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Recent advances in nanosized drug delivery systems for overcoming the barriers to anti-PD immunotherapy of cancer

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ABSTRACT

Checkpoint blockade immunotherapy revolutionizes the field of cancer therapy, among which anti-programmed cell death (anti-PD) therapy is the most successful one in clinic. Despite the tremendous advancement, anti-PD therapy is still limited by barriers such as low response rate and immune related adverse effects (AEs), which are mainly resulted from poor intratumoral infiltration of cytotoxic T lymphocytes and off-tumor distribution of the anti-PD drugs. To address these issues, nanosized drug delivery systems (NDDS) have been fabricated to realize synergistic application with anti-PD drugs for anti-tumor immunity priming or enable targeting delivery of anti-PD drugs with versatile chemical properties. In this review, we summarize the major barriers for successful anti-PD therapy, highlight current advances in NDDS with improved anti-PD therapeutic benefits, and discussed the possible directions of the field.

Contents

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Introduction

Immune checkpoint blockade is a field of rapid progression in cancer therapy, showing durable clinical benefits in a subset of cancer patients [1–3]. Among all the known immune checkpoints, programmed cell death (PD) pathway including PD receptor 1 (PD-1, CD279) and PD receptor 1 ligand (PD-L1, CD274) is the most promising target to restore the functions of cytotoxicity T lymphocytes (CTLs) [4,5]. PD-1 is one of the co-inhibitory receptors of the CD28 superfamily, which can be induced in T cells, B cells and myeloid cells in response to inflammation. PD-L1 is the ligands for PD-1, which is similar to the B7 molecules and also upregulated by pro-inflammatory cytokines especially interferon-gamma (IFN-γ). Typically, PD-L1 is expressed on lymphocytes, antigen presenting cells (APCs), endothelial cells, glial cells in inflamed brain and pancreas β cells. The ligation of PD-1 and PD-L1 constrains the function of CTLs to maintain a homeostasis of immunity [1,6], via suppressing the production of immune cytokines such as interleukin 2 (IL-2) and IFN-γ and inhibiting the proliferation of T cells [7]. However, the expression of PD-L1 is dynamic and inducible, which is extremely sensitive to T cell derived IFN-γ. Many types of cells including tumor cells express PD-L1, keeping themselves from being identified by CTLs so as to avoid immunologic surveillance [8,9]. Given the essential role of PD pathway in CTL activity control, PD pathway blockade is expected to reverse the immunosuppressive tumor microenvironment and reinvigorate the exhausted T cells [10–13]. Indeed, anti-PD cancer therapy with antibodies against PD-1 or PD-L1 has been approved by US FDA (Food and Drug Administration) for the treatment of melanoma, lung cancer, colorectal cancer, renal cell carcinoma, prostate cancer, large B cell lymphoma and follicular lymphoma, eliciting long-term remissions of tumor in partial patients [14–19].

Despite the impressive clinical benefits of anti-PD immunotherapy, there are still barriers that limited the clinic outcomes of anti-PD therapy [20–23]. Firstly, increasing evidences indicate that the response to anti-PD therapy is limited to only a subset of patients with certain types of tumor [24,25]. For instance, the total response rates to nivolumab (PD-1 inhibitor) are 28%, 27% and 18% in patients with melanoma, renal cell carcinoma, and advanced non-small cell lung cancer (NSCLC), respectively [26]. A lack of sufficient number of pre-infiltrated $CD8^+$ T cells in the tumors may be the main reason leads to the low response rate [27]. Secondly, systemic administration of anti-PD drugs always results in "on target but off tumor" distribution and unexpected immune related adverse effects (AEs) [28–32]. It was reported that grade 3–4 AEs were found in 7%–12% patients with single-dosage of anti-PD antibodies. Blocking the function of checkpoint molecules can result in a disruption in immunologic tolerance and induce unchecked immune response, which lead to autoimmune-like or inflammatory side-effects. Undesired side-effects on normal organ systems such as hepatic, pulmonary, gastrointestinal, mucocutaneous and endocrine systems have been found post anti-PD therapy [33,34]. Thirdly, tumors can develop resistance against anti-PD therapy by exploring multiple complementary immunosuppressive mechanisms [35,36], and simultaneous inhibition of different pathways is necessary for maximizing therapeutic outcomes. Finally, current anti-PD drugs in clinic are monoclonal antibodies targeting either PD-1 or PD-L1, which are costly, immunogenic, unstable and not convenient for storage and transportation. Candidate molecules such as nucleotide-, peptide-based anti-PD drugs are emerging but require effective transporters before clinic translation.

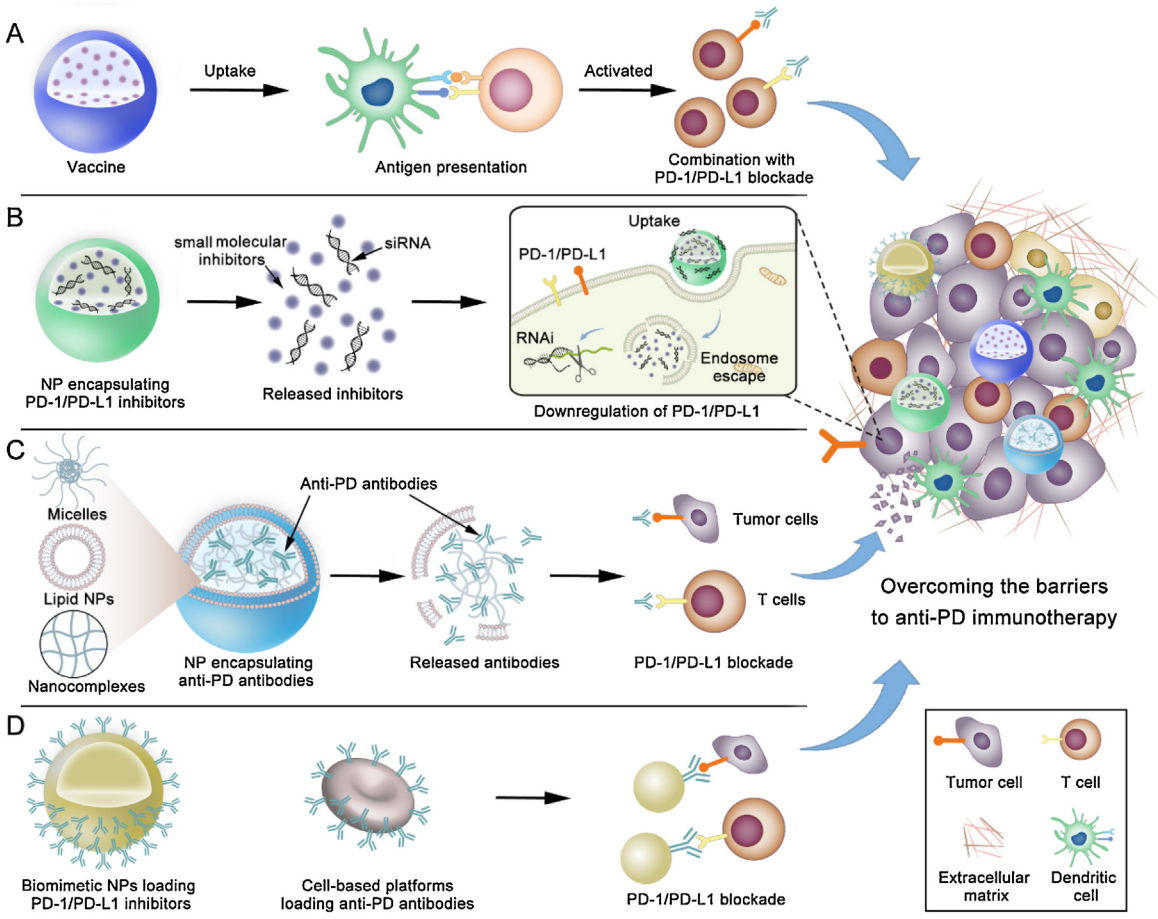
Encouragingly, these barriers that impede the efficacy of anti-PD therapy can be potentially addressed by nanosized drug delivery systems (NDDS) [37,38]. NDDS can reduce the "on target but off tumor" effect of anti-PD drugs by delivering them specifically into tumors, taking advantages of their passive or active targeting capability after proper modification and engineering [39–50]. With their ability in combined drug delivery, NDDS can regulate the tumor microenvironment by priming anti-tumor immunity, relieving tumor immunosuppressive microenvironment and improving intratumoral infiltration of CTLs, which could potentially improve the response rate of immune tolerant tumors [51]. There have been several important reviews overviewed the recent progresses of biomaterials or nanoparticles for cancer immunotherapy [52–55], which provide inspired and forward-looking insights into the design of nanomaterials that are applied in main classes of cancer immunotherapy. However, a brief overview of NDDS focusing on PD-1/PD-L1 pathway, a vital drug target with six US FDA approved antibodies, is still needed to promote anti-PD therapy. Moreover, increased immune related AEs were reported following the broad application of anti-PD inhibitors in clinic [26]. Discussing designed NDDS that have the potential to reduce the toxicity of anti-PD drugs also shows great priority. Herein, we summarize recent advances in NDDS for overcoming the barriers to anti-PD therapy (Scheme 1), with a focus on NDDS design and their mechanisms of action in achieving durable therapeutic effects and decreasing immune related AEs. We also discussed the challenges faced by NDDS-based anti-PD therapy and the possible directions of the field in the future.

Potentiating anti-PD therapy with NDDS

Successful anti-PD therapy requires the pre-existence of tumor infiltrating $CD8^+$ T cells that can recognize tumor-associated antigens (TAAs). TAAs are kinds of peptides, or proteins overexpressed on tumor cells, which play important role in marking cancer cells for immune recognition. However, many tumors can evade immunosurveillance by inhibiting the function of APCs and the infiltration of tumor-specific CTLs. NDDS are able to priming anti-tumor immunity by delivering TAAs to APCs or inducing immunogenic cell death (ICD) of cancer cells.

NDDS as cancer vaccines

Vaccines can protect subjects from certain disease via establish adoptive immune protection against non-self-antigens such as TAAs. Unfortunately, many vaccines only induce strong humoral immunity, while the cellular immunity is usually mild. NDDS as carriers for TAAs can significantly elevate the extent of cellular immunity, after their physicochemical properties and composition such as co-stimulating adjuvants are properly optimized to maximize lymph node accumulation and APCs uptake. Anticancer vaccine alone is not very effective due to the presence of immunosuppressive microenvironment, which make it ideal for combination use with anti-PD therapy. For instance, combining a nanosized multipeptide vaccine with PD-1 blockade could enhance the antitumor efficiency by increasing the population of memory T cells and decreasing $PD\text{-}1^+$ dendritic cells (DCs) [56]. Neoantigens produced during cancer mutations may exert better functions for more specific properties [57]. Neoantigens are a class of identified peptide antigens that are derived by the genome mutation of an individual tumor, which are entirely absent from the normal tissues. According to previous studies, the neoantigen-specific T cell activity displays vital role in successful personalized immunotherapy [58,59]. In order to induce strong tumor-specific cellular immunity, personalized vaccine nanodiscs have been fabricated by Moon and colleagues via leveraging high-density lipoprotein to deliver TLR-9 agonist CpG-ODN and tumor neoantigen peptides (Fig. 1A). The nanodiscs post subcutaneous injection can accumulate in draining lymph nodes, and elicited 9-times higher levels of antigen presentation at 24 h than free peptides + CpG-ODN. The prolonged antigen presentation led to robust cross-priming



Scheme 1. Schematic illustration of NDDS for potentiating anti-PD therapy or delivering anti-PD inhibitors. (A) Eliciting anti-tumor immunity with NDDS-based vaccines to potentiate the efficiency of anti-PD antibodies. (B) Integrating anti-PD inhibitors such as siRNA or small molecules in NDDS to block PD-1/PD-L1 pathway. (C) Encapsulating anti-PD antibodies in NDDS for PD-1/PD-L1 blockade. (D) Biomimetic nanovehicles or cell-based plaforms with surface modified anti-PD antibodies for enhancing anti-PD therapy.

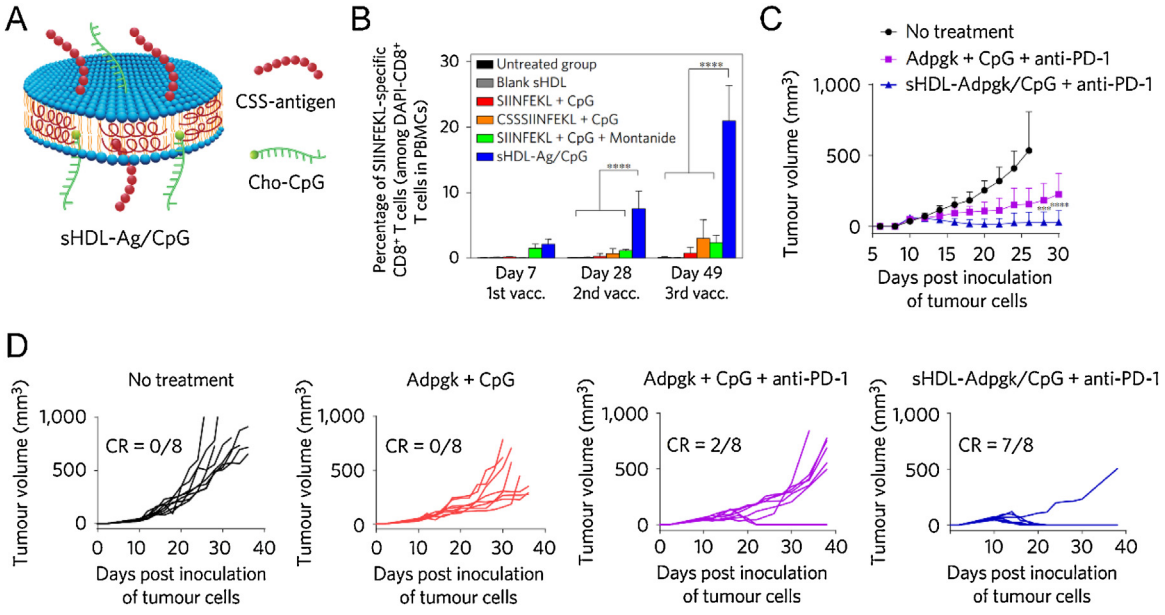


Fig. 1. (A) Schematic of engineered sHDL nanodisc loading cysteine-modified neoantigens (CSS-antigen) and cholesterol modified CpG (Cho-CpG). (B) Frequency of $OVA_{257-264}$ specific $CD8^+$ T cells in peripheral blood 7 days post each vaccination. The C57BL/6 mice were vaccinated with various suspensions containing 15.5 nmol antigen peptide and 2.3 nmol CpG on day 0, day 21 and day 42, and then examined by flow cytometry. (C) Average and (D) individual MC-38 tumor growth plots were shown. The C57BL/6 mice were treated with equal Adpgk peptide and CpG on day 10 and day 17, and intraperitoneally injected with anti-PD-1 on day 1 and day 4 post each vaccination. Images reproduced from [60] with permission from Springer Nature, Copyright 2017.

of T cells and ~21% antigen-specific CTLs was detected in the formulation group after the third vaccination (Fig. 1B). Although vaccination with the formulation elicited antigen specific CD8α$^+$ T cells and inhibited the growth of MC-38 tumor, no tumor rejection was found in control and treated groups possibly due to the suppressive effects of PD-1/PD-L1 in tumor microenvironment. Combination the nanodiscs with anti-PD-1 therapy could eradicate established MC-38 and B16-F10 tumors in more than 85% of mice (Fig. 1C, D). The results suggested that the cytotoxic capability of T cells could be totally unleashed by combining neoantigens and PD-1/PD-L1 checkpoint inhibitors. The vaccination efficiency of neoantigens regulated the tumor immune microenvironment and elicited neoantigens specific CTLs, improving the therapeutic benefits of anti-PD therapy [60]. In addition to TLR family, stimulator of interferon genes (STING) is another important pathway for DC maturation and CD8$^+$ T cell priming. Gao and co-workers synthesized a pH-sensitive polymer 2-(hexamethyleneimino)ethyl methacrylate (PC7A) for lymph node targeted delivery of antigens. The suitable particle size of PC7A nanoparticles facilitated their accumulation in the draining lymph nodes, and PC7A improved antigen cross-presentation and stimulated STING after being endocytosed by DC cells. The anti-tumor study on human papilloma virus E6/7 TC-1 tumors showed that 50% of mice were free from tumors in 60 days after the treatment of $E7_{43-62}$-PC7A nanoparticles. Similarly, anti-PD therapy alone was barely to enhance the tumor growth inhibition effect in B16-OVA melanoma or TC-1 tumor models as previous reports. However, the combination of anti-PD therapy with PC7A nanovaccine synergistically improved the rate of tumor free mice to 90%. These results indicate that PC7A vaccine synergy with anti-PD inhibitors [61].

Although synthetic neoantigen-based vaccines showed promising activity, their applications heavily rely on exon sequencing which is still challenging. In order to address this issue, necrotic tumor cells mimetic nanoparticles were utilized to enhance APC presentation of personalized TAAs and induce anti-tumor immunity [62,63]. To achieve potent anti-tumor immunity, Zhang and co-workers loaded PLGA nanoparticles with TLR-9 agonist CpG-ODN and enveloped the nanoparticles with cancer cell membrane (Fig. 2). The resulting nanoparticles offer an available approach for co-delivery of tumor antigens and immune adjuvants. Besides, effective lymph nodes trafficking of PLGA nanoparticles enables efficient antigen presentation to promote the maturation of bone marrow-derived dendritic cells (BMDCs). Despite the nanovaccine facilitating the immunity of appropriate antigens, the cytotoxic effector of CTLs may not be boosted by the vaccine formulations due to the immunosuppressive factors in vivo. Cytotoxic T lymphocyte antigen 4 (CTLA-4) is another notable immune checkpoint, which has higher binding affinity with CD80 and CD86 in contrast to CD28. So the expressed CTLA-4 on activated T cells inhibits the co-stimulation of CD28 with CD80 and CD86 on APCs [64]. Blocking CTLA-4 and PD-1 checkpoints by antibodies performed moderate antitumor ability. In contrast, combining the vaccine formulation with a mixture of anti-CTLA-4 and anti-PD-1 antibodies significantly inhibited tumor growth [65]. To further improve APC-targeting efficiency, Liu and colleagues developed a core-shell nanoparticle with a PLGA core loaded with TLR-7 agonist R837 and a mannose modified cancer cell membrane shell (Fig. 3). The designed nanoparticles effectively accumulated in lymph nodes and were uptake by dendritic cells (DCs) via mannose receptor-mediated pathway. It was found that the nanovaccine could elicit improved generation of CD3$^+$CD8$^+$CD107a$^+$ T cells and increase IFN-γ production. Due to the vaccination properties in priming antitumor immunity, immune checkpoint inhibitors may be combined to further amplify the therapeutic effect. Anti-PD-1 therapy alone inhibited tumor growth in early stage but failed later. The nanovaccine in cooperation with anti-PD-1 antibody mediated significant melanoma regression, indicating the combinational strategy is rather attractive for cancer immunotherapy [66]. The strategy utilizing membranes derived from endogenous cancer cells for immune stimulation offers wider antigens exposure than single antigen treatment. By taking advantages of biomimetic nanotechnology, the fabricated nanovaccines realize multi-antigenic immune responses and is suitable for personalized immunotherapy

In summary, although PD-1/PD-L1 blockade immunotherapy are able to regulate the immunosuppressive factors of CTLs, the therapeutic efficiency is still hampered by the intrinsic anti-tumor immune responses and low infiltration of CTLs in solid tumors. By combining PD-1/PD-L1 blockade with NDDS-based cancer vaccines, durable therapy benefits can be achieved. Rationally designed nanovaccines could enhance the response rates as well as durable therapeutic benefits of PD-1/PD-L1 blockade immunotherapy.

Inducing immunogenic cell death

NDDS-based chemotherapy

Chemotherapeutics have been widely used in cancer treatment. Although effective, chemotherapy usually leads to severe side effects and systemic immune suppression due to the non-specific distribution of drugs. Immunogenic cell death (ICD) is a series of defined temporal changes occurred in cell surface contents and specific soluble mediators release, which operate on the receptors of APCs to potentiate T cell priming [67]. Recently, several drugs such as doxorubicin (DOX), paclitaxel (PTX), gemcitabine and oxaliplatin have been found to induce ICD and subsequently promote surface exposure of calreticulin protein, adenosine triphosphate (ATP) and high-mobility group box 1 protein (HMGB1) release, holding great promise to combine with anti-PD therapy [68–70]. Due to the systemic immune suppression effect of these drugs, tumor targeted drug delivery with NDDS is therefore necessary to promote anti-tumor immunity and potentiate anti-PD therapy [71–73].

Chemotherapy induced ICD can sensitize the response to the anti-PD therapy by promoting the pre-infiltration of immune supportive cells in tumor microenvironment. For instance, Lin and coworkers have designed coordination polymer core-shell nanoparticles for co-delivery of oxaliplatin (OxPt) and dihydroartemesinin (DHA) to treat colorectal tumor [74]. The particle core was formed by OxPt prodrug and Zn, and then coated with cholesterol-DHA based lipid bilayer. The synergetic OxPt/DHA induced strong anti-tumor immune responses as confirmed by the surface exposure of calreticulin (CRT) and high mobility group box 1 (HMGB-1) protein release. Myeloid-derived suppressor cells, which constitute above 50% of CD45 positive lymphocytes in MC38 tumor, lead to highly immunosuppressive microenvironment. ICD induced by OxPt/DHA promoted engulfment of phagocytes and sequentially the infiltration of CTLs in lymph nodes and tumors, ameliorating the low immunogenicity of MC38 tumor for anti-PD-L1 immunotherapy (Fig. 4). In another study, Gao et al. synthesized a MMP-2 responsive and hyaluronic acid (HA) based polymeric prodrug of Dox, which could self-assemble into polymeric vehicles for prolonged blood circulation [75]. The prodrug strategy modified the pharmacokinetic properties of Dox, and efficiently reduced its damage to immune cells, creating a favorable environment for the subsequent PD-1 blockade therapy. Both the ICD and PD-L1 expression of tumor cells increased, indicating that the combination of DOX prodrug with PD-1 blockade showed great potential. As a result, the combined administration of DOX prodrug and anti-PD-1 synergistically induced immune response to regress primary tumor and inhibit tumor metastasis. Similar results were found in another study reported by Li and coworkers [76]. They prepared low molecular weight heparin-D-α-tocopheryl succinate micelle to encapsulate DOX and toll-like receptor 7 agonist imiquimod for chemo-immunotherapy. The micelles elevated the matura-

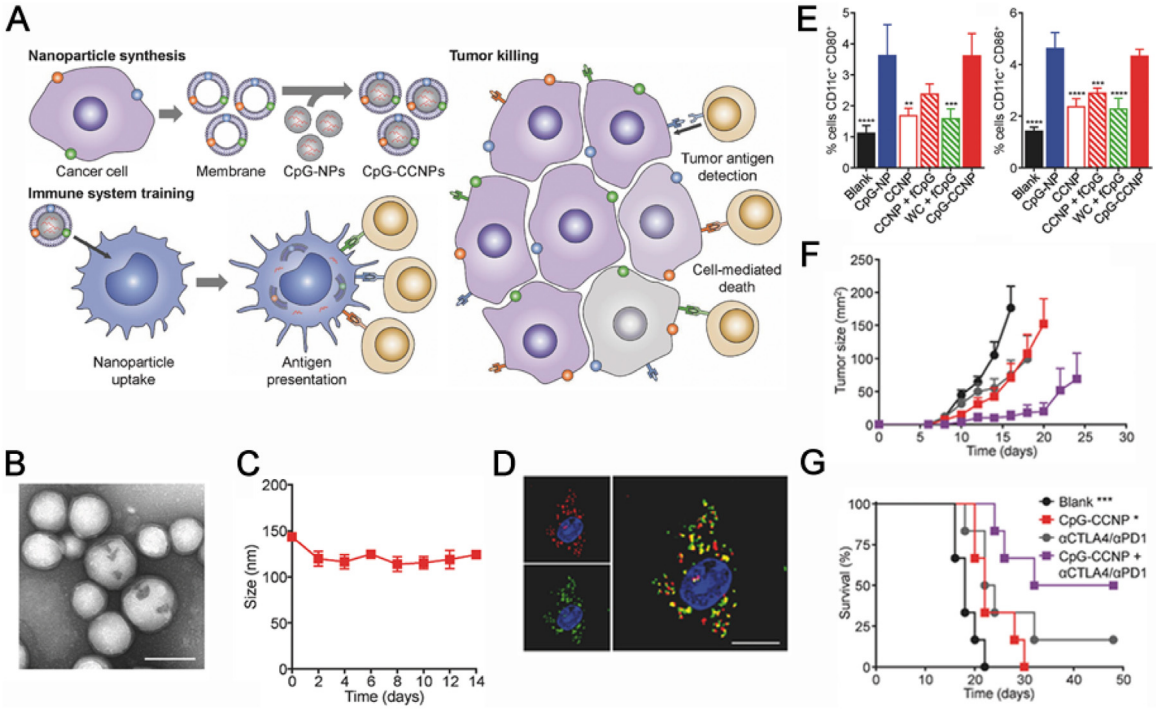


Fig. 2. (A) Fabrication of CpG loaded nanoparticulate anticancer vaccine CpG-CCNPs. The CpG loaded nanoparticle cores were coated by cancer cell membrane, which contains abundant associated tumor antigens for vaccination. (B) TEM examination of CpG-CCNP (Scale bar =100 nm). (C) Stability of CpG-CCNP as a function of time in 10% sucrose (n = 3). (D) Intracellular localization of CpG-CCNP (green) with membrane protein (red) in BMDC. (E) Frequency of matured DCs ($CD11c^+CD80^+$/ $CD11c^+CD86^+$) after incubated with final or control suspensions. (F) Average growth curve and (G) survival of B16-F10 tumors bearing mice after treated by CpG-CCNP based cocktail therapy. The mice were treated on days 1, 2, 4 and 7 (n = 6). Images reproduced from [65] with permission from Wiley-VCH, Copyright 2017.

tion of DCs as well as the $CD8^+$ T/Treg and $CD4^+$ Teff/Treg ratio, further enhancing the therapeutic efficiency of anti-PD-L1 antibody. The Chemotherapy could also alter the immunosuppressive microenvironment. A polymer micelle nanoparticle with stromal modulation effects has been synthesized to overcome anti-PD-1 resistance in pancreatic ductal adenocarcinoma [77]. The nanoplatform was constructed by co-encapsulating cyclopamine (CPA) and PTX (Fig. 5A). Given that tumor microenvironment with infiltrated $CD8^+$ T cells could benefit PD-1 blockade, the strategy developed here synergized a sonic hedgehog inhibitor with PTX to selectively normalize tumor capillary density, promoting infiltration of $CD8^+$ T cells while without regulatory T cells or myeloid-derived suppressor cells. Moreover, the intratumoral concentration of IFN-γ was increased after the treatment of micelles, which could coordinate with a low dose PTX to eliminate tumor cells and strengthen the ICD effects of PTX. Further combination with anti-PD-1 antibody regulate the immunosuppressive microenvironment, leading to significant elimination of pancreatic cancer cells (Fig. 5B, C). In summary, drug delivery systems with chemotherapeutics can prime anti-tumor immunity by inducing ICD, facilitating the combination with PD-1/PD-L1 blockade immunotherapy.

NDDS-based phototherapy

Phototherapy such as photodynamic therapy (PDT) and photothermal therapy (PTT) has recently been widely investigated for tumor treatment. PTT is a noninvasive strategy to elevate the temperature in tumor regions by using NIR light and photo-thermal conversion agents. PDT involves a photosensitizer that can transform oxygen in tumors into reactive oxygen species (ROS) or singlet oxygen. Both PTT and PDT can induce necrosis and apoptosis of cancer cells as well as neoplastic vessels.

Except direct cancer cell killing, PDT can also induce ICD to sensitize checkpoint blockade immunotherapy. A biocompatible core-shell nanoplatform namely ZnP@pyro has been developed to combine immunotherapy with PDT, inducing innate and adaptive immune response (Fig. 6A, B) [78]. Zn and pyrophosphate were polymerized to construct the core of ZnP@pyro, and then coated by a cocktail of DOPC, pyrolipid and DSPE-PEG. Pyrolipid specifically accumulated in tumor owing to the EPR effect, and DSPE-PEG prevented the platform from fast clearance by the reticular endothelial system (RES). ZnP@pyro mediated PDT induced an immunogenic tumor phenotype as confirmed by increased proinflammatory cytokines (Fig. 6C, D), thus promoting antigen presentation and subsequently priming T cells. When used in conjunction with anti-PD-L1 antibody, enhanced tumor control was achieved.

Similarly, PTT also induced significant immune response to facilitate anti-PD therapy. Nanoparticles used in PTT could be of intrinsic photothermal property or those loaded with photothermal agents [79,80]. For example, a multifunctional nanoplatform Fe_3O_4-R837 was fabricated by encapsulating R837 loaded Fe_3O_4 with an amphiphilic copolymer PLGA-PEG [81]. Fe_3O_4 superparticles function as the photothermal agent and drug depots for R837, realizing spatiotemporally controlled drug release upon NIR laser irradiation. With the guidance of an additional outside magnetic field, Fe_3O_4-R837 can mitigate to the tumor specifically to exert tumor ablation effects upon irradiation. Tumor associated antigens were released to activate DCs and prime anti-tumor immunity, which was further enhanced by R837 and anti-PD-L1 treatment. As a result, increased infiltration of $CD45^+$ leukocytes was achieved by the synergistic effects of Fe_3O_4-R837 mediated PTT and PD-L1 blockade therapy, leading to primary tumors regression and metastasis prevention. Overall, PDT/PTT induced immune response contributes to maintain an immunogenic microenvironment for anti-PD-1/PD-L1 therapy, potentially increasing the infiltration of CTLs in immune tolerant tumors. The potent immunity induced by the combination therapy can lead to abscopal effects to eliminate tumor metastasis.

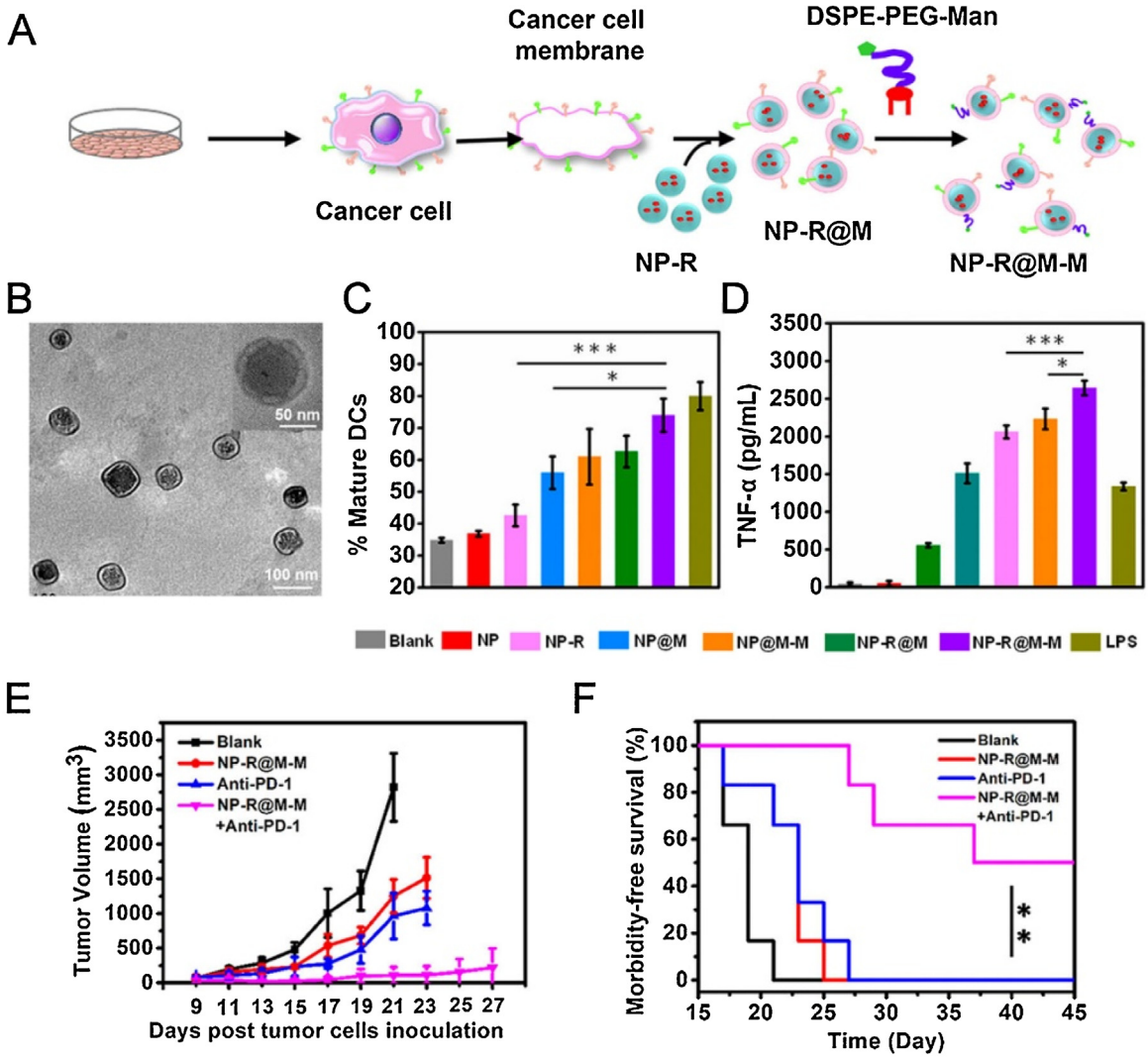


Fig. 3. (A) Schematic illustration to prepare the cancer membrane coated nanovaccine NP-R@M-M. (B) TEM image of NP-R@M-M (Scale bar =100 nm). (C) Representative DC maturation after various nanoparticle suspensions treatment. (D) Secretion of TNF-α from DCs in control and treated groups. (E) Anti-tumor growth effect of NP-R@M-M when combined with anti-PD-1 checkpoint blockade. (F) Morbidity-free survival of B16-OVA tumor bearing C57BL/6 mice in control and treated groups. Images reproduced from [66] with permission from American Chemical Society, Copyright 2018.

NDDS assisted radiotherapy

Radiotherapy is a common strategy for cancer treatment in clinic. The radiotherapy can induce anti-tumor immunity by promoting antigens exposure and releasing damage-associated molecular patterns (DAMPs) [82]. However, solid tumors may develop immune tolerance during radiation, which results in cancer relapse and metastasis. Previous studies have shown that the expression of PD-L1 on tumor cells are upregulated after radiation, which indicated that a combination between radiotherapy and anti-PD therapy will be beneficial for more effective tumor growth control [83,84]. In addition, combined radiation and anti-PD-L1 therapy can lessen the immunosuppressive state in tumor microenvironment, promoting T cell infiltration as well as reducing MDSCs [85]. Further combination use with anti-CTLA-4 antibodies could also improve the outcome [86]. Nevertheless, radiotherapy always needs high X-ray doses due to low tumor target ability. A wide range of nanoparticles are fabricated to potentiate radiotherapy for more efficient treatment and minimized side effects. With the help of nanoparticles, a locally increased irradiation dose in the tumor site is achieved to distinguish tumor from normal tissues for better radiotherapy [87,88]. Besides, penetrable nanoparticles as radiosensitizers can be designed to enhance the efficacy of radiotherapy in deep tissue and reduce toxicities related with high dose of X-ray [89].

In addition to improve the radiotherapy-induced ICD, NDDS could also potentiate anti-PD therapy via improving DC maturation and T cell priming. A variety of biodegradable antigen-capturing nanoparticles (AC-NPs) were developed to deliver antigens released during radiation process. AC-NPs were fabricated by PLGA with different surface functionalized modifications to bind with tumor-derived protein antigens (TDPAs) through different interactions. With the help of AC-NPs, a variety of TDPAs and DAMPs were exposed to the immune system in a more efficient manner than single radiotherapy, thereby remarkably enhanced the therapeutic efficacy of anti-PD-1 therapy. Besides, the immunosuppressive tumor microenvironment was reversed by enhancing population of $CD8^+$ T cells and $CD8^+$ to Treg ratio, leading to local tumor control as well as metastatic tumor inhibition [90]. Anti-PD therapy is able to overcome the immunosuppressive microenvironment of tumor, and potentiate the anti-tumor immunity induced by radiotherapy, which can be further improved with the assistance of NDDS. Besides that, radiation dose and administration sequence are vital for combination therapeutic strategies. A research demonstrated that executing radiotherapy and anti-PD-L1 therapy concurrently

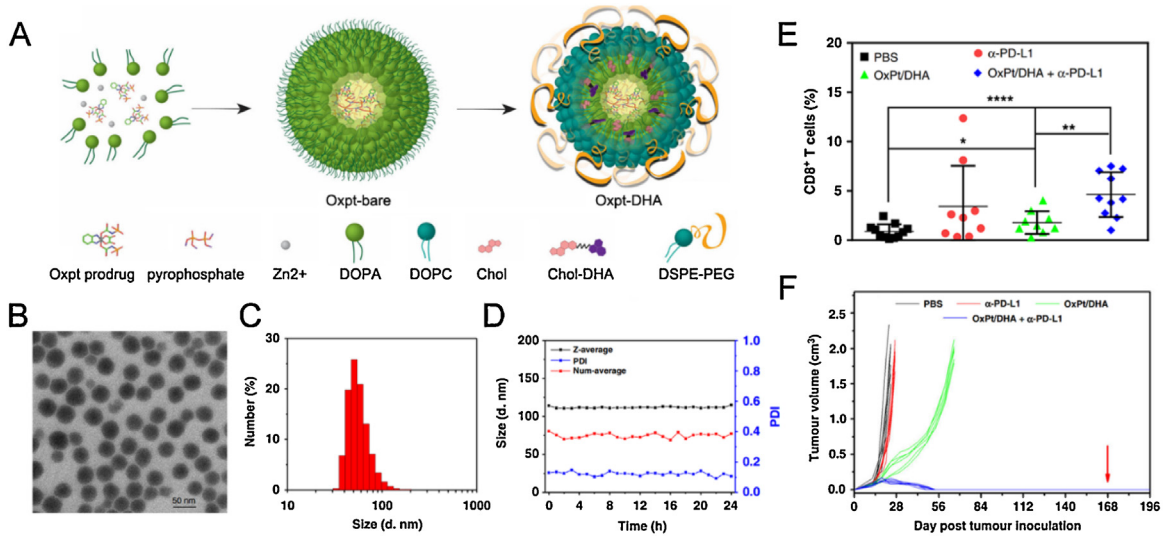


Fig. 4. (A) Preparetion of OxPt and DHA loaded core-shell nanoparticles. The nanoparticle core was formed by the coordination between OxPt prodrug and Zn^{2+} ions, and the shell was formed by chol-DHA. (B) TEM of OxPt/DHA nanoparticle. (C) Dynamic light scattering examination of the number-average size of the nanoparticle. (D) Stability of OxPt/DHA over time at 37° in 5 mg/ml of BSA. (E) Frequency of tumor infiltrated $CD8^+$ T cells was examined 12 days post the first treatment of various suspensions. (F) Growth kinetics of CT26 tumors after treated with the nanoparticle formulation or combined with anti-PD-L1 antibody. Images reproduced from [74] with permission from Springer Nature, Copyright 2018.

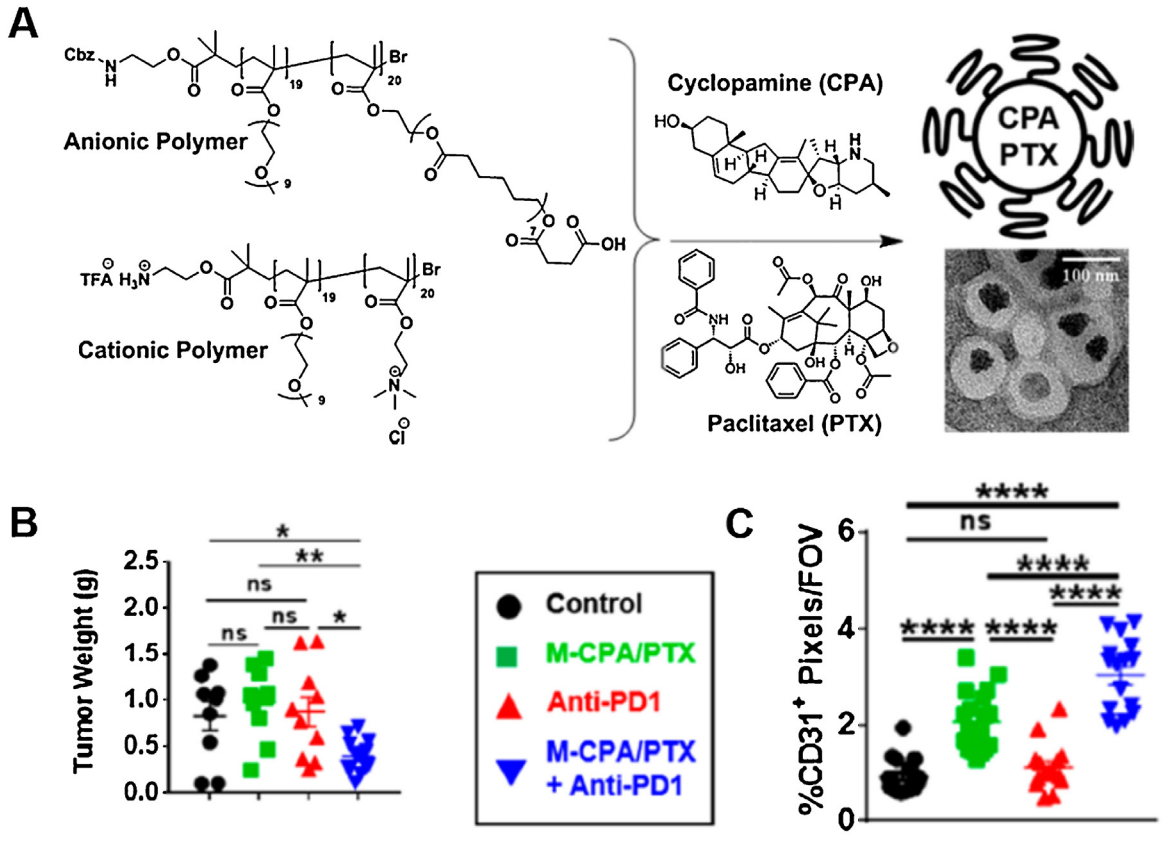


Fig. 5. (A) Chemical structure of the anionic and cationic block polymers fabricating the cyclopamine (CPA) and paclitaxel (PTX) co-loaded nanoplatform M-CPA/PTX. The M-CPA/PTX was spherical nanoparticles with a diameter between 50 and 100 nm by transmission electron micrograph examination. (B) Tumor weight was quantified at the end of anti-tumor study (n = 10). (C) Corresponding quantifications of CD31 IHC staining in tumors in control and treated groups. Images reproduced from [77] with permission from American Chemical Society, Copyright 2018.

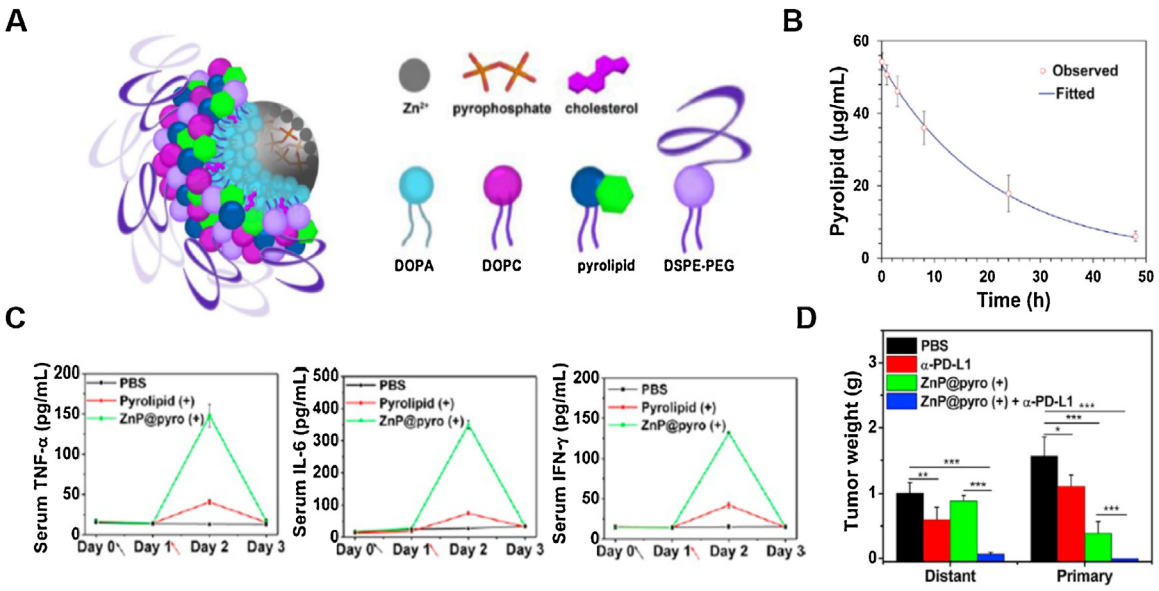
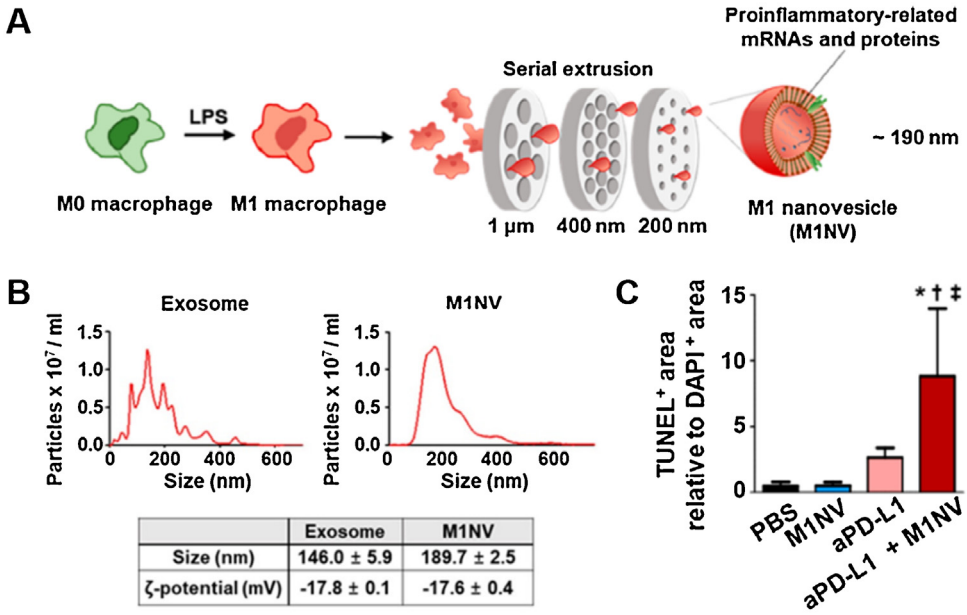


Fig. 6. (A) The ZnP@pyro was composed by Zn-pyrophosphate core and asymmetric lipid bilayer shell. (B) Concentration kinetics of pyrolipid (6 mg/kg) in blood along with time post i.v. treatment of ZnP@pyro. (C) Secretion of pro-inflammatory cytokines in the sera of mice treated with ZnP@pyro along with time. The nanoparticles were administrated on day 0 and irradiation on day 1. "(+)" refers to laser irradiation. Data are expressed as means ± s.d. (n = 3). (D) Tumor weight of primary or distant 4T1 tumors at the end of anti-tumor study. Images reproduced from [78] with permission from American Chemical Society, Copyright 2016.



	Exosome	M1NV
Size (nm)	146.0 ± 5.9	189.7 ± 2.5
ζ-potential (mV)	-17.8 ± 0.1	-17.6 ± 0.4

Fig. 7. (A) Fabrication of M1 macrophage-derived nanovesicles (M1NVs) and the therapeutic effects of M1NVs to potentiate the anticancer efficacy of anti-PD-L1 antibody. (B) Particle size and zeta potential of M1NVs and M1 macrophage-derived exosomes (n = 3). (C) Apoptotic tumor cells were stained by TUENL assay at the end of anti-tumor study. The TUNEL positive area was evaluated relative to the DAPI positive area to show the apoptotic inducing efficiency of anti-PD-L1 and M1NV combined therapy. Images reproduced from [95] with permission from American Chemical Society, Copyright 2018.

instead of sequentially contributed to more satisfied outcomes [82]. More researches however are needed before the clinic translation of radio-based combination therapy.

NDDS regulating multiple immune checkpoints or metabolic pathways

Given that tumors may develop immunological tolerance via various checkpoint pathways such as T cell immunoglobulin domain and mucin domain-3 [91], combined use of antibodies like CTLA-4, TIM-3 and LAG-3 with anti-PD drugs is beneficial. Indeed, a phase I clinical trial demonstrated that combination of ipilimumab (anti-CTLA4) and nivolumab (anti-PD-1) is well-tolerated by melanoma patients. Moreover, interactions between cytotoxic T cells and other relevant cells like APCs, MDSCs, Tregs have crucial impacts on the efficacy of anti-PD therapy. Targeting infiltrated MDSCs in metastatic castration-resistant prostate cancer using multikinase inhibitor showed minimal anti-tumor effects. While depleting immunosuppressive MDSCs with synergized anti-PD-1 and anti-CTLA-4 therapy led to effective responses in tumors, suggesting a new clinical path for treatment of cancer by combining checkpoint inhibitors with MDSC-targeted therapy [92]. Additionally, agonists of co-stimulatory receptors can be combined with anti-PD-1/PD-L1 therapy to induce potent immune

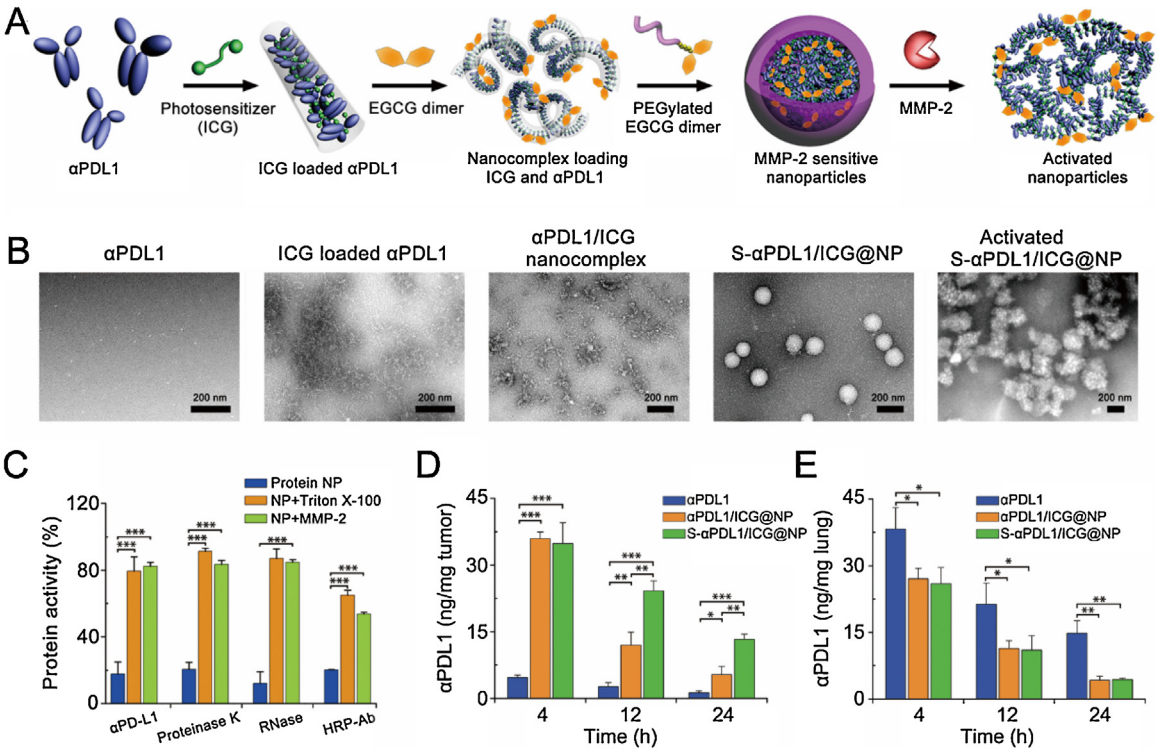


Fig. 8. (A) Fabrication of MMP-2 responsive nanoparticles co-loading photosensitizer ICG and anti-PD-L1 antibody. (B) TEM imaging of the fabrication process from free anti-PD-L1 antibody to the antibody nanoparticles. (C) Protein activity post the fabrication process with or without MMP-2 treatment. (D) Quantified in vivo distribution of the antibody nanoparticle in tumors. (E) In vivo distribution of antibody nanoparticles in lungs. Images reproduced from [96] with permission from American Association for the Advancement of Science, Copyright 2019.

responses. Besides dual-checkpoints blockade, nanoenabled modulation of carbohydrate metabolic pathway can also restore the function of CTLs and potentiate anti-PD therapy [93,94]. A cationic lipid-assisted PLGA nanoparticle has been fabricated to deliver siRNA for lactate dehydrogenase a downregulation in tumor cells [94]. The nanoparticles reprogrammed the pyruvate metabolic pathway and reversed the acidity microenvironment in tumor, leading to increased infiltration of $CD8^+$ T cells and NK cells and decreased immunosuppressive T cells in tumor. As a result, the neutralization of tumor pH activated infiltrating immune cells and potentiated anti-PD-1 therapy. In another study, repolarizing the immunosuppressive M2 phenotypic of macrophages to an immunostimulatory M1 type could boost the efficacy of anti-PD-1 therapy [95]. Nanovehicles derived from M1 macrophages (M1NVs) displayed similar membrane and size characteristics with exosomes to imitate their signal conducting functions for effective transfection (Fig. 7). M1NVs possessed high amounts of pro-inflammatory cytokines and M1 phenotype related mRNAs that were essential for the induction of phenotypic conversion of macrophages. The immunosuppressive action of macrophages in tumor microenvironment was strongly attenuated by M1NVs and the resulted M1 macrophages could produce pro-inflammatory cytokines to improve function of T cells. The anti-tumor effects of M1NVs synergized well with the subsequent anti-PD-1 treatment. In summary, NDDS regulating metabolic pathways can serve as an alternative strategy to improve anti-PD therapy by lifting immunosuppressive factors.

Integrating anti-PD drugs in NDDS to reduce immune related AEs

Cooperating PD-1/PD-L1 blockade with NDDS showed encouraging therapeutic benefits. However, the treatments of NDDS and anti-PD drugs were separated in most cases discussed above, with anti-PD drugs systemically treated. Systemic exposure of checkpoint inhibitors induces serious immune related AEs due to its "on target but off tumor" dissemination in major organs and tissues. NDDS show tremendous advantages in improving specific distribution and controllable release of drugs within the area of interest, thus reducing the "on target but off tumor" effects of anti-PD drugs. In the meanwhile, NDDS are feasible to deliver various kinds of drugs such as proteins, peptides, nucleotides and small molecules, further expanding the therapeutic library for PD-1/PD-L1 blockade. So, we focus on NDDS loading anti-PD drugs in the second part of this review. It is quite different from the traditional separated treatment methods, which can realize spatiotemporal control of anti-PD drugs in targeted areas. Engineered NDDS prevent anti-PD drugs from binding with the ligands expressed on normal cells, automatically reducing the drug induced immune related AEs.

Delivering anti-PD antibodies by NDDS

Encapsulating

Anti-PD antibodies can be carried by nanoparticles to realize higher tumor selectivity and lower toxicities, taking advantages of the passive and active targeting capability of NDDS. An engineered anti-PD-L1 antibody-based nanoparticle has been reported by us (Fig. 8) [96]. The antibody nanoparticles kept inert in blood circulation to weaken its interaction with PD-L1 expressed in livers and lungs, while activated in the present of matrix metalloproteinase protein 2 (MMP-2) within the tumor. The nanoparticle was rationally designed by noncovalently compressing anti-PD-L1 antibody and indocyanine green (ICG) with MMP-2 liable PEGylated (−)-epigallocatechin-3-O-gallate (EGCG). The binding affinity of anti-PD-L1 antibody was barely influenced by manufacturing process and significantly restored to ~80% post MMP-2 treatment. The

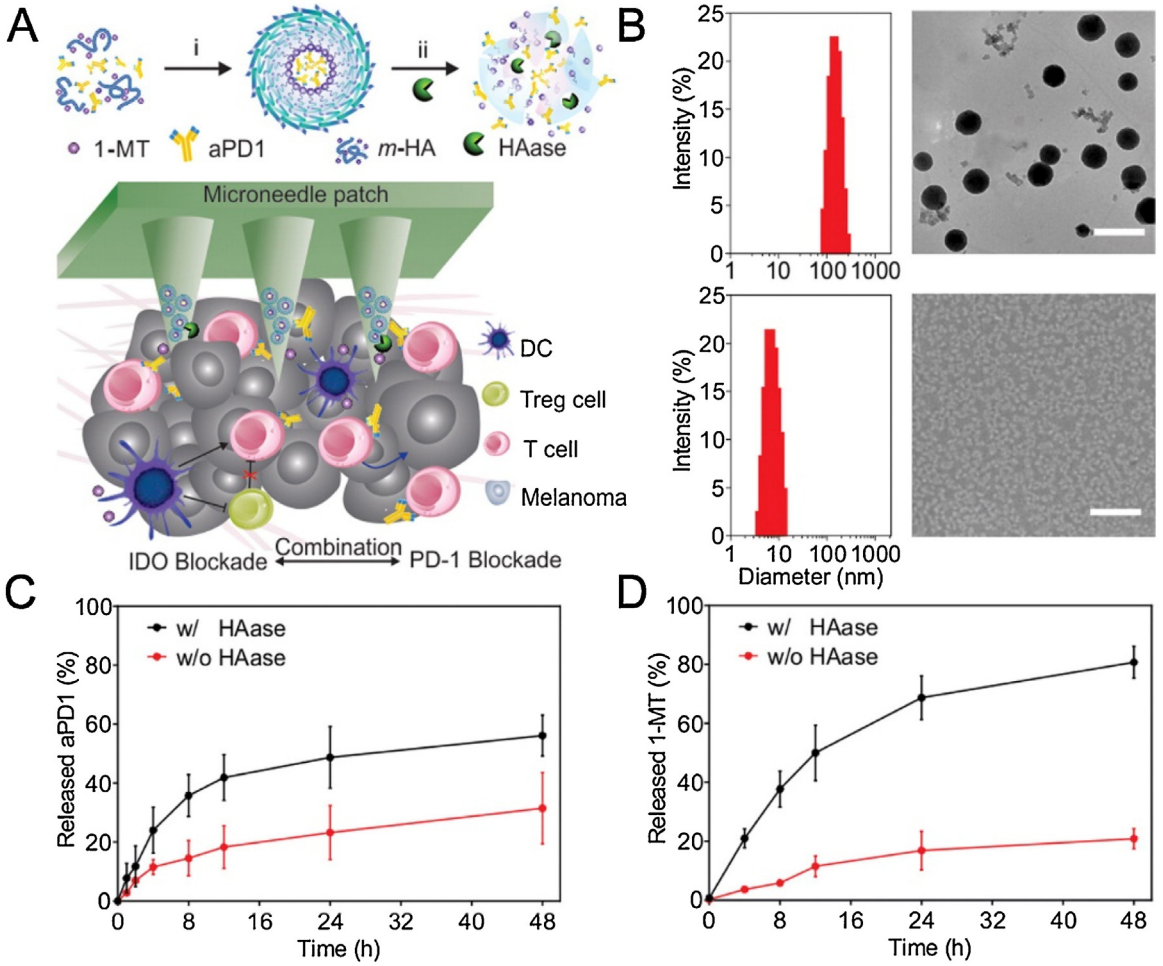


Fig. 9. (A) Fabrication of a microneedle-based transcutaneous platform loading enzyme-responsive nanocarriers. (B) Average hydrodynamic size and TEM images of enzyme-responsive nanoparticles with or without enzyme treatment. (C) Percentage of anti-PD-1 antibody released from the nanocarriers with HAase. (D) Percentage of 1-MT released from the nanocarriers with HAase. Images reproduced from [102] with permission from American Chemical Society, Copyright 2016.

nanoparticle showed 10.7-fold higher intratumoral accumulation than free antibody group, while was lower than free antibody in mice lungs. The results indicated that the antibody nanoparticle efficiently suppressed anti-PD-L1 antibody distribution in normal organs while specifically targeting the tumor, thus probably reducing "on target but off tumor" effects of anti-PD-L1 antibody. Unlike conventional therapeutic strategies using checkpoint inhibitors alone, this nanoparticle can spatiotemporally deliver payloads to tumor mass for reduced AEs and enhanced anti-tumor immunity. Although the nanoparticle performed promising potential to combat advanced tumors, more studies are still needed to declare how the antibody interact with the tumor microenvironment. And it is of great priority to investigate the systemic biosafety to determine whether the antibody nanoparticles suppress the immune related AEs of ICB therapy.

Another classic innovative delivery carrier encapsulating anti-PD-1 antibody has been reported by Gu and co-workers [97]. Anti-PD-1 antibody was firstly loaded in CpG containing DNA nanococoon, and then attached with restriction enzyme HhaI caged triglycerol monostearate (TGMS) nanoparticles. TGMS can be digested by MMPs to trigger HhaI release, and sequentially active the release of CpG and anti-PD-1 antibody. MMPs are over-expressed in inflammatory conditions. So, the cargo release will be precisely controlled in the inflammatory surgical site of tumor. The results showed that the CpG DNA-based carrier triggered sustained release of anti-PD-1 and CpG in post-surgical site, which could inhibit the potential risk of toxic peak dosage of anti-PD-1 antibody in vivo.

Immune related AEs of anti-PD antibodies not only can be reduced via tumor targeting NDDS, but also can be decreased by in situ administration. Although sustained drug release properties were found in most hydrogels [98–100] or microneedle patches [101], chemical or physical stimulation responsive release of payloads can be achieved by locally injected nanoparticles. For example, self-assembly nanoparticles using 1-methyl-$_{DL}$-tryptophan (1 MT) grafted hyaluronic acid were fabricated for anti-PD-1 antibody encapsulation, and the resulting nanoparticles were delivered by microneedle patch and dissociated in tumors in the presence of hyaluronidase (Fig. 9) [102]. The platform co-delivering indoleamine 2,3-dioxygenase (IDO) inhibitor and anti-PD-1 antibody enhanced the retention time of payloads in tumors and potentially decreased the immune related side effects of systematic administration. Another example reported by the same group performed an anti-PD-1 antibody, glucose oxidase and catalase encapsulated pH-sensitive dextran nanoparticle via double-emulsion method [103]. The nanoparticles were in situ delivered by microneedle patch. The glucose oxidase was used to generate gluconic acid from blood glucose and oxygen (O_2). Catalase promoted glucose oxidation by eliciting O_2 and consuming excess hydrogen peroxide (H_2O_2). With help of the enzymatic system immobilized inside the nanoparticles, the enzyme induced production of gluconic acid could promote the

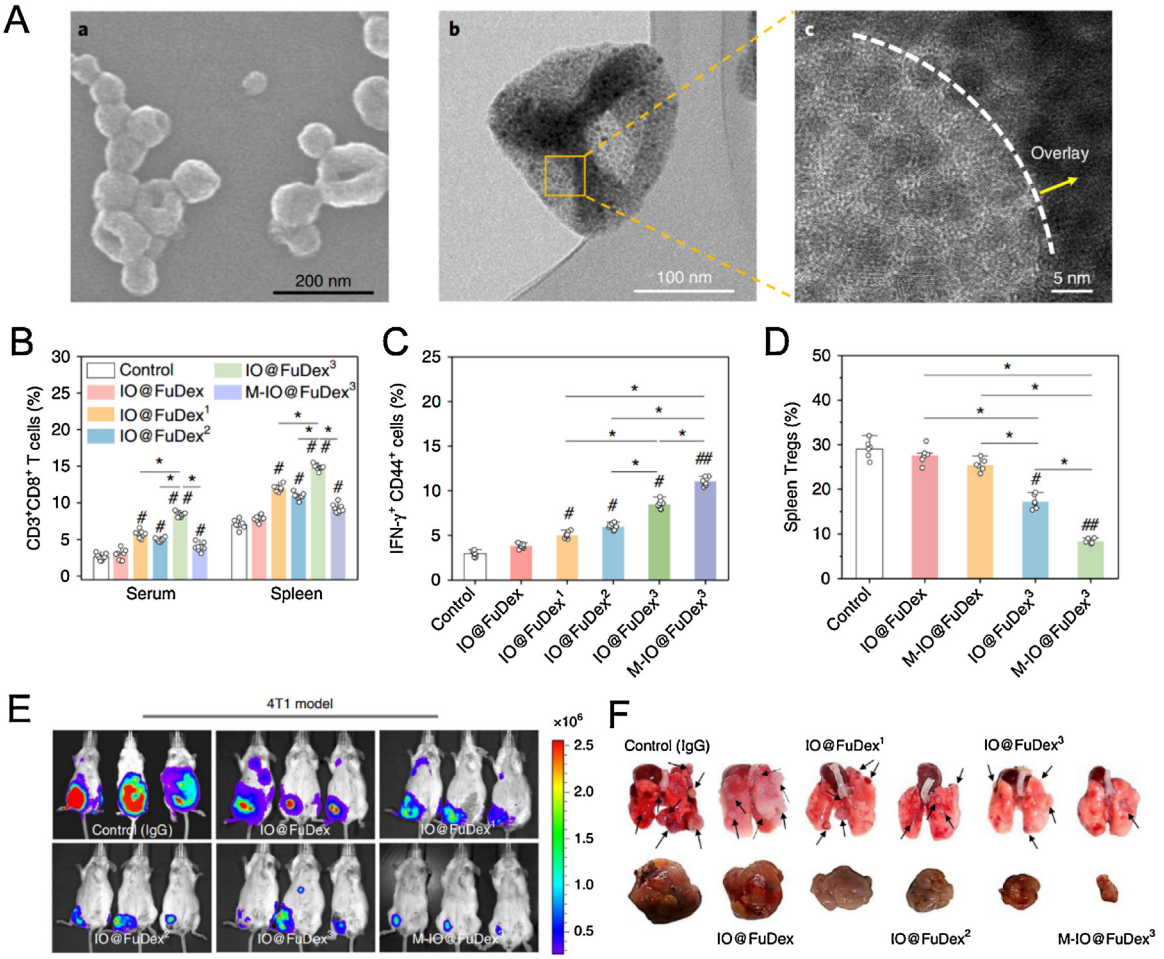


Fig. 10. (A) SEM examination (a) and TEM image (b) of IO@FuDex. (c) Zoomed TEM image of IO@FuDex. (B) Frequency of $CD8^+$ T cells in serum or spleens of mice after treated with various IO@FuDex formulations. (C) Population of IFN-γ^+CD44^+ cells in tumor infiltrated lymphocytes with distinct formulations. (D) Frequency of spleen Tregs in different groups. (E) Bioluminescent images of 4T1 tumor bearing mice with various treatments (n = 3). (F) Representative photographs of metastatic lungs and primary tumors in control and treated groups. Images reproduced from [104] with permission from Springer Nature, Copyright 2018.

gradual self-dissociation of nanoparticles, leading to sustained release of anti-PD-1 antibodies within three days. Such minimally invasive strategy can enable long-lasting action of PD-1 blockade, minimizing the dose amount as well as dosage-dependent autoimmune disorders. These local injection method shows great advantages in preventing dissemination and systemic exposure of anti-PD drugs, thus potentially reducing immune related AEs by avoiding "on target but off tumor" effects.

Surface modifying

Surface modification is also a feasible way to load anti-PD antibody on NDDS, which was fully reported in several studies. A magnetic nanoplatform (IO@FuDex^3) possessing inherent therapeutic effects has been fabricated by integrating anti-PD-L1 antibody and two activators of T cell (anti-CD3 and anti-CD28 antibodies) (Fig. 10) [104]. The nanoplatform is mainly composed of superparamagnetic iron oxide nanoparticles (IO), which can localize to the tumor site with the guidance of a magnetic field. Besides, fucoidan, a negative charged polymer with immunostimulatory functions and antitumor ability, was also added into the formulation to improve the therapeutic effects. For the modifications of anti-PD-L1, anti-CD3 and anti-CD28 antibodies, dextran was incorporated to provide active sites to conjugate antibodies on the surface of the nanoparticles. IO@FuDex^3 specifically localized in the tumor site to reverse the tumor microenvironment. IO@FuDex^3 promoted the activation and proliferation of immune supporting T cells, and depleted immunosuppressive lymphocytes. The IO@FuDex^3 showed high tumor inhibition efficacy with improved tumor selectivity and reduced systemic toxicity in both 4T1 breast and CT-26 colon tumor models. Besides, owing to the high antibody delivery efficiency of IO@FuDex^3, greatly reduced dosage of anti-PD-L1 antibody in IO@FuDex^3 exhibited comparable effects with soluble anti-PD-L1. Overall, IO@FuDex^3 can be navigated to the tumor under a magnetic field to decrease the systemic distribution of anti-PD-L1, reducing toxicities induced by off-target effects.

Besides targeting the tumor tissues, NDDS with surface modified anti-PD antibody is applicable to target T cells expressed corresponding ligands. For example, Schmid et al. explored the use of anti-PD-1 antibody as targeting ligand of nanoparticles, and fabricated a modular nanoplatform that enables small molecule immunomodulators specifically delivered to PD-1^+ T cells [105]. To prepare the nanoplatform, the F(ab')2 fragments of anti-PD-1 antibody was acquired through IdeS-mediated cleavage and conjugated to the surface of PEG-PLGA nanoparticles, endowing PD-1 targeting ability. The conjugation of anti-PD-1 fragments on PEG-PLGA nanoparticles exhibited both specific PD-1 targeting and checkpoint blockade ability (Fig. 11A, B). In addition, hydrophobic immunomodulators were encapsulated in the core of the PEG-PLGA nanoparticles for targeting delivery. Firstly, the platform was designed to deliver an inhibitor of TGF-β pathway to PD-1^+ T cells. The nanoparticles remarkably reversed TGF-β medi-

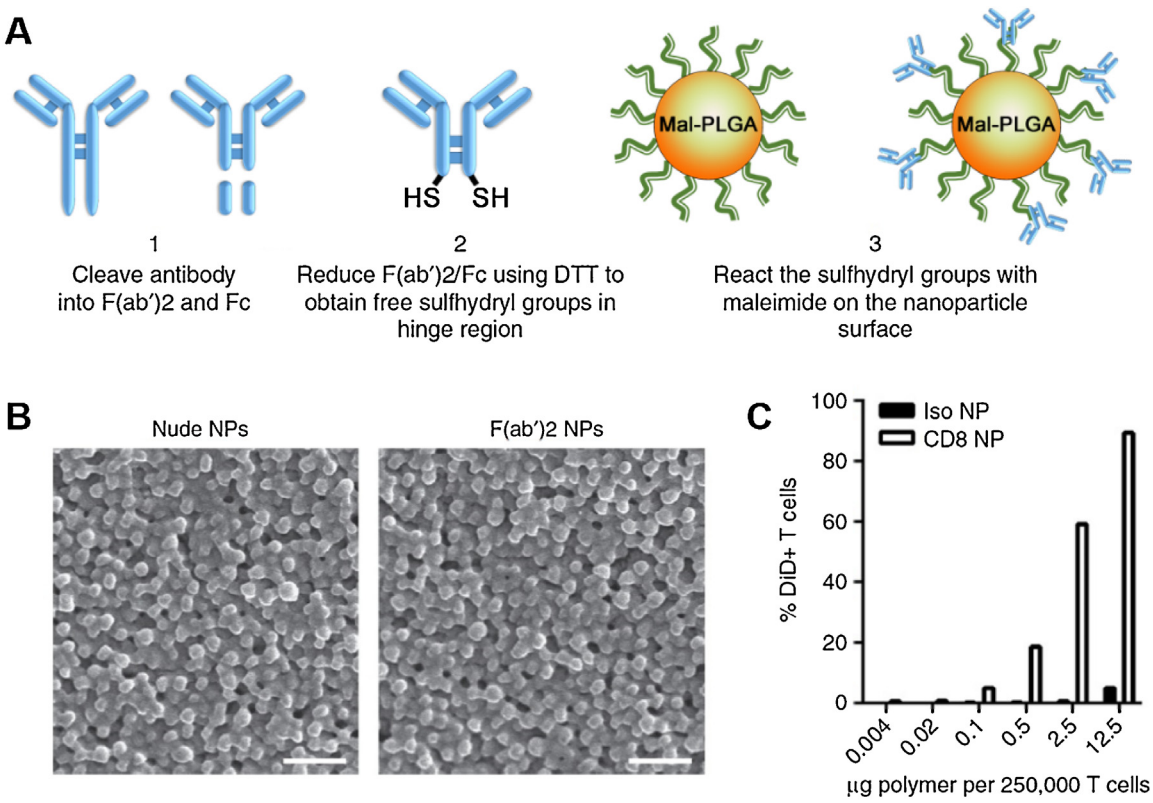


Fig. 11. (A) Scheme showing the conjugation of antibody fragment to the surface of pre-formulated maleimide-modified PLGA nanoparticles (NPs). (B) SEM images of nude NPs and F(ab')2 conjugated NPs (scale bar =500 nm). (C) After co-incubation with spleen derived $CD8^+$ T cells for 30 min, DiD loaded CD8a targeting NPs could bind to the cell surface; data representative for more than 4 experiments. Images reproduced from [105] with permission from Springer Nature, Copyright 2017.

ated immunosuppression and restored the functions of T cells for tumor prevention, thus avoiding cancer metastasis. Secondly, the nanoplatform was used to deliver a toll like receptor7/8 (TLR7/8) agonist to PD-1^+ T cells, leading to activation of innate immunity and expansion of lymphocytes in the low T cell infiltrating tumor microenvironment (Fig. 11C). The nanoplatform efficiently resolved some challenges in anti-PD therapy. Modification of anti-PD-1's F(ab')2 fragments getting rid of Fc on the surface of the nanoparticles can avoid clearance of phagocytic cells. Moreover, the nanoplatform can exert comparable effects with much lower dose than free anti-PD-1 antibodies, decreasing immune related toxicities. In addition, targeting T cells instead of tumor cells enhances the delivery effects of immunomodulators, leading to improved therapeutic index. To sum up, delivery anti-PD antibodies by using tumor specific NDDS can leverage the inherent advantages of nanocarriers while minimize the side effects with decreased dosages.

Inspired by the advances in biological engineering, biomimetic drug delivery system is gaining more and more interests [106]. Two main forms of biomimetic drug delivery system, namely whole cells and cell membrane based vehicles, have been conjugated with anti-PD antibodies for enhanced delivery efficiency. Covalent conjugation by chemical linkers is a promising approach for cell-based modification, which depends on the glycoprotein derived amino residues on cell surface. Zhang et al. fabricated cell membrane derived nanovehicles with stable PD-1 receptor expression for PD-1 blockade [107]. The nanovehicles (NVs) were further improved by loading 1-methyl-tryptophan for IDO inhibition (Fig. 12). It proved that PD-1 on the NVs can specifically interact with PD-L1 on the tumor cells to mediate efficient PD-1/PD-L1 pathway blockade. The NVs could reverse inhibition and exhaustion effects on T cells by regulating immunosuppressive pathways, holding great potential to enhance the response rate of immunotherapy. Conjugating anti-PD-1/PD-L1 to vectors derived from inherent cells enables long blood circulation, improving drug distribution in tumors and reducing immune related adverse effects.

Lots of intrinsic biological cells can be used to realize more efficient delivery of therapeutics. Cell-based delivery systems display lower immunogenicity than other artificial stealth strategy, which contribute to decreased risk of immune disorders post PD-1/PD-L1 blockade [108]. The nature formation also endows the system better compatibility and lower toxicity than synthetic materials. Besides, it is hopeful to use cell-based platforms to overcome biophysical barriers for delivering therapeutics [109]. Specific targeting function can be realized via adherent tendency between homologous cells [110,111]. It should be noted that this property is more favorable when it comes to personalized therapy.

Cell-based platforms can improve the targeting efficiency of conjugated anti-PD antibodies to desired tissues by taking advantages of their intrinsic migration ability, leading to reduced off-target toxicity. For instance, engineered platelets expressed PD-1 have been developed for PD-L1 blockade and loading cyclophosphamide (CP) to deplete regulatory T cells. The combination therapy efficiently reverted exhausted $CD8^+$ T cells and greatly reduced tumor recurrence after surgery [112]. Moreover, anti-PD-L1 antibody bounded platelets were also fabricated through a covalent conjugation process to target residual tumor lesions after surgery and prevent tumor recurrence (Fig. 13). It was demonstrated that anti-PD-L1 was conjugated to platelets by a maleimide linker and responsively released by activated platelet-derived microparticles. Due to the intrinsic properties of platelets to specifically accumulate at the wound site, anti-PD-L1 antibodies were delivered to residual tumor lesions with the help of platelets, thus depleting residue tumors and preventing secondary tumor occurrence. Interestingly, platelets are able to recognize circulating

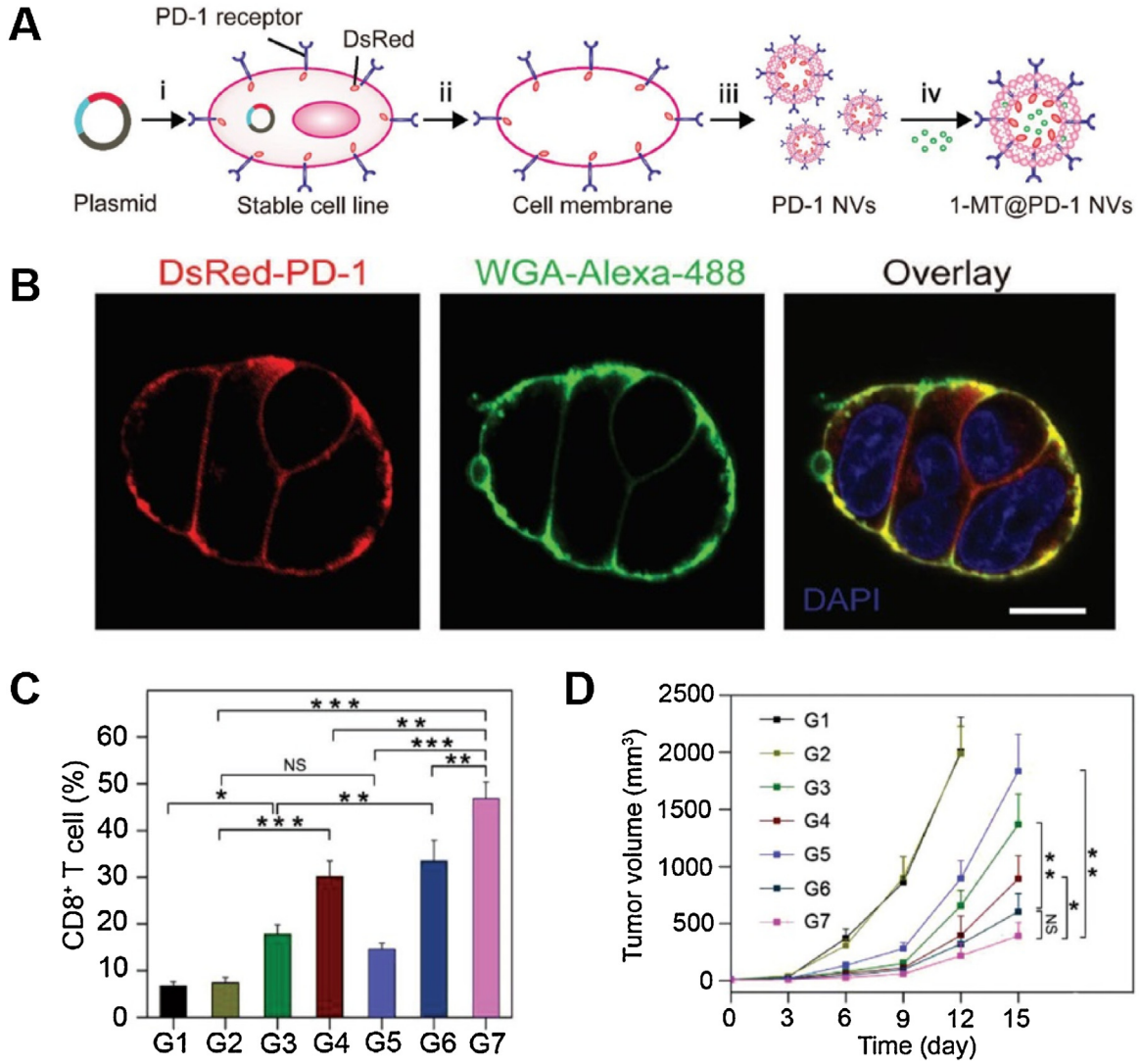


Fig. 12. (A) Fabrication process of PD-1 receptor expressed nanovehicles (NVs) loading 1-MT. PD-1 receptor expressed cell membrane were collected for Engineered HEK 293 T cell. PD-1 NVs were prepared by extrusion and then loaded with 1-MT. (B) Confirmation of PD-1 receptor expression on HEK 293 T cell line by co-localizing WGA Alexa-Fluor 488 dye on cell membrane. (Scale bar =10 μm). (C) Frequency of $CD8^+$ T cells in tumors after different treatments (gate on $CD3^+$, $n = 3$). (D) Average tumor growth kinetics of the treated mice in indicate groups ($n = 7$). G1, PBS control; G2, free-NVs; G3, 1-MT; G4, PD-1 NVs; G5, 1-MT@NVs; G6, aPD-L1 + 1-MT and G7, 1-MT@PD1- NVs. Images reproduced from [107] with permission from Wiley-VCH, Copyright 2017.

tumor cells (CTCs) derived from primary tumors, so that anti-PD-L1 can capture CTCs in the bloodstream to block tumor metastasis through interfering the mitigation stage. In addition, activation of platelets could trigger cytokines release and recruit immune cells to create a pro-inflammatory environment, which further strengthen the function of immune cells when coupled with anti-PD-L1 therapy. Overall, platelet conjugated anti-PD-L1 achieved better accumulation at surgery bed, higher response rate and lower off-target binding cases than free antibodies [113]. Interestingly, a recent study reported by Wang et al. found that the platelet-based anti-PD-L1 vehicle could also migrate and distribute into physical treated (PTT, PDT, ultrasound or radiotherapy) tumors [114], which could further potentiate the broad application of this biomimetic vehicle.

Delivering nucleotides for PD-1/PD-L1 blockade

Antibodies against PD pathway is currently among the most promising approaches in clinic, however the application of antibody drugs is limited due to the production cost and immunogenicity. Despite great efforts have been devoted in the use of anti-PD antibodies, nucleotides which regulate the PD-1/PD-L1 pathway show extensive potential in checkpoint blockade immunotherapy. First, nucleotides are cost-efficiency and readily available through chemical synthesis. Second, nucleotides can downregulate the total expression of PD-1 or PD-L1 before protein transcription process, which is prior to the antagonism effects of antibodies. Third, various of well-studied non-viral gene vectors have been developed for the delivery of nucleic acid drugs with optimized pharmacokinetic properties, thus enhancing the blocking effect of PD-1/PD-L1 pathway by nucleotides.

Inspired by the superiorities of gene therapy for regulating protein expression in cells, a multifunctional micelle was constructed for combination of photodynamic therapy and PD-L1 RNA interference (RNAi) [115]. The platform was composed by co-assemble of acid-responsive and cationic polymers (Fig. 14A). One is a photosensitizer pheophorbide A (PPa) conjugated diblock poly(ethylene glycol)-block-poly(diisopropanol amino ethyl methacrylate-co-hydroxyethyl methacrylate) (PDPA), which is responsible to acidic condition (pH ≤ 6.3) due to protonation of its tertiary amines. The

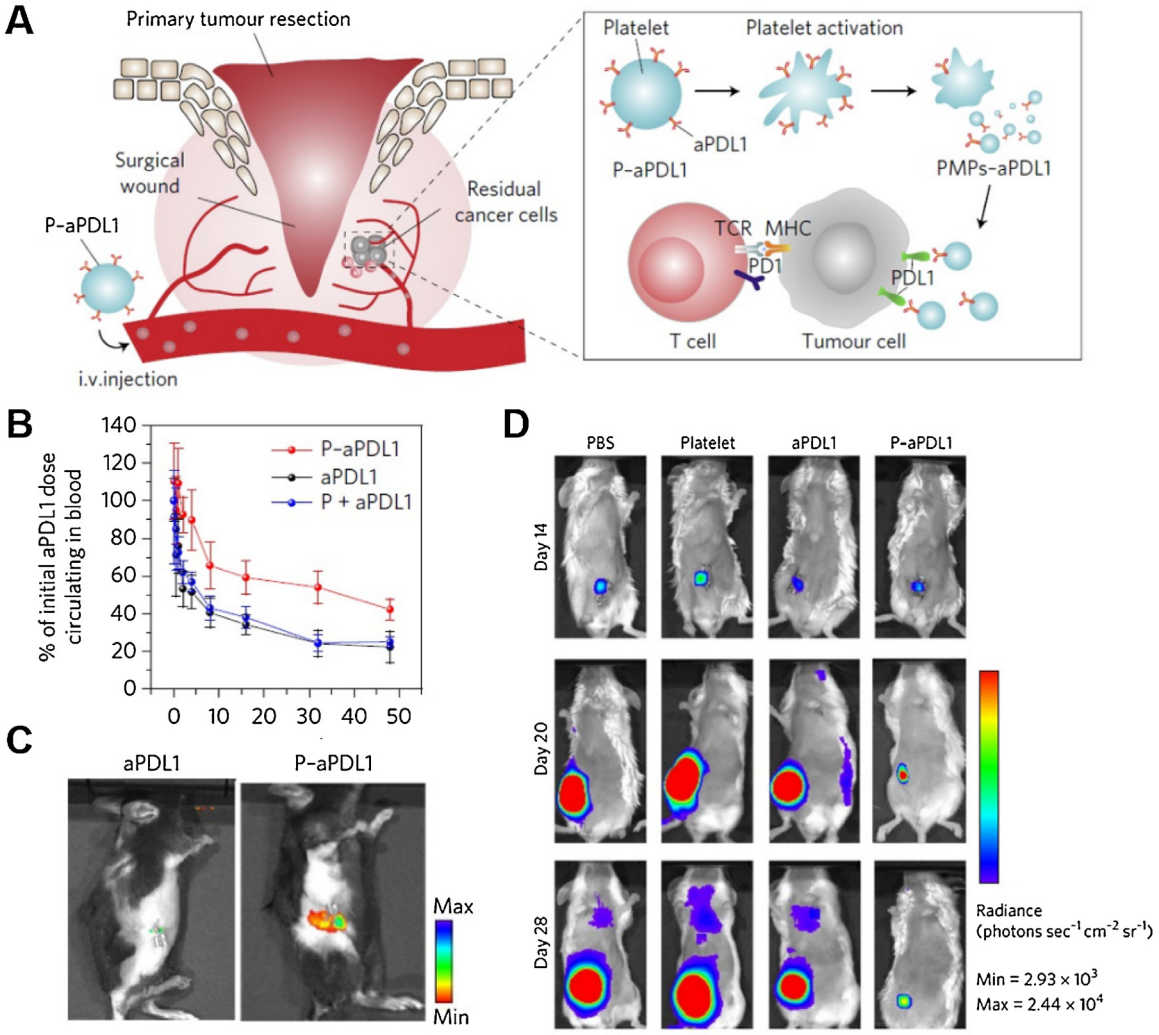


Fig. 13. (A) Modifying anti-PDL1 antibody (aPDL1) on platelet (P-aPDL1) for in situ activation of PDL1 blockade at surgical wound (TCR, T cell receptor; MHC, major histocompatibility complex). (B) Blood concentration of aPDL1 levels along with time after mice were treated by P-aPDL1, aPDL1 or platelets plus aPDL1 mixture (n = 3). (C) Fluorescence imaging of aPDL1-Cy5.5 based formulations in vivo 2 h post i.v. injection of P-aPDL1 or equal aPDL1. (D) Bioluminescence imaging of 4T1 tumor recurrence in BALB/c mice after different treatments. Images reproduced from [113] with permission from Springer Nature, Copyright 2017.

other is an amphiphilic cationic polymer with strong siRNA loading capability, leading to the downregulation of PD-L1 expression by RNAi. The acid-activatable micelles remained inert during blood circulation to prevent siRNA degradation and leakage, while disassembled in the acidic endosome of tumor cells (Fig. 14B, C). Since the endosome/lysosome escape ability of siRNA was improved, the micelles significantly downregulated the expression of PD-L1 in B16-F10 melanoma. It was demonstrated that recruitment of $CD8^+$ T cells and pro-inflammatory cytokines mediated by PDT were further enhanced through PD-L1 blockade. The combination therapy led to tumor regression in both primary and metastatic mouse models (Fig. 14D). Overall, innate and adaptive immune responses stimulated by PDT can be synergized with PD-L1 RNAi therapy for treatment of metastatic tumor.

In order to sensitize epithelial ovarian cancer (EOC) cells to adaptive T cell immunotherapy, folic acid (FA) modified polyethylenimine (PEI) and PEG were synthesized for targeted delivery of PD-L1 siRNA [116]. The high positive surface charge of PEI made it an effective carrier for nucleic acid delivery. FA or PEG-FA modification on PEI could minimize toxicity by decreasing the cationic charge density. In addition, a disulfide bond mediated conjugation between FA or PEG-FA and PEI realized enhanced siRNA transfection efficiency for glutathione-responsive disassembly. As a result, specific endocytosis of FA/siRNA or PEG-FA/siRNA by ovarian cancer cells resulted in about half knockdown of PD-L1 expression. Moreover, it is verified that PD-L1 downregulation in EOC cells makes the cancer cells more sensitive to cytotoxicity T lymphocytes (CTLs), which is meaningful for clinical treatment. Chen et al. prepared a plasmid DNA loaded acidity responsive PEG[(PLG/PEI)/DNA nanoparticle (shPD-L1@NPs) for PD-L1 gene silencing [117]. The nanoparticles could efficiently silence PD-L1 gene with the help of HAase to degrade the HA in tumor. This study possessed that preinjection of HAase could improve the penetration of shPD-L1@NPs in solid tumors, and sequentially reduce PD-1/PD-L1 interaction via gene-silencing method. Such combinational treatment inhibited the growth of B16-F10 tumor, providing useful information for nucleotide-based PD-1/PD-L1 blockade immunotherapy.

Except for polymeric micelles, lipid nanoparticle (LNP) has long been perceived as a useful platform for the delivery of drugs. The main component of LNP is phospholipid, which has analogous characteristics with cell membrane, endowing the carrier well-tolerant property [118]. Cationic liposomes and ionized lipids are two main forms for the delivery of nucleic acid drugs. LNP is often shielded with water-soluble polymers such as PEG and HA to reduce interaction with negative charged plasma proteins, thus avoiding the capture by reticuloendothelial system (RES) [119]. As reported,

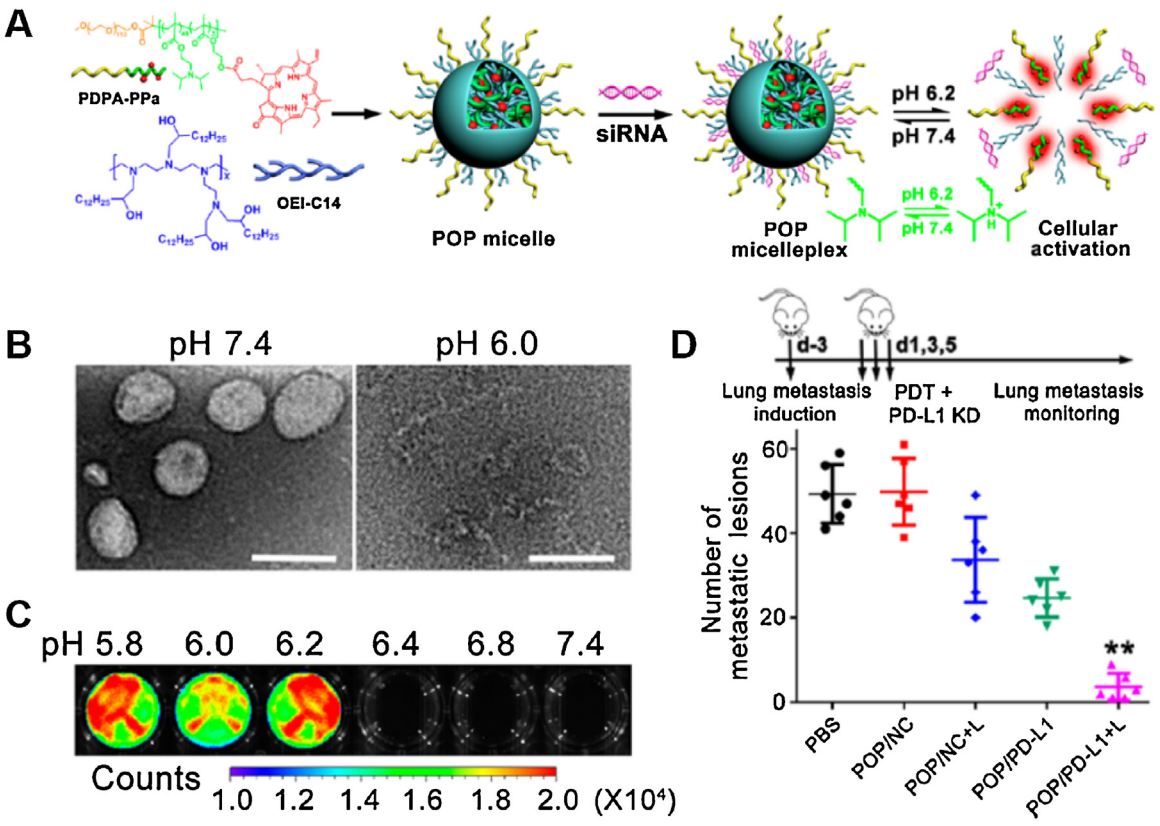


Fig. 14. (A) Schematic illustration of preparation of the pH responsive micelles (POP) loading siRNA. (B) TEM images of POP micelles in pH 7.4 and 6.0 buffer (Scale bar =100 nm). (C) Fluorescent images of POP micelles along with the increase of pH values (Ex640 nm, Em 680 nm). (D) Quantification of metastatic lesions on B16-F10 lung metastatic tumor-bearing mice after indicated treatments. Images reproduced from [115] with permission from American Chemical Society, Copyright 2016.

pDNA, mRNA and siRNA targeting PD-1/PD-L1 can be entrapped into lipid nanoparticles for efficient transfection to regulate the PD-1/PD-L1 pathway.

Targeting molecule modified biocompatible LNP can specifically deliver the nucleic acid of PD-1/PD-L1 to desired site with minimized adverse effects. A lipid-protamine-DNA (LPD) nanoparticles loading PD-L1 coding plasmid DNA (pDNA) can transiently and locally generate PD-L1 trap in the tumor site to disrupt the interaction between tumor cells and T cells with minimize side effects (Fig. 15) [70]. Further combination with oxaliplatin, which induces ICD to activate dendritic cells and stimulate T cell responses, led to synergistic tumor inhibition. Compared with free antibodies, the engineered PD-L1 trap displayed a thousand times higher binding affinity for PD-1. Moreover, local delivery of the PD-L1 trap plasmid with the assist of lipid nanoparticles could diminish the systemic side effects such as autoimmune disorders caused by free anti-PD antibodies, as evidenced by no significant accumulation of Th17 cells in spleens. Besides that, encapsulated by LPD nanoparticles protected PD-L1 plasmid from fast degradation and enhanced its accumulation in tumor region, resulting in more efficient transfection. Ultimately, tumor-selective PD-L1 blockade coupled with immune stimulation mediated by oxaliplatin successfully induced potent immune response to fight against colorectal cancer cells.

Endosomal escape mediated by LNP contributes to efficient and successful transfection of PD-1/PD-L1 nucleic acid. Goodwin et al. delivered two traps targeting PD-L1 and CXCL12 by using a lipid nanoparticle (Fig. 16). The nanoparticles with calcium phosphate core modulated the immunosuppressive tumor environment of liver hepatocytes [120]. Additional combination with a model cancer specific vaccine allowed for potent immune response with increased $CD8^+$ T cell infiltration in the metastatic region. The carrier possesses a PEGylated core-membrane structure with high stability. The $Ca_3(PO_4)_2$ core was coated by lipids, cholesterol and galactose conjugated DSPE-PEG to facilitate specific hepatocytes uptake. The $Ca_3(PO_4)_2$ core was dissolved in the acidic environment, which helps with effective lysosomal escape and nucleic acid release. Overall, the lipid nanoparticles led to specific and efficient blockade of PD-L1 and CXCL12, relieving the suppression of T cells and resolving the immunosuppression related ectopic lymphoid structures separately. The lipid nanoparticles can serve as a powerful tool for gene immunotherapy. And the therapeutic strategy represents an effective immunotherapy approach that manipulates the immunosuppressive environment from different aspects. Another study also took the advantages of the same lipid nanoparticles to specifically deliver an mRNA antigen and a PD-L1 siRNA to DCs [121]. The nanoparticles efficiently accumulated in the lymph nodes through lymph node drainage, releasing the brake of T cells activation through blocking the PD-1/PD-L1 interaction between DCs and T cells and synergistically promoting immune responses with the mRNA vaccine. Many other delivery systems have also been reported to interrupt the PD-1/PD-L1 pathway via nucleotide-based anti-PD drugs [122,123], showing encouraging anti-tumor effects by blocking the checkpoints and remodeling anti-tumor immunity. These researches show that regulation of PD-1/PD-L1 pathway via nucleotides can recover the functions of T cells, thus facilitating the therapeutic benefits of anti-PD therapy.

Delivering peptide antagonists of PD pathway by NDDS

Alternative easily synthesized and cost-efficient peptide antagonists targeting PD-1/PD-L1 pathway have been developed for anti-PD therapy. Gao et al. created the first protease-resistant D-peptide antagonist for PD-L1 blockade [124]. The antagonist could bind to PD-L1 with low micromolar affinity, inhibit CT26 tumor

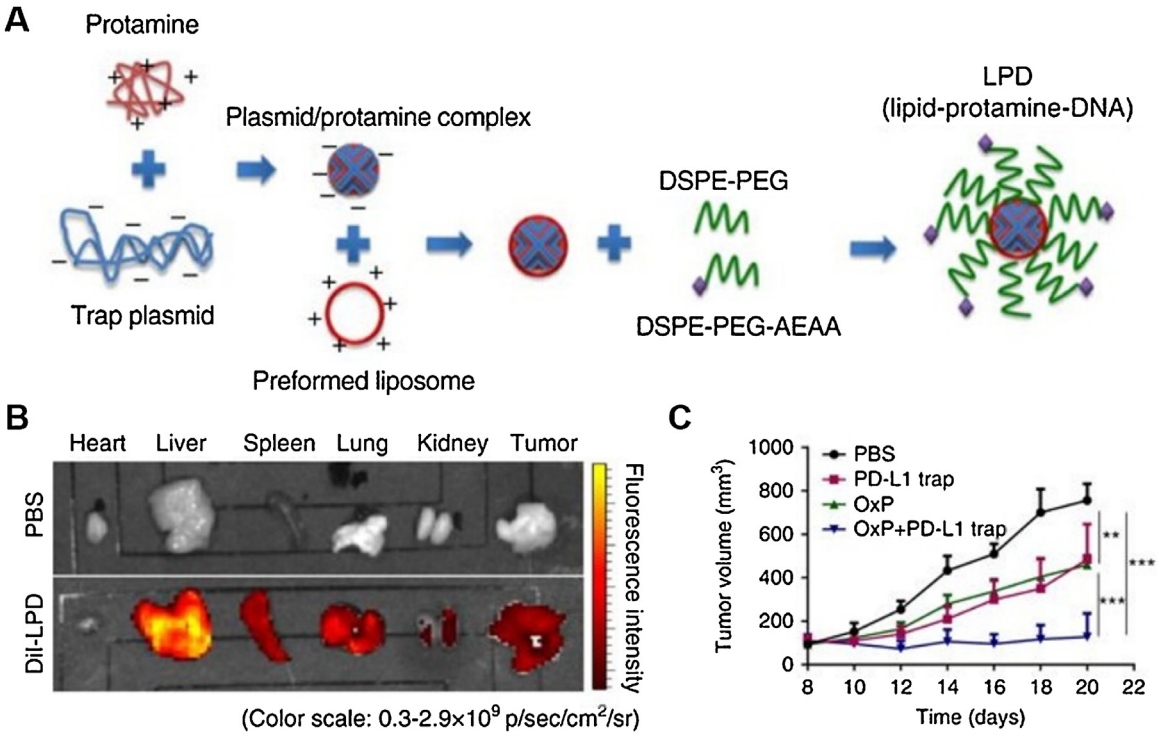


Fig. 15. (A) Scheme showing the fabrication of PD-L1 trap plasmid loaded nanoparticles (LPD). (B) Fluorescence images performing the distribution of Dil-loaded LPD in tissues. (C) Average tumor growth plots in control and treated groups (n = 4). Images reproduced from [70] with permission from Springer Nature, Copyright 2018.

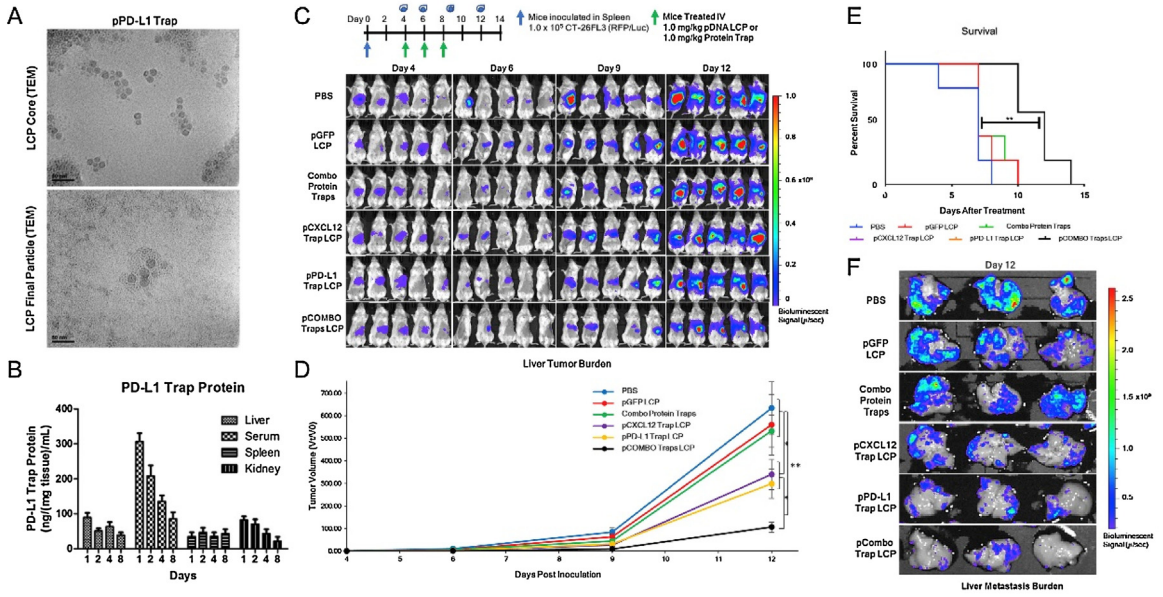


Fig. 16. (A) TEM images of LCP core and LCP pTraps. (B) The expression of PD-L1 Trap protein in all major organs with i.v. injected LCP formulations. (C) Bioluminescence examination of the colorectal liver tumor on day 4, day 6, day 9 and day 12 after treated by various formulations according to the dosage schedule (n = 5). (D) Quantifying the tumor burden in all groups (n = 8). (E) Survival curve of mice by considering body weight, activity and life quality after treated by pPD-L1 trap LCP or combined traps LCP (n = 5). (F) Bioluminescence analysis of the liver metastasis CT-26 FL3 tumor burden on day 12 in control and treated groups (n = 3). Images reproduced from [120] with permission from Elsevier Ltd, Copy right 2017.

growth and prolong mice survival. Zhang and coworkers designed a lipid zinc phosphate hybrid nanoparticle for delivering toll-like receptor 4 agonist and tumor lysate to APCs [125]. The nanovaccine efficiently promoted DC maturation and elicited CTLs in lymph nodes, achieving synergetic anti-melanoma effect when combined with PD-L1 antagonist D-peptide antagonist ^{D}PPA-1. This peptide has also been used to combine with photo/chemotherapy [126]. In another study, a convenient and safe strategy to synergize photothermal therapy (PTT) with perdurable PD-1 blocking has been developed to combat metastatic tumors [127]. Hollow gold nanoshells (HAuNS) with photothermal transformation efficiency and an anti-PD-1 peptide (APP) were co-loaded into PLGA nanoparticles (Fig. 17A, B). The fabricated delivery platform effectively protected the APP from rapid clearance and exhibited persistent release up to 40 days, maintaining an effective APP concentration in the bloodstream to avoid frequently dosage of free APP.

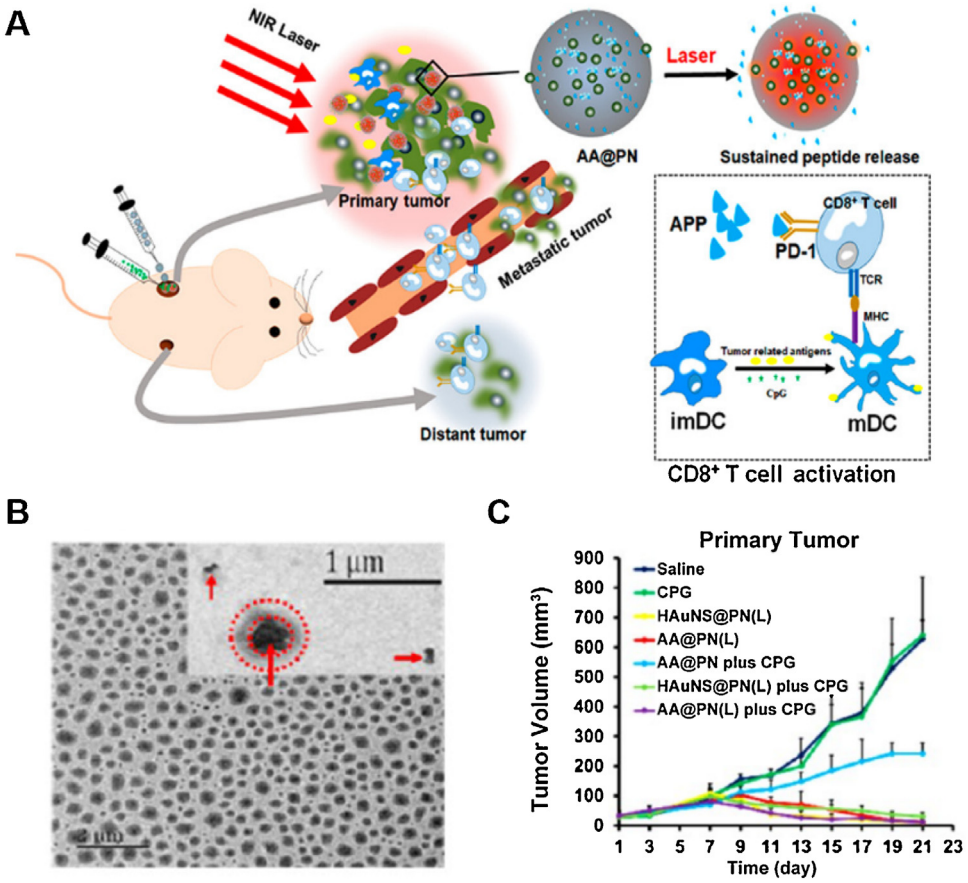


Fig. 17. (A) Scheme showing the action mechanism of anti-PD-1 peptide encapsulated PLGA nanoparticles (AA@PN). (B) TEM examination of AA@PN (Scale bar =1 μm). (C) Average growth kinetics of primary 4T1 tumors treated with indicated formulations (n = 7). Images reproduced from [127] with permission from American Chemical Society, Copyright 2018.

Moreover, the release profile of the PD-1 peptide was controlled spatiotemporally with the assistance of PTT. Besides alleviated immunosuppression mediated by durable PD-1 blocking, PTT coupled with an immune adjuvant CpG oligonucleotide displayed immunostimulation effects by promoting antigen presentation and maturation of DCs. Particularly, local administration of nanoparticles rendered more security assurances with minimized systemic adverse effects (Fig. 17C). As a result, the strategy conducted effective regression of both primary and metastatic tumors, and prevented tumor recurrence with memory T cells.

The hydrolysis-resistant anti-PD-L1 peptide ^{D}PPA was also combined with PPa mediated PDT and ROS-responsive prodrug of PTX for better anti-tumor and anti-metastasis efficacy [128]. An enzyme-responsive and size-reducible nanoparticle (mCAuNCs@HA) was synthesized to promote tumor penetration, retention and controlled release of payloads. It was found that the surface exposure of CRT and HMGB1 release were increased in tumor cells when treated by nanoparticles plus laser irradiation, indicating the nanoparticle mediated PDT induced obvious ICD. When combined with ^{D}PPA to alleviate the immunosuppressive environment, the delivery system elicited obvious anti-tumor immune responses. The frequency of $CD8^+$ T cells, $CD8^+Ki67^+$ T cells was significantly increased in final treated group in contrast to the corresponding controls, and the secretion of TNF-α, IL-12 was also enhanced by the triple-combined therapy. It was proved that satisfied tumor growth and metastasis inhibition could be achieved via the chemo-, PDT and PD-L1 blockade triple-combined therapy. These present research put forward an effective strategy for advanced cancers by using peptide-based PD-1/PD-L1 inhibitors.

Conclusion and outlook

The development of NDDS provides promising strategies for overcoming the barriers to successful anti-PD therapy. In this review, we summarized the recent progress in NDDS that have been explored to potentiate the therapeutic effect of anti-PD therapy, via strategies such as improving the specificity of anti-PD drug, boosting anti-tumor immunity, and enabling delivery of novel entities targeting the PD pathway. By virtue of drug delivery systems, therapeutics can be released in a controllable manner with prolonged retention in tumors, decreasing undesired distribution to normal tissues and reducing dosage frequency. It is noteworthy that NDDS based combination therapies modulating various immune pathways are more preferable for the ultimate therapeutic effects. Moreover, demands can be derived from clinical trials to guide the design of NDDS, meeting the needs for sequentially dosage and combinational therapy.

Despite the attractive advantages of NDDS for anti-PD therapy, there are still some challenges for achieving satisfied therapeutic benefits and finally clinic translation. First, components of certain NDDS explored so far are not biodegradable and may cause toxicities on normal tissues. The development of functional NDDS with good biocompatibility and biodegradability is of great priority for the clinical translation of NDDS. Second, as many nanoparticles with intrinsic immunomodulation capabilities may also develop auto-immune disease or immune tolerance, the interaction between NDDS and the immune system should be carefully investigated. Thirdly, due to the heterogeneous tumor microenvironment of individual patients, designed NDDS for per-

sonalized treatments may further improve the therapeutic benefits of NDDS. Finally, combining anti-PD therapy with additional therapeutic strategies is urgently needed and the mechanisms behind those applications should be carefully investigated. As the advancement of nanotechnology, detailed interactions between NDDS, tumor microenvironment and PD pathway will be clarified. We believe that nanotechnology promoted PD-1/PD-L1 checkpoint blockade will largely contribute to the next generation of cancer immunotherapy.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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