]
			ICG/PEG-Epacadostat NPs combined with NIR light plus Anti-PD-L1 Ab administration led to 1.9- and 2.2-fold higher ratio of $CD8^+$ T/T_{reg} cells in the secondary tumors than ICG/PEG-Epacadostat NPs plus Anti-PD-L1 Ab and ICG/PEG-Epacadostat NPs plus NIR light, respectively.	
			ICG/PEG-Epacadostat NPs combined with NIR light, with or without Anti-PD-L1 Ab administration, led to the eradication of the primary tumor.	
			ICG/PEG-Epacadostat NPs combined with NIR light plus Anti-PD-L1 Ab administration induced the strongest reduction in the growth of the secondary tumor.	
PEGylated Fe_3O_4 nanoparticles	Anti-CTLA-4 Ab (non-loaded)	Fe_3O_4 nanoparticles	PEGylated Fe_3O_4 nanoparticles combined with NIR light plus by Anti-CTLA-4 Ab administration induced the eradication of the primary tumor and prevented the establishment of the secondary tumors.	[101]
CpG/PEI/Prussian blue nanoparticles	CpG ODNs; Anti-CTLA-4 Ab (non-loaded)	Prussian blue nanoparticles	Prussian blue NPs combined with NIR light, with and without CpG/Anti-CTLA-4 Ab, induced the eradication of the primary tumor.	[75]
			CpG/PEI/Prussian blue NPs combined with NIR light plus Anti-CTLA-4 Ab could induce the eradication of the majority of the secondary tumors.	
Ce6 and Aluminum hydroxide loaded BSA nanoparticles	Aluminum hydroxide; CpG ODNs (non-loaded)	Ce6	Ce6/Aluminum hydroxide/BSA NPs combined with laser radiation, with or without CpG ODNs administration, led to the highest levels of $CD8^+$ T cells in the primary and secondary tumors.	[33]
			Ce6/Aluminum hydroxide/BSA NPs combined with laser radiation plus CpG ODNs administration, can induce the eradication of the primary tumor and reduced the growth of the secondary tumor.	
			Ce6/Aluminum hydroxide/BSA NPs combined with laser radiation plus CpG ODNs administration reduced the occurrence of lung metastases.	

[a]Poly(ethylenimine); [b]Single-walled carbon nanotubes; [c]Zn-pyrophosphate; [d]Poly(caprolactone); [e]Protoporphyrin IX; [f]Alkyl triphenylphosphonium; [g]Graphene oxide; [h]DSPE: 1,2-distearoyl-3-phosphatidylethanolamine; [i]Poly(L-lysine).

CTLA-4 blockade did not enhance the levels of T_{EM}, and hence the re-inoculated tumors in this group grew at a rate comparable to the control.[30]

In another work, treatment with R837 and ICG loaded PLGA nanoparticles combined with NIR light plus Anti-CTLA-4 Ab administration increased the levels of T_{EM} cells in the spleen by 2.3-fold (compared to the control) (**Figure 5**).[29] In turn, the same treatment regimen without CTLA-4 blockade (R837 and ICG loaded PLGA nanoparticles plus NIR light) just enhanced the T_{EM} cells levels by 1.7-fold.[29] Despite the ability of these two treatment regimens to increase the levels of T_{EM} cells, only the re-inoculated tumors of mice treated with the nanomaterials' mediated PTT and R837 delivery combined with CTLA-4 blockade had a meaningfully slower growth.[29] The combined effect of the PTT/PDT mediated by Ce6 loaded PEGylated UCNP/polydopamine nanoparticles and Anti-PD-1 Ab administration was capable of inducing 1.5- and 2.5-fold higher levels of T_{EM} cells in the spleen than the nanomaterials' mediated PTT/PDT (Ce6/PEG/UCNP/polydopamine nanoparticles plus NIR light) and PD-1 blockade (Ce6/PEG/UCNP/polydopamine nanoparticles plus Anti-PD-1 Ab), respectively.[81]

Furthermore, the nanomaterials' mediated PTT/PDT combined with i) immunostimulants,[31] ii) immunostimulants and PD-1/PD-L1 blockade,[70] and iii) PD-1/PD-L1 blockade and IDO1 inhibition[83] could also generate an effect that delayed/impaired the growth of the re-inoculated tumors. Together these studies suggest that the nanomaterials' mediated PTT/PDT and

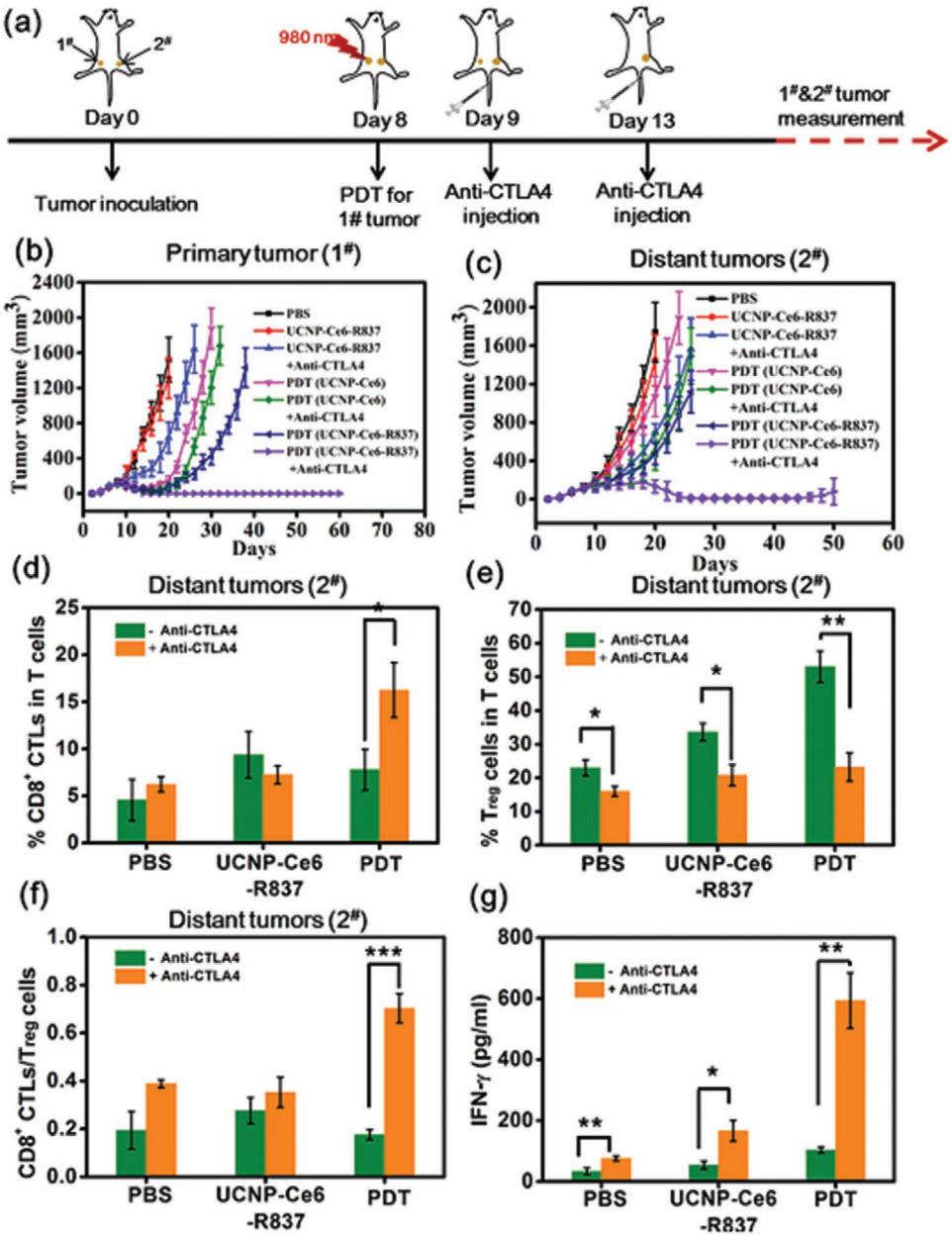

Figure 4. PDT mediated by R837 and Ce6 loaded PEGylated UCNPs in combination with Anti-CTLA-4 Ab administration. a) Representation of the experimental approach. Growth curves of b) the primary and c) secondary tumors after the different treatments. Percentage of d) $CD8^+$ T cells, e) T_{regs} cells, and f) ratio of $CD8^+$ T/T_{regs} cells in the secondary tumor after the different treatments. g) IFN-γ levels in the sera of mice after the different treatments. Reproduced with permission.[30] Copyright 2017, American Chemical Society. UCNP-Ce6-R837: R837 and Ce6 loaded PEGylated UCNPs; PDT: R837 and Ce6 loaded PEGylated UCNPs plus NIR light.

the immunomodulation induced by ICIs/immunostimulants are both required to achieve immune memory that can meaningfully prevent/delay tumor recurrence (Table 3).

4. Conclusion and Outlook

In this progress report, the potential of nanomaterials' mediated PTT/PDT in combination with ICI-based immunotherapies for treating metastatic cancer was analyzed.

In general, the nanomaterials' photothermal/photodynamic effects can per se trigger several events that unlock a venue for an improved antitumoral immunotherapy. The hallmarks of nanomaterials' mediated PTT/PDT include their ability to induce the release of TAA and DAMPs, as well as their capacity to relieve tumor hypoxia and trigger a pro-inflammatory response.

By combining the nanomaterials' photothermal/photodynamic effects with the action of immunostimulants, greater levels of DCs' maturation could be achieved. In many cases,

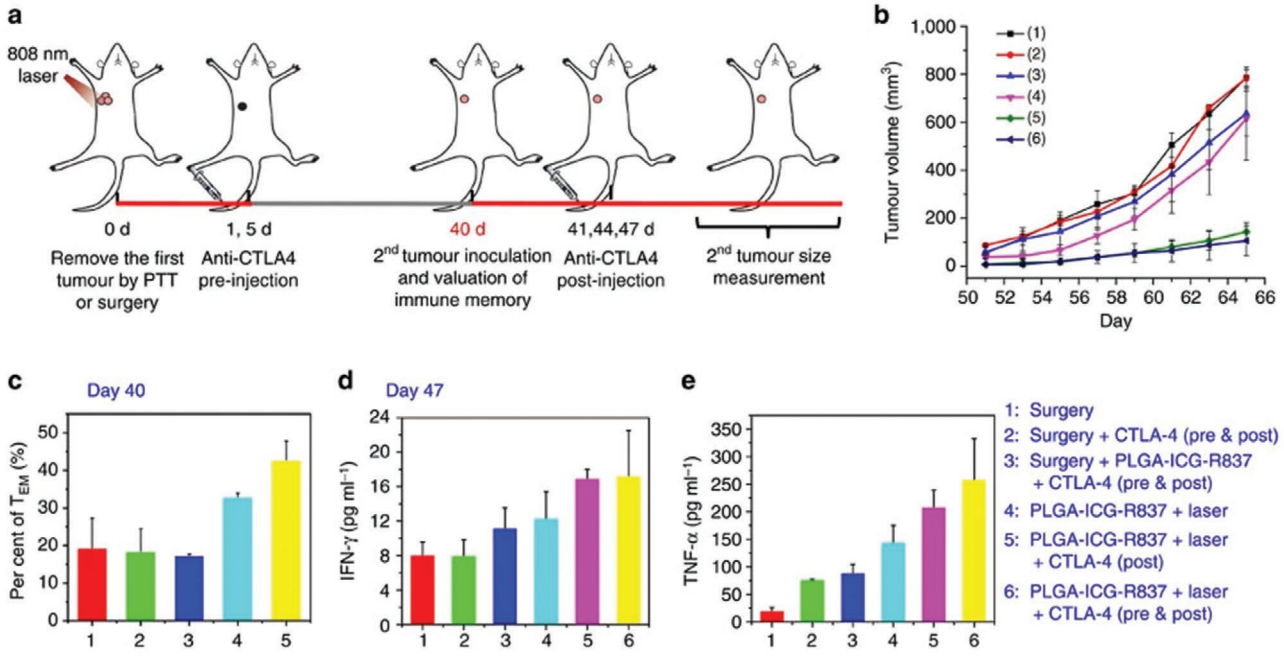

Figure 5. The effect of the PTT mediated by R837 and ICG loaded PLGA nanoparticles in combination with Anti-CTLA-4 Ab administration in the creation of immune memory. a) Representation of the experimental design. b) Growth curves of the re-inoculated tumors after the elimination of the primary tumors (through surgery or PTT mediated by R837 and ICG loaded PLGA nanoparticles) and after several Anti-CTLA-4 Ab administrations. c) Percentage of T_{EM} cells in the spleen before tumor re-inoculation. d) IFN-γ and e) TNF-α levels in the sera of the mice after tumor re-inoculation. Reproduced under the terms of the Creative Commons Attribution 4.0 International License.[29] Copyright 2016, The Authors, published by Springer Nature. PLGA-ICG-R837: R837 and ICG loaded PLGA nanoparticles; laser: NIR light irradiation.

the nanomaterials' photothermal/photodynamic effect alone could also eradicate the primary tumor. However, in order to achieve an abscopal effect on the secondary tumor/metastases, the additional inclusion of ICIs was fundamental to abrogate the immunosuppressive actions induced by CTLA-4, IDO1, and PD-1/PD-L1. Such enabled effective responses mediated by $CD8^+$ T cells against the secondary tumors/metastasized cancer cells. On the other hand, the nanomaterials' mediated PTT/PDT and the immunomodulation induced by ICIs/immunostimulants greatly improved the levels of T_{EM} cells, which are crucial for preventing/delaying tumor recurrence.

Despite the nanomaterials' ability to coordinate the delivery of ICIs and immunostimulants to the tumor site, the ICIs/immunostimulants were in many studies administered in their non-encapsulated form. Such may be related to the water solubility of some ICIs (antibodies against PD-1/PD-L1 and CTLA-4) and immunostimulants (CpG ODNs). This factor renders the encapsulation of these agents in nanomaterials a complex process since most nanostructures have a hydrophobic core, thus excelling in the encapsulation of hydrophobic agents (e.g., R837, NLG919). On the other hand, the different events occurring in cancer immuno-PTT/PDT have a non-overlapped character in the temporal scale. For instance, first, the nanomaterials' photothermal/photodynamic effect must occur in order to trigger the release of TAA/DAMPs. Only after that is the optimal time point for immunostimulants' action (to aid in DCs' maturation), and later the most appropriate timeframe for the blockade of the immune checkpoints (to enable/recover antitumoral T response). In this way, in the future, the incorporation of nanomaterials, immunostimulants, and ICIs in macroscale delivery systems (e.g., injectable hydrogels, and microneedles) can pave the way for a sequential delivery of these agents in a rationale fashion. Considering that some nanomaterials display a poor tumor-homing capacity after systemic administration,[84] their local delivery using these macroscale delivery systems may also be fundamental for their future translation into the clinic. Such strategy can also enable the use of simpler nanomaterials (i.e., with fewer complex assemblies/structures), hence facilitating their production at a larger scale. Another important consideration is related to the long-term safety of the nanomaterials employed in cancer PTT/PDT. In this context, long-term biocompatibility studies must be performed, and the use of biodegradable and/or easily cleared nanomaterials should be favored. Moreover, the use of peptides (e.g., ^{D}PPA-1,[85] TPP-1[86]) that can perform immune checkpoint blockade is also a future perspective that can reduce the costs associated with the use of antibodies, hence contributing to the translation of this therapeutic strategy. To achieve an even better therapeutic outcome, the combination of nanomaterials' mediated immuno-PTT/PDT with additional modalities (e.g., chemotherapy,[87] radiotherapy[88]) is an exciting approach that has been showing promising results. Similarly, combinatorial strategies involving the use of pyroptotic agents may lead to an enhanced immune system activation, thus deserving further investigations.[89]

Overall, the continuous investigation of the synergies occurring between the nanomaterials' mediated PTT/PDT and the

Table 3. The effect of PTT/PDT mediated by nanomaterials in combination with ICIs/immunostimulants on immune memory creation and tumor recurrence prevention.

Nanoformulation	Immuno-agent	Photo-agent	Observations	Ref.
Prussian blue nanoparticles	Anti-CTLA-4 Ab (non-loaded)	Prussian blue nanoparticles	The mice treated with Prussian blue NPs combined with NIR light plus Anti-CTLA-4 Ab administration resisted tumor re-inoculation.	[102]
R837 and ICG loaded PLGA nanoparticles	R837; Anti-CTLA-4 Ab (non-loaded)	ICG	R837/ICG/PLGA NPs combined with NIR light plus Anti-CTLA-4 Ab administration led to 1.3- and 2.5-fold higher levels of T_{EM} cells in the spleen than R837/ICG/PLGA NPs plus NIR light and R837/ICG/PLGA NPs plus Anti-CTLA-4 Ab, respectively.	[29]
			The re-inoculated tumors of mice treated with R837/ICG/PLGA NPs combined with NIR light plus Anti-CTLA-4 Ab administration presented a delayed growth.	
R837 and Ce6 loaded PEGylated UCNPs	R837, Anti-CTLA-4 Ab (non-loaded)	Ce6	R837/Ce6/UCNPs combined with NIR light plus Anti-CTLA-4 Ab administration led to 2.7-fold higher levels of T_{EM} cells in the spleen than R837/Ce6/UCNPs plus Anti-CTLA-4 Ab.	[30]
			The mice treated with R837/Ce6/UCNPs combined with NIR light plus Anti-CTLA-4 Ab administration resisted tumor re-inoculation.	
AUNP12 and HAuNS loaded PLGA based nanoparticles	AUNP12; CpG ODNs (non-loaded)	HAuNS	The re-inoculated tumors of mice treated with AUNP12/HAuNS/PLGA NPs combined with NIR light plus CpG ODNs administration presented the slowest growth.	[70]
Ce6 loaded PEGylated UCNP/polydopamine nanoparticles	Anti-PD-1 Ab (non-loaded)	Ce6, polydopamine	Ce6/PEG-UCNP/polydopamine NPs combined with NIR light plus Anti-PD-1 Ab administration induced 1.5- and 2.5-fold higher levels of T_{EM} cells in the spleen than Ce6/PEG/UCNP/polydopamine NPs plus NIR light and Ce6/PEG/UCNP/polydopamine NPs plus Anti-PD-1 Ab, respectively.	[81]
1MT-IR820 nanoparticles	1MT; Anti-PD-L1 Ab (non-loaded)	IR820	1MT-IR820 NPs combined with laser radiation plus Anti-PD-L1 Ab administration suppressed the establishment of lung metastases after re-inoculation with cancer cells.	[83]
R848 loaded chitosan-polyaniline nanoparticles	R848	Polyaniline	The mice whose primary tumors were eradicated by R848/chitosan-polyaniline nanoparticles plus NIR light resisted tumor re-inoculation.	[31]

immunostimulants' and ICIs' actions brings closer the gap for attaining a therapeutic strategy that can not only ablate the primary and the distant tumors, but that can also contribute to the establishment of memory that can prevent tumor recurrence.

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Conflict of Interest

The authors declare no conflict of interest.

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