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Mini-review

Emerging strategies in cancer therapy combining chemotherapy with immunotherapy

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ABSTRACT

Cancer immunotherapy holds great potential to battle cancer by exerting a durable immunity effect. However, this process might be limited by various constraints existing in the tumor microenvironment (TME), such as the lack of available neoantigen, insufficient T cells from the naive repertoire, or immunosuppressive networks in which immunogenic tissue is protected from immune attacks. Certain chemotherapeutic drugs could elicit immune-potentiating effects by either inducing immunogenicity or relieving tumor-induced immunosuppression. Some also leave tumors directly susceptible to cytotoxic T cell attacks. Mounting evidence accumulated from preclinical and clinical studies suggests that these two treatment modalities might be mutually reinforcing as an effective "chemo-immunotherapy" strategy. Herein, we reviewed the latest advances in cancer immunotherapy and related mechanisms involved in chemotherapeutic-mediated immune activation. The emerging combination strategies and synergistic effects in response to chemo-immunotherapy are highlighted. We also discuss the challenges and critical considerations in its future development.

1. Introduction

The immune system is gradually coming to be known as an important component of tumor destruction. With a better insight into molecular drivers of tumorigenesis, immunotherapy has developed into a novel tactic to overcome established cancers [1]. By activating a long-lasting T cell-mediated immune response, durable remission and eradication of micrometastases are likely to be achieved [2]. However, the overall survival (OS) of cancer patients has still far only marginally improved with a relatively low objective response rate (ORR), which is attributed to a lack of available neoantigen, insufficient T cells from the naive repertoire, and immunosuppressive tumor microenvironment (TME) in which immunogenic tissue is protected from immune attacks [3].

Chemotherapy remains a cornerstone of oncotherapy for decades. Current studies have revealed that apart from direct cytotoxicity of these anti-cancer agents, they also elicit immune-potentiating effects by releasing tumor antigen, disrupting tumor-induced immunosuppressive networks and sensitizing tumor cells to immune attacks [4,5]. All these factors turn the "cold tumor" into "hot tumor", so as to facilitate the full interplay with immunotherapy. The integration of these potentially complementary methods provides a new dimension to cancer care and suggests an intensified research on combined therapies be more effective than either modality alone.

Over the past few decades, mounting evidence accumulated from preclinical and clinical studies suggested that these two treatment modalities might be mutually reinforcing, and therefore their combination represents an effective anti-tumor strategy. Whether cancer vaccines at an early stage, or fast-growing immune checkpoint blockers (ICBs) in the near future, are in attempting to be integrated with conventional chemotherapeutics, which is interpreting a new therapeutic concept of "chemo-immunotherapy" [6,7]. While relieving the tumor debulk, it enhances the action of immunologic surveillance by leveraging the body's immune activation. In this review, we descriptively present latest advances in cancer immunotherapy and related mechanisms involved in chemotherapeutic-mediated immune activation. Furthermore, we propose a rationale for the design of combination therapy, and discuss how strategies are currently being harnessed with

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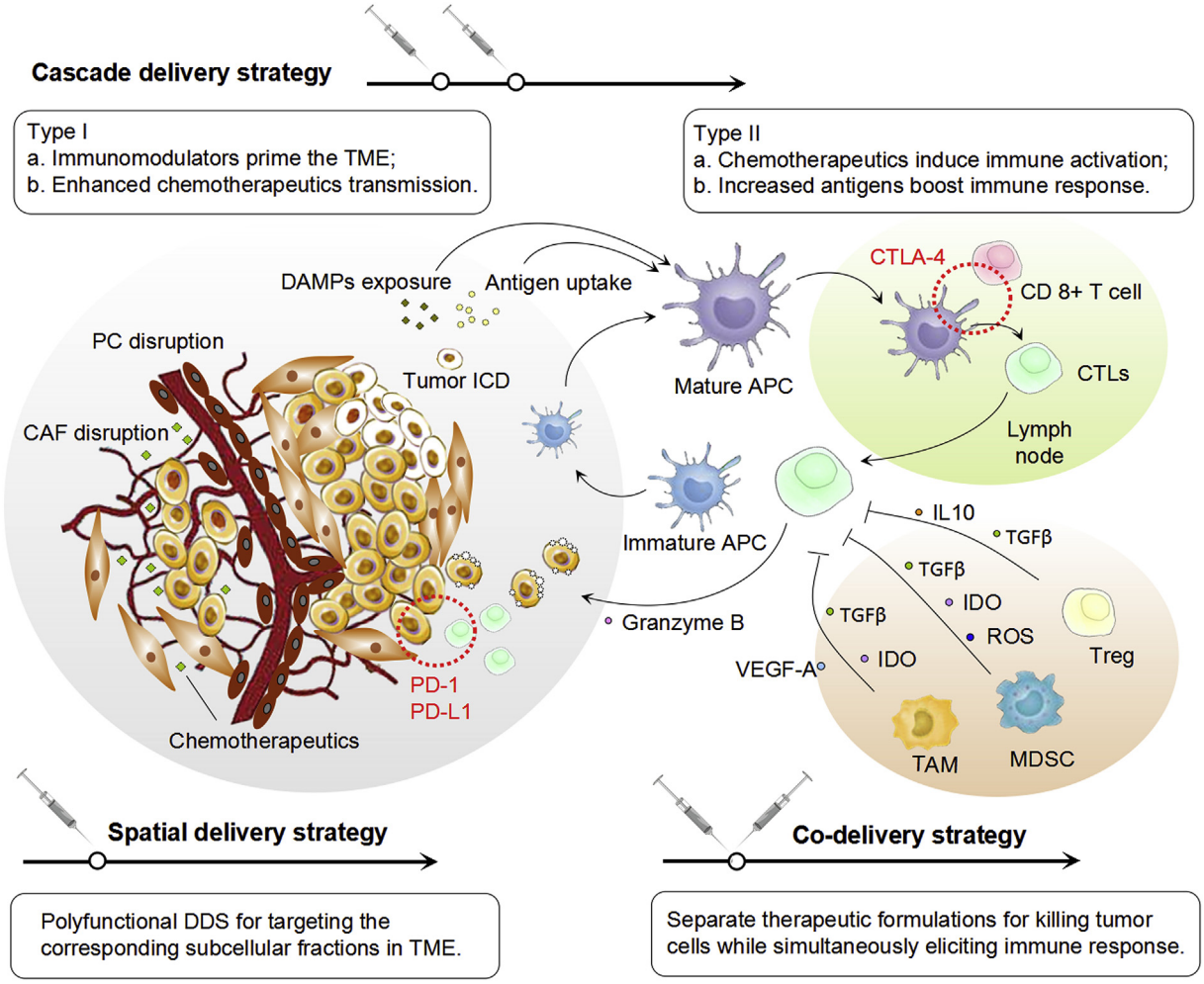

Fig. 1. Schematic representation of strategies in cancer combination therapies using chemotherapy with immunotherapy. PC, pericyte; CAF, cancer-associated fibroblast; DAMPs, damage-associated molecular patterns; APC, antigen presenting cell; CTL, cytotoxic lymphocytes; IDO, indoleamine-2,3-dioxygenase; TAM, tumor-associated macrophage; MDSC, myeloid-derive suppressor cell; Treg, regulatory T cell; TGFβ, transforming growth factor-β; VEGF-A, vascular endothelial growth factor A.

synergistic superiority in clinical or relevant laboratory models. Finally, we also highlight the challenges and future perspectives of integrating the rational therapeutic combination therapies (Fig. 1).

2. Cancer immunotherapy

Cancer immunotherapy helps stimulating the immune system to attack tumors in a general way, which involved in white blood cells, organs and tissues of the lymph system. Certain immunotherapies could label tumor cells in order to induce the differentiation and maturation of the cytotoxic T cell. Others boost the immune response to work better against cancer. The main types of immunotherapy now being used to treat cancer include: (a) immune checkpoint blocker; (b) cytokine therapy; (c) cellular immunotherapy; (d) oncolytic virus; and (e) polysaccharides.

2.1. Immune checkpoint blocker

In the past few years, immense progress has been made in cancer immunotherapy for treating certain cancers. One active area that has been extensively studied is the field of immune checkpoint blockers (ICBs). The therapy targets molecules on certain immune cells that act as a signal of “off switch” to help keep T cells from attacking normal cells in the body (Fig. 2) [8–10]. Currently approved checkpoint inhibitors block cytotoxic T-lymphocyte antigen 4 (TLA-4), programmed death 1 (PD-1) and programmed death ligand 1 (PD-L1).

CTLA-4 is a transmembrane protein expressed on the surface of activated T cells. The activation of CTLA-4 transmits an inhibitory signal to T cells and prevents the rise of memory T cell [10]. Researchers have found that CTLA-4 assists tumors in immune escape once binds to CD80 or CD86 on the surface of antigen-presenting cells (APCs) [11]. Ipilimumab, as the first approved CTLA-4 checkpoint inhibitor, has been verified to synergize with chemotherapy in preclinical murine tumor models as well as clinical trials [12]. In a phase III trial, ipilimumab plus dacarbazine improved OS versus dacarbazine alone in patients with advanced melanoma [13]. On-going studies are evaluating ipilimumab in combination with gemcitabine or cisplatin for treating urothelial carcinoma [14].

PD-1 and PD-L1 inhibitors are novel ICBs holding a great deal of promise as cancer treatments. The binding of PD-1 and its ligand PD-L1 mediates the co-suppression of T cell activity. Therefore, antibodies that specifically block PD-1/PD-L1 axis promote the capacity of T cells to kill tumors by potentially inducing tumor immunological surveillance. Several PD-1 and PD-L1 inhibitors are being trialed within clinic for usage in a multiple types of cancer [15]. By the end of 2017, PD-1/PD-L1 inhibitors such as permbrolizumab, nivolumab, and lately approved durvalumab, have been approved for the treatment of nine forms of cancer. Several clinical trials are underway for PD-1 inhibitors in combination with chemotherapies in order to explore a better curative effect (Table 1). For example, a phase II study is assessing the activity of

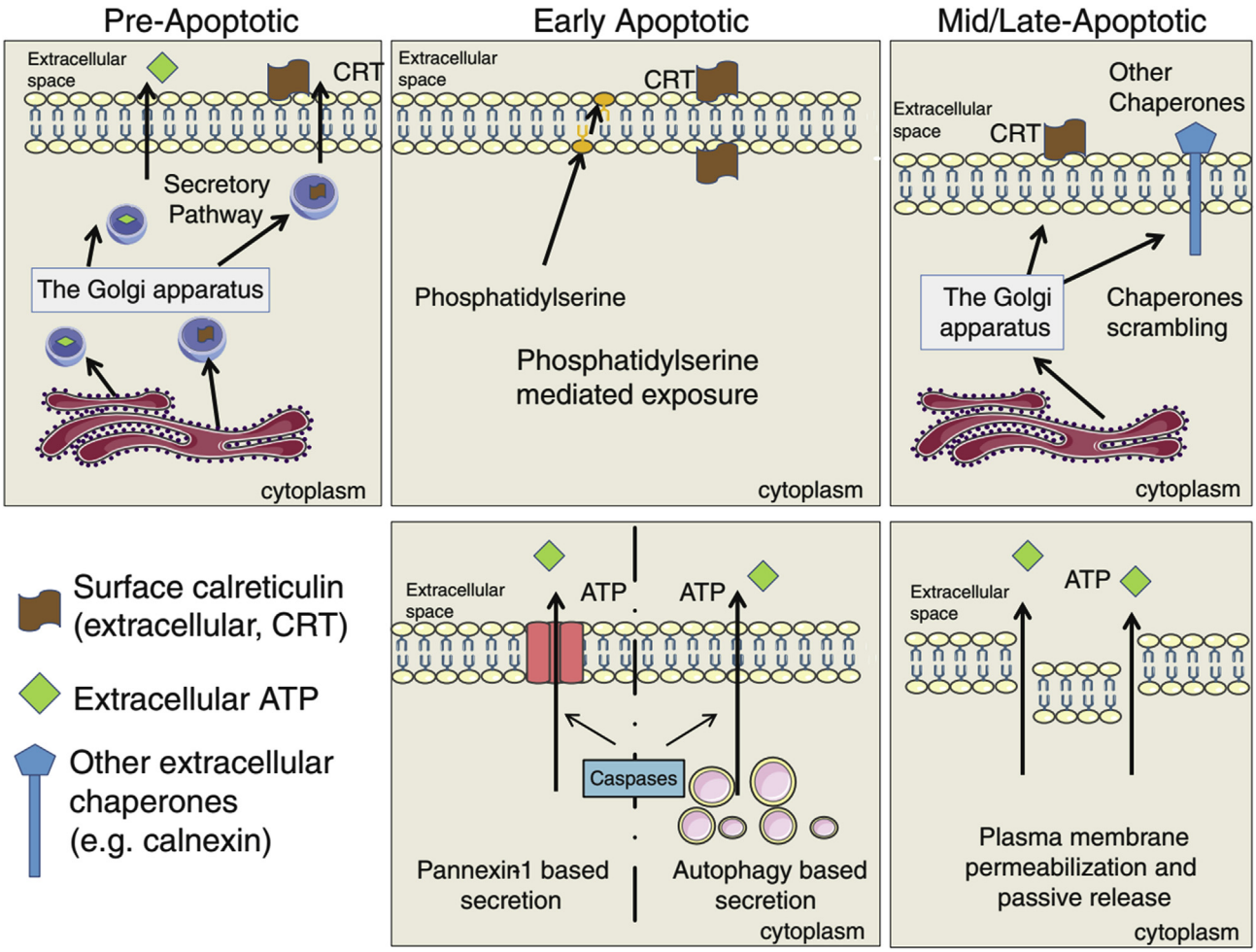

Fig. 2. An overview of the danger signaling pathways involved in surface CRT exposure and ATP secretion and their relation to different apoptotic stages. Signaling pathways responsible for surface exposure of CRT and secretion of ATP depend on immunogenic cell death stimuli. Adapted from J. Rios-Doria et al. [51].

Table 1
Currently On-Going Clinical Trials of PD-1/PD-L1 inhibitors Combining with Chemotherapeutics.

Identifier No.	Immune checkpoint inhibitors	Chemotherapeutic drugs	Condition or disease	Phase
NCT02967133	Nivolumab Ipilimumab	Nab-Paclitaxel Gemcitabine	NSCLC	2
NCT02181738	Nivolumab Ipilimumab	Doxorubicin Vinblastine Dacarbazine	Hodgkin Disease	2
NCT02041533	Nivolumab Ipilimumab	Gemcitabine Cisplatin Carboplatin Paclitaxel Pemetrexed	Stage IV or Recurrent NSCLC	3
NCT01905657	Pembrolizumab	Docetaxel	NSCLC	2, 3
NCT02963090	Pembrolizumab	Topotecan	Small Cell Lung Cancer	2
NCT02710396	Pembrolizumab	Carboplatin Nab-paclitaxel Pemetrexed	NSCLC, Head and Neck Cancer, Urinary Bladder Neoplasms, Esophageal Squamous Cell Carcinoma	2
NCT03018080	Pembrolizumab	Paclitaxel	Breast Cancer	2
NCT02775435	Pembrolizumab	Paclitaxel Nab-paclitaxel Carboplatin	NSCLC	3
NCT02335411	Pembrolizumab	Cisplatin 5-FU Capecitabine	Gastric Adenocarcinoma, Gastroesophageal Junction Adenocarcinoma	2
NCT02578680	Pembrolizumab	Cisplatin Carboplatin Pemetrexed	NSCLC	3
NCT03111732	Pembrolizumab	Oxaliplatin Capecitabine	Biliary Tract Neoplasms, Cholangiocarcinoma Bile Duct Cancer, Liver Cancer, Gallbladder Cancer	2
NCT02142738	Pembrolizumab	Paclitaxel Carboplatin Pemetrexed Cisplatin Gemcitabine	NSCLC	3
NCT03562871	Pembrolizumab	Carboplatin Pemetrexed	NSCLC	1, 2
NCT02039674	Pembrolizumab	Paclitaxel Carboplatin Bevacizumab Pemetrexed	NSCLC	1, 2
NCT02807636	Atezolizumab	Carboplatin Gemcitabine Placebo Cisplatin	Urothelial Carcinoma	3
NCT02425891	Atezolizumab	Nab-Paclitaxel	Triple Negative Breast Cancer	3
NCT02763579	Atezolizumab	Carboplatin Etoposide	Small Cell Lung Carcinoma	3
NCT02716038	Atezolizumab	Carboplatin Nab-paclitaxel	NSCLC	2
NCT02628132	Durvalumab	Paclitaxel	Breast Cancer	1, 2
NCT03390595	Avelumab	Carboplatin gemcitabine	Metastatic Urothelial Cancer	2

single agent chemotherapy, including docetaxel (DTX), gemcitabine (GEM) and pemetrexed, combined with nivolumab in squamous or non-squamous NSCLC subjects with primary resistance to prior PD-1 or PD-L1 inhibitor [16]. Furthermore, utilization of combination triweekly carboplatin/weekly paclitaxel (PTX) with intravenous pembrolizumab (for 6 cycles) is under study in first line treatment of patients with advanced ovarian cancer post surgery with any residual disease [17].

Other monoclonal antibodies (mAb) targeting stimulatory checkpoint of tumor necrosis factor (TNF) receptor family, such as CD134 and CD40, are under development for the promotion of tumor-specific T cell activation and proliferation. They remain experimental or available to cancer patients principally through participation with combined chemotherapy in clinical trials [18].

2.2. Cytokine therapy

Cytokines are a broad category of small proteins (~5–20 kDa) released by cells that have critical effect on cell signaling as immunomodulating agents. These agents can be used to enhance anti-tumor activity by triggering an immune response. Two commonly used cytokines, as drugs, are interferons (INFs) and interleukins (ILs).

INFs are released by host cells in response to the presence of tumor cells. They are typically divided into three classifications of INFs depending on the type of receptor through which they signal: type I (IFNα and IFNβ), type II (IFNγ) and type III (IFNλ). In general, IFNs are responsible for activating immune response by upregulating major histocompatibility complex molecules (MHC) [19,20]. High MHC class I and II expression increase the presentation of antigens to cytotoxic lymphocytes (CTLs) and helper T cells, respectively, that co-ordinate the activity of other immune cells. Specifically, IFNα has been approved for the treatment of hairy-cell leukaemia, chronic myeloid leukaemia and melanoma. IFNγ was shown to be clinically effective, on the basis of its *in vitro* effect on tumor growth inhibition caused by cellular apoptosis or autophagy [21]. Besides, IFNλ directly activates immune cells, such as macrophages and natural killer (NK) cells, showing benefits in anti-tumor effects. Dijkgraaf EM and his team demonstrated that IFNα (branded as Peg-Intron®) was feasible and safe in epithelial ovarian cancer (EOC) patients treated with carboplatin/doxorubicin and tocilizumab [22]. This combination therapy has been recommended for a further phase II evaluation based on advantageous immune parameters [22].

Interleukins (ILs) play an important role in regulating the growth and differentiation of most hematopoietic cell types [23]. IL-2 (branded as Proleukin®) is applied to the treatment of malignant melanoma and renal cell carcinoma in large intermittent doses or continuous doses extensively, through regulating both effector T cells and Tregs [24]. IL-10 exerts multiple and pleiotropic effects, such as suppressing host's immune response or tolerance *in vivo*. It is reported that immunomodulatory nanoparticles (NPs) containing a small interfering RNA (siRNA) effectively initiated the activation and proliferation of $CD8^+$ T cells. As a result, the combination of chemotherapy using the PTX-loaded hyaluronic acid complex (HA/PTX) and siRNA-loaded NPs efficiently inhibited tumor growth and increased the animal survival rate [25]. Intriguingly, IL-10 also performs immunostimulatory function. More recently, PEGylated recombinant human IL-10 (branded as Pegilodecakin®) has been shown to augment the systemic immune activation, $CD8^+$ T cell invigoration and polyclonal T cell expansion in patients with multiple oncology indications. The FDA also granted Pegilodecakin® in combination with FOLFOX regimen (FOL-Folinic acid, F— Fluorouracil, OX-Oxaliplatin) as a second-line therapy in patients with pancreatic cancer. Moreover, it also increases the secretion of the cytotoxic molecules granzyme B (GrzB) and perforin [26].

2.3. Cellular immunotherapy

Cellular immunotherapy, also known as adoptive T cell transfer (ACT), attempts to induce normal T cells to become T cells that are capable of recognizing target cells through *in vitro* modification, thereby triggering immunity against tumors. Currently, cellular immunotherapy is focus on dendritic cells (DCs) therapy and chimeric antigen receptor (CAR)-T cell therapy [27].

DCs are important APCs that act as messengers between the innate and the adaptive immune system. Antigen-loaded DCs initiate the differentiation of antigen-specific T cells into effector T cells and display unique functions and cytokine profiles, which is eventually associated with a wide variety of cellular changes. Consequently, their capacity of capturing, processing and presenting antigens to T cells makes it an essential component of cancer vaccine. One important method of activating DCs is by vaccination with autologous tumor lysates [28]. Besides, DCs can also be activated *in vivo* by immunogenic cell death (ICD) caused by some cytostatic agents such as anthracyclines, OX and bortezomib [29]. The only approved cellular cancer therapy based on DCs is sipuleucel-T, for the treatment of asymptomatic or minimally symptomatic metastatic castration-resistant prostate cancer. Recently, new design composed of DCs and NK cell cluster together demonstrated an excellent prognostic tool for T cell-directed immunotherapy [30]. Furthermore, direct injection of immature DCs (iDCs) into tumor tissue along with either chemotherapy or radiotherapy, has been used to treat advanced malignancies and suggests anti-tumor efficiency in the form of chemo-immunotherapy [31].

CAR-T cell therapy is to genetically engineer T cells with the new ability to a specific antigen that is present on the surface of tumor. CARs are core components of CAR-T therapy that are typically composed of three regions: the ectodomain, the endodomain, and the transmembrane domain [32]. The ectodomain is the region of the receptor that is responsible to interact with tumor-associated antigen (TAA). The endodomain is the internal cytoplasmic end of the receptor that perpetuates signaling inside the T cell. The transmembrane domain is a structural component, which is essential for the stability of the CAR.

Technically, CAR-T cell therapy involves a one-time infusion of a patient's own immune cells with a desired epitope specificity. The particular given property is capable of specifically recognizing their target through binding domain, resulting in great progress in hematological malignancies. So far, two types of CAR-T cell therapy have been approved by the FDA, namely Tisagenlecleucel® and Axicabtagene ciloleucel®. They are used to treat lymphoma and diffused large B-cell lymphoma (DLBCL), respectively. Both of them target cells that express a molecule called CD19 on their surfaces.

Furthermore, the exploration of CAR-T cell therapy on solid tumors has also been carried out by binding antigenic targets in the TME, including proteins, carbohydrates or glycolipids [33,34]. The clinical trial are also widely expanded with specific receptor for mesothelin, a protein that is overexpressed by chemotherapy refractory metastatic PDAC cells. This therapy induced a spreading of antibody responses against multiple proteins, including immunoregulatory molecules (eg. PD-1 and PD-L1), playing decisive roles in conquering the TME barriers and the final anti-tumor efficacy [35].

2.4. Oncolytic virus

Oncolytic virus (OVs) are live viruses selectively toxic to cancer cells [36]. They are found not only to cause direct destruction of tumor cells, but also to stimulate the host's anti-tumor immune response [37]. Consequently, increasing attention has been given to making use of newly developed virions to help destroy the remaining tumors [38]. There is a growing interest in their combination with cytotoxic agents, which might result in a highly synergistic anti-tumor efficacy compared to each modality alone. For instance, reovirus and GEM combination treatment was demonstrated to postpone peritoneal carcinomatosis development and prolong the survival of cancer-bearing hosts. The advantages of their combined action were reflected in the following two aspects. On the one hand, GEM benefited the downregulation of

myeloid-derive suppressor cell (MDSC)-related factors and accelerated the development of T cell responses. On the other hand, the complementation of revirus further potentiated virus-initiated anti-cancer immunity and enhanced the efficacy of oncotherapy [39].

2.5. Polysaccharides

Certain compounds, primarily polysaccharides, possess anti-cancer properties by activating the immune system. Beta-glucans such as lentinan have been investigated as immunologic adjuvants in clinical trials, as they demonstrate the ability of stimulating macrophage, NK cells, T cells and certain cytokines [40]. Moreover, fucoidins were subjects of preliminary studies exploring for potential effects on immunomodulatory and anti-cancer activity [41]. A recent study indicated that with the modification of fucoidin, doxorubicin (DOX) loaded NPs exhibited elevated immunocompetency and quantifiable antiproliferative activity against breast cancer [42]. The treatment generated an immunotherapeutic response with gradual increment of plasma IL-12 and TNF-α levels, and reversed the polarization of TAMs towards M1 subtype, that retarded tumor growth [42].

3. Chemotherapy induced immune activation

Standard chemotherapy provides a major treatment option as a single regimen. Emerging evidence suggests that the clinical success of conventional chemotherapy is not only attributed to its cytotoxic effect, but also caused by the activation of immunosurveillance, which promotes a proimmunogenic milieu within the tumor capable of stimulating host cancer-specific immune responses [43]. In this section, the mechanisms related to the immune activation induced by chemotherapy are discussed in details from three aspects: (i) improving immunogenicity of tumor cells; (ii) eliminating immunosuppressive networks; and (iii) sensitizing tumor cells to immune attacks.

3.1. Improving immunogenicity of tumor cells

Immunogenic cell death (ICD) or immunogenic apoptosis is a form of cell death caused by radiotherapy, photodynamic therapy, or some cytostatic agents. Unlike normal apoptosis, which is mostly non-immunogenic or even tolerogenic, immunogenic apoptosis of cancer cells can induce an effective anti-tumor immune response through activation of DCs and the subsequent specific T cell response [44].

ICD induced by different drugs shares common features of releasing a series of damage-associated molecular patterns (DAMPs), such as calreticulin (CRT), ATP and high mobility group box 1 (HMGB1) that promote APCs maturation and activate CTLs to kill tumor cells [45]. Exposure of CRT and heart-shock protein (HSP) on the cell surface is likely to stimulate DCs to engulf, and facilitate the crosspresentation of antigens derived from tumor cells on MHC class I molecule, which then leads to $CD8^+$ T cell response [46]. The release of HMGB1 promotes DCs to steadily bind with dying tumor cells and induces specific T cell-mediated anti-tumor immune response [47]. Recent researches have found that chemotherapeutics can cause autophagy of tumor cells, accompanied with distinguishing feature of ATP release, which functions as a “finding me” signal to recruit DCs and T cells to enter into tumor bed. Correspondingly, in autophagy deficient tumor cell lines, inhibition of extracellular ATP degradation enzyme and augment of extracellular ATP concentration can recruit immune cells and restore the therapeutic effect of anti-cancer drugs (Fig. 2) [48].

The use of ICD inducers alone or in combination with other anti-cancer therapies has yet been proven to be effective in preclinical models of cancer and now is underway in the clinical trials [49]. Fend and co-authors found that an engineered oncolytic vaccinia virus could be further potentiated by co-administration of ICD-inducing chemotherapy or ICBs [50]. In addition, liposomal doxorubicin (Doxil) was documented to induce tumor-infiltrating cells and elicit a costimulatory phenotype capable of activating an anti-tumor T-cell response [51]. These results uncover the potent innate and adaptive immunity caused by ICD, especially in combination with immune-based therapies. The synergistic benefit of the anti-tumor activity, in terms of reduced tumor cell proliferation, is associated with the increased immune response rates due to an increased exposure of tumor antigen.

3.2. Elimination of immunosuppressive networks

In the process of the interaction with immune system, distinct immunosuppressive networks are often found in tumor parenchyma, consisting of Tregs, MDSCs, and M2-type tumor associated macrophages (TAMs). They aggregate around tumor cells, forming a microenvironment conducive to tumor growth, which limits the ability of immune cells to infiltrate and recognize tumor cells. Beyond that, Tregs are known to contribute to an inhibitory microenvironment by secreting high amounts of TGFβ and IL10 that significantly suppress the function of CTLs and APCs [52]. While the M2-type TAM is mostly involved in mediating tissue repair with immunosuppressive traits and produces several anti-inflammatory cytokines and modulators, including IL10, TGFβ, IL1 receptor antagonist (IL1rα), IL2rα and arginase I [53]. MDSCs, on the other hand, mediate the recruitment and expansion of tumor-specific Tregs and actively favor the differentiation to M2-type macrophages [54]. Some chemotherapeutics potentially provide a favorable microenvironment of anti-tumor immunity by directly eliminating these immunosuppressive cells or cytokines. For example, a dose-dense regimen of cisplatin (CIS) plus PTX was shown to significantly reduce MDSCs in ovarian tumor-bearing mice compared with a maximum tolerated dose [55]. PTX has been proved to alter the cytokine network within the tumor sites in the Lewis lung carcinoma model by reducing the expression of monocyte chemoattractant protein 1 (MCP-1) [56]. Another study demonstrated that multiple injection of GEM resulted in a decrease in the proportion of MDSCs and Tregs in both splenic and tumor tissue compared to the control group which treated with phosphate-buffered saline [57]. In a majority of cases, cyclophosphamide (CTX) has always been administered with the primary aim of inhibiting or depleting Tregs. Along similar lines, low dose of CTX (100 mg/kg) is capable of depleting cycling $CD4^+$ $CD25^+$ Tregs and inhibiting their immunosuppressive activity [58]. In addition, the administration of CTX also suppresses M2-type macrophage polarization, which is essential to facilitate effector T cell infiltration and the subsequent tumor elimination [59].

3.3. Sensitizing tumor cells to immune attack

In addition to recruiting activated immune cells to TME, chemotherapy may also make tumor cells more vulnerable to immune attacks. It is reported that after treated with DOX, CIS or PTX, tumor cells tend to be more susceptible to the cytotoxic effect of CTLs through a substantial increase in permeability to the intracellular GrzB, a serine protease released by the CTLs during antigen-specific interaction with tumor cells [60]. This effect was mediated via the upregulation of mannose-6-phosphate receptors on the surface of tumor cells. When combined with chemotherapy, activated CTLs interacting with tumor antigen could release GrzB that were able to penetrate into neighboring tumor cells and induce cellular apoptosis without a cell-cell contact. More importantly, chemotherapy allows for bypassing a requirement for antigen recognition by CTLs, hence large numbers of tumor cells including those that do not express specific antigen would be sensitive to the immune attack of CTLs [60].

4. Emerging strategies in the combination treatment of chemotherapy and cancer immunotherapy

The rationale for this dual-combined strategy is that chemotherapy has the capacity to debulk the primary tumor mass, whereas immune-

based therapy has the latent potential to eradicate disseminated disease by manipulating the body's immune system. In the context of the aforementioned mechanisms, the combinatory approach represents a parallelism between cytotoxic and immunological effects that prompts potential synergies without any apparently additional toxicities. The combination strategies in this section are divided into three categories, namely (i) cascade delivery strategy; (ii) spatial delivery strategy; and (iii) co-delivery strategy of chemo-immunotherapy.

4.1. Cascade delivery strategy

Cascade delivery of chemo-immunotherapy is performed through the sequential administration with different waves, in order to improve the overall clinical efficacy. To this end, immunomodulators could be employed in the first wave that remodels the TME for higher perfusion and lower resistance. Based on this, the second wave of chemotherapeutics might achieve an enhanced tumor transmission. The TME modulators can be small molecule kinase inhibitors that target and block the key factors associated with supportive stroma cells, tumor angiogenesis, reactive fibroblasts, and an altered extracellular matrix (ECM) [61].

Taking advantage of nanodrug delivery system, the integration of a variety of regimens becomes possible with a diversity of therapeutic mechanisms. An engineered NPs proposed by Leaf Huang's group achieved high-efficiency tumor inhibition against pancreatic ductal adenocarcinoma (PDAC) by sequential delivery of different therapeutics. The first wave targeted pericyte (PC) coverage of vasculatures through a small molecule inhibitor that blocked the TGFβ signaling pathway, followed by a second wave of GEM loaded liposomes [62]. Compared with mice receiving liposomal administration alone, there was more abundant and homogeneous liposome distribution in BxPC3 xengrafts by means of the TGFβ inhibitor. This two-wave approach successfully disrupted the ECM of PDAC and improved tumor blood perfusion through immunomodulating kinase that allowed an vascular access, thereby providing effective shrinkage of the tumor xenografts [62].

On the other hand, as previously mentioned, immunogenic apoptosis of cancer cells caused by several types of chemodrugs produce tumor-derived antigens [47–49,63]. These antigenic substances can be efficiently internalized by APCs and hence increase the activation of CTLs, serving as potential candidates for assisting the combination with cancer immunotherapy. Therefore, sequential delivery strategy may also improve the therapeutic effect by firstly releasing tumor antigens, and then boosting combined immune response to efficiently attack and eliminate the solid tumor. For instance, a synthetic high-density lipoprotein (sHDL)-mimicking nanodiscs was designed for stimuli-responsive delivery of DOX and sequentially synergized with ICBs [64]. Compared with free DOX therapy, the ultrasmall size of sHDL-DOX enabled the intratumoral delivery of DOX. Whatsmore, priming tumors with DOX-carrying nanodiscs elicited robust anti-tumor $CD8^+$ T cell responses while broadened their epitope recognition to tumor-associated antigens (Fig. 3) [64]. Combination with the subsequent anti-PD-1 therapy then induced complete regression of established colon carcinoma tumors in 80–88% of animals and protected survivors from tumor recurrence [64].

In another study, two types of PLGA NPs were fabricated with a toll-like receptor (TLR) ligand to activate bone marrow-derived dendritic cells (BMDCs) or a siRNA to silence IL-10 [25]. The combined regimen of these polyfunctional NPs caused an increase in the index of Th1/Th2 cytokine (IL-12/IL-10) ratio, which is critical to stimulate an anti-tumor immune response [25]. After primary injection of HA/PTX, tumor-associated antigen was released and taken up by tumor-recruited BMDCs. Followed up by immunomodulatory NPs, BMDCs became activated and migrated to the tumor-draining lymph nodes [25]. As a result, the sequential treatment displayed excellent potential to activate APCs at the injection site and naive T-cells in the lymph nodes, thereby generating a potent synergistic effect against solid tumors. Likewise, the anti-tumor efficacy of CIS or PTX were investigated followed by anti-PD-1 therapy and glucocorticoid-induced TNF receptor related protein (GITR) mAb in murine ID8 ovarian cancer and 4T1 breast cancer models, respectively [65]. The administration of CIS or PTX before immunotherapy shifted an immunosuppressive tumor milieu to an immunostimulatory state in peritoneal cavity, by increasing IFNγ producing effector T cells and decreasing Tregs and MDSCs. Consequently, a synergistic effect was realized with improved anti-tumor efficacy with a long-term of tumor-free survival [65]. The increasing tumor antigenicity and a further decrease in immunosuppressive cells by chemotherapeutic drugs may partially account for their synergism.

4.2. Spatial delivery strategy

The TME is a complex community composed of surrounding blood vessels, immune cells, fibroblasts, lymphocytes, signaling molecules and ECM. However, the subpopulation of cells are located with different distribution patterns in tumor tissues, extending stereoscopically to the marginal, middle and deep areas of TME [66]. Spatial delivery strategy, carrying different therapeutics into one multifunctional drug delivery system (DDS), is aimed at different subcellular groups in the TME.

TAMs, account for up to 50% of tumor mass, are enriched in well-perfused areas such as perivascular regions, whereas tumor cells spread throughout the bulk of tumor mass [67]. In response, an immunostimulatory nanocarrier was developed with BLZ-945 and a platinum (Pt) based prodrug. These co-existing therapeutics were selectively delivered to the corresponding TAMs and tumor cells in the TME [68]. Pt prodrug conjugated small particles with a size less than 10 nm could penetrate into poorly vascularized areas and induce apoptosis of tumor cells. This was highly in consistent with the result from a previous research showing that 10 kDa particles (~7 nm) most efficiently targeted cancer cells through passive accumulation and tissue penetration [69]. However, it was found that Pt prodrug significantly increased the ratio of TAMs in tumor-infiltrating leukocytes compared with PBS group, which blunted the effectiveness of chemotherapy by suppressing adaptive immunity [68]. To solve this dilemma, BLZ945 was integrated into the delivery system and preferentially taken up by TAMs in the perivascular region, where it disturbed the CSF-1/CSF-1R signaling pathway. The combination of BLZ945 then blocked the recruitment of TAMs precursor and induced TAMs depletion from tumor tissues [68]. This administration strategy showed a significant increase in $CD8^+$ T cells/Tregs ratio, indicating a positive shift of T cells towards cytotoxic effector T cells and a declined tendency of tumor-promoting Tregs through TAMs depletion (Fig. 4).

Multifunctional nanohybrid system contained CIS prodrug and indoleamine-2,3-dioxygenase inhibitor (IDOi) was provided to boost the immune activity of conquering cervical tumor especially that responded poorly to conventional chemotherapeutics [70]. By converting L-tryptophan (trp) to L-kynurenine (kyn), IDO restricts Trp availability in tumor cells and innate immune cells; this triggers effector pathways that interfere in the development of cytotoxic T cells, while inducing Tregs [71]. In terms of the overall structure of NPs, the former prodrug was reduced to CIS and bind to genomic DNA to induce cancer cell apoptosis. Meanwhile, the latter IDOi blocked the pathway of kyn production, leaving more Trp in the TME for T cell proliferation and development [70]. Consequently, spatial targeting delivery system not only accommodates multiple cargos, but also optimizes their delivery profiles, which is desirable for the combination cancer therapy.

Analogously, IDOi (indoximod, IND) was conjugated to a phospholipid as a prodrug to be incorporated into a lipid bilayer (LB) that encapsulates mesoporous silica nanoparticles (MSNP) [72]. The porous MSNP interior allows contemporaneous delivery of OX. Kaplan-Meier plots confirmed that while OX/IND-MSNP improved survival, the dual-delivery carrier had a significant survival benefit. Furthermore,

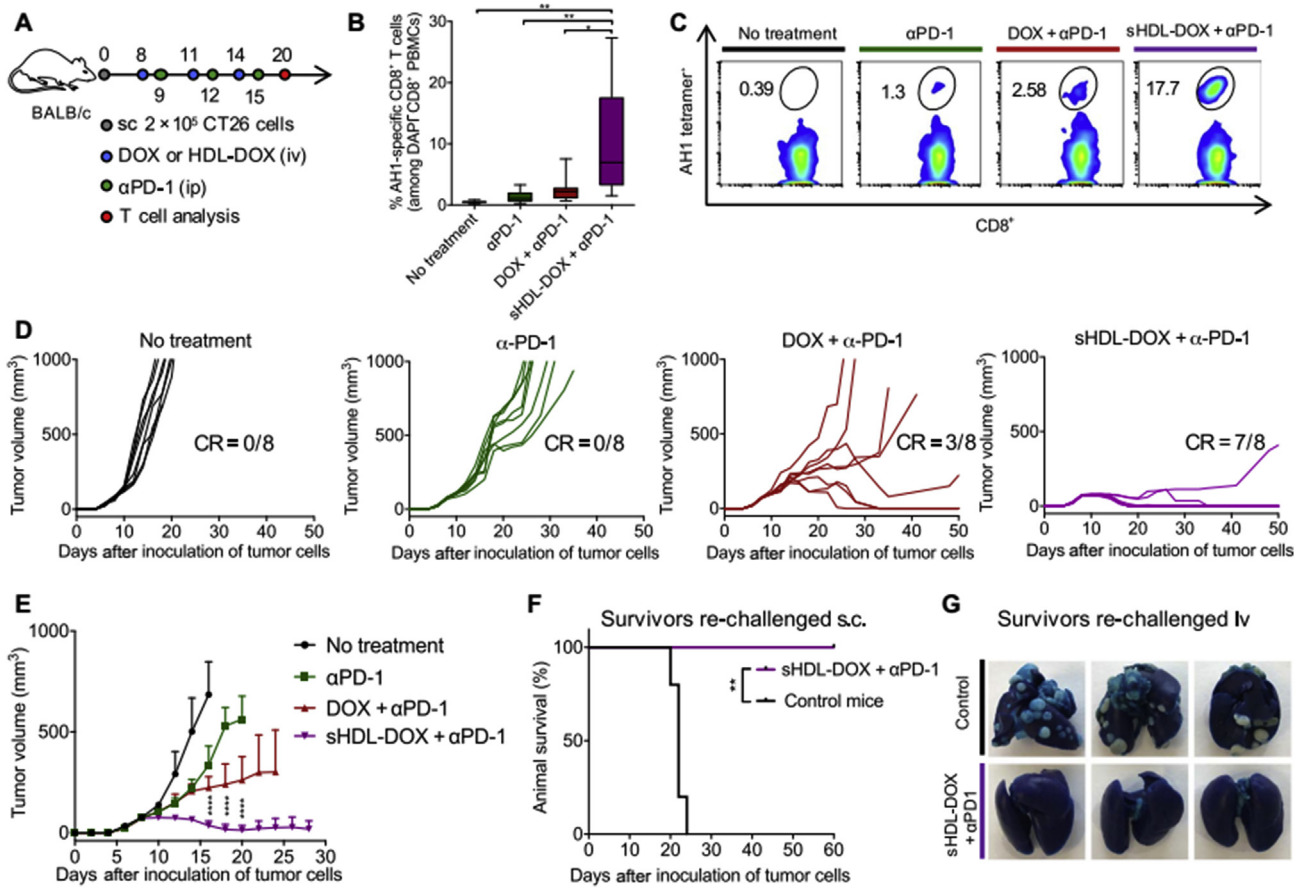

Fig. 3. Potentiation of αPD-1 immunotherapy with sHDL-DOX for treatment of CT26 tumors. (A) BALB/c mice were subcutaneously inoculated with 2×10^5 CT26 cells on day 0. On day 8, 11, and 14, tumor-bearing mice were treated with indicated formulations containing DOX (4 mg/kg). αPD-1 was injected intraperitoneally at 100 mg per dose on day 9, 12, and 15. (B) The percentage of CT26 tumor antigen AH1-specific $CD8^+$ T cells among PBMCs on day 20, and (C) the representative scatterplots. (D) Individual growth curves for mice treated with indicated formulations. (E) The average tumor growth curves for mice treated with indicated formulations. (F and G) On day 60, sHDL-DOX + αPD-1-treated animals in (E) were re-challenged by subcutaneous or intravenous injection of 2×10^5 CT26 cells. For the control groups, naive BALB/c mice were re-challenged with the same number of CT26 cells. Shown are the animal survival (F) and lung metastasis (G) of CT26 cells on day 22 after re-challenge. Adapted from K. Rui et al. [62]. sHDL, synthetic high-density lipoprotein; PMBCs, peripheral blood mononuclear cells.

immuno-positron emission tomography (immuno-PET) confirmed anti-PDAC immunity, quantitatively tracking the presence and abundance of $CD8^+$ and other immune cell subsets by using a ^{89}Zr-desferrioxamine-labeled anti-CD8 cys-diabody. Compared to tumors of saline-treated animals, there was a remarkable increase of 2.5- and 7.5-fold in radioactivity in the interior tumor tissues of OX/LB-MSNP and OX/IND-MSNP treated animals, respectively, indicating an augment infiltration of $CD8^+$ T cells. There was also a remarkable increase in signal intensity in the spleen and tumor draining lymph node (Fig. 5) [72]. All considered, based on the synergistic effect of OX and IND delivery, an effective systemic anti-PDAC immune response was generated by recruiting CTLs, concomitant with downregulation of Tregs.

4.3. Co-delivery strategy

Co-delivery strategy of chemo-immunotherapy consist of separate therapeutic formulations, but simultaneously administrated into the body. Evidence showed that the single agent for tumor cell-specific targeting and killing is not sufficient to overcome microenvironment barriers [73]. As a result, the co-delivery of chemo-immunotherapy offers a convenient and universal strategy for killing cancer cells while elicits broad anti-tumor T cell responses.

In recent study, Doxil acted as a broad booster of anti-tumor immunity when co-delivered with a variety of cancer immunotherapies [51]. In CT26 syngeneic models, the combination of Doxil with anti-PD-1, PD-L1, CTLA-4 antibodies and GITR ligand led to high rate of complete response (CR). A decreased expression of PD-L1 induced by Doxil may explain the higher sensitivity of tumors to this combination that further increased the percentage of $CD8^+$ T cells and decreased the amount of Tregs [51]. Moreover, mice that achieved CR with Doxil treatment rejected tumors upon rechallenge, demonstrating that Doxil also generated an immunological memory T-cell response [51].

Strong synergy with promising anti-tumor activity was also observed in the combination of chemotherapy and cytokine therapy. Interleukin 7 (IL-7) serves as a growth factor that signals through the C-chain to affect T cell proliferation, development and homeostasis. The *in vivo* administration of IL-7 combined with OX remarkably inhibited the growth of tumors in lung and abdomen metastases models of colon cancer, while IL-7 alone had no effect on chemosensitivity in culture or tumor growth [74]. Compared with IL-7 or OX alone, the co-delivery therapy involved a marked increase in tumor-infiltrating activated $CD8^+$ $CD69^+$ positive cells and suppression of $CD4^+$ FoxP3+ Tregs, altogether defending against tumor development and progression [74].

5. Synergistic effects of chemo-immunotherapy

One reason why tumor thrives is that they are able to hide from the immunological surveillance. According to the results of preclinical and clinical studies, there is an outstanding performance brought by the combination of chemotherapy and immunotherapy. This could be

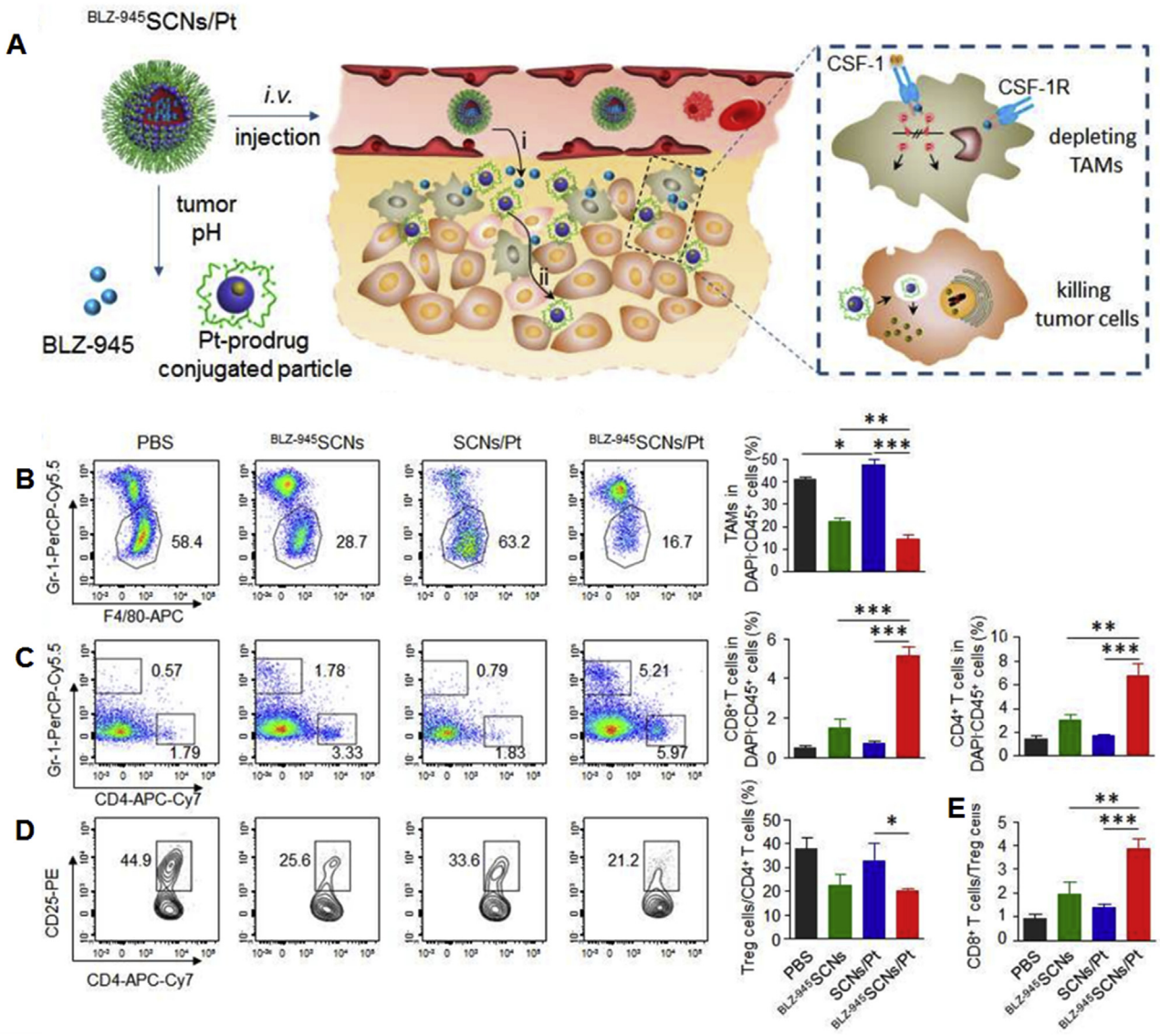

Fig. 4. Spatial delivery mechanism of BLZ-945 and Pt-prodrug to TAMs and tumor cells and an *in vivo* analysis of immune cells in tumor tissues. (A) Schematic illustration showing the mechanism of spatial delivery of BLZ-945 and Pt-prodrug to TAMs and tumor cells, respectively. (B–D) Relative abundance of various immune cells in 4T1 tumor tissues at the end of treatment by flow cytometry. These cells included CD45 $^{+}$ CD11b + Gr1-F4/80 + TAMs (B), CD45 $^{+}$ CD11b- CD8 $^{+}$ T cells and CD45 $^{+}$ CD11b- CD4 $^{+}$ T cells (C), and Treg cells (D). (E) The ratio of CD8 $^{+}$ cells/Treg cells at the end of treatment. Adapted from J. Wang et al. [68]. CSF-1 and CSF-1R, colony stimulating factor 1 and its receptor; BLZ945, a highly selective inhibitor of CSF-1R; SCNs, sensitive cluster nanoparticles.

summarized in four aspects as follows, including: (i) elevated anti-tumor efficacy; (ii) anti-invasion and metastases; (iii) reversing drug-resistance; and (iiii) preventing recurrence.

5.1. Elevated anti-tumor efficacy

Several studies have been certified to present a robust effect on lengthening tumor burden through synergistic chemo-immunotherapy. As mentioned earlier, Doxil administration increased CD80 expression on mature DCs, monocytic and granulocytic myeloid cells. In either CT26 or MCA205 tumor models, the combination of efficacious doses of Doxil and cancer immunotherapies accounted for the maturation of tumor-infiltrating APCs, which elicited a costimulatory phenotype to activate an anti-tumor T-cell response [51].

Cancer vaccines have been applied to chemo-immunotherapy, during which the abundant infiltration of polyfunctional vaccine-induced CD8 $^{+}$ T cells could be synergized with chemodrugs to promote tumor cell death [75]. Upon combined treatment of synthetic long peptides (SLP) and chemotherapeutics, tumor cell proliferation was significantly decreased rather than a single therapy [76]. SLP vaccines induced only a temporal decrease in tumor size in most mice and complete tumor eradication in 16% of the mice. Comparatively, the combination with chemotherapeutics like topotecan, carboplatin, GEM or CIS clearly exhibited a better survival. Notably, of these chemotherapeutics, CIS synergized best with SLP vaccination in tumor eradication without the requirement for a maximum-tolerated dose (MTD). The *in vivo* cell death analyzed by the TUNEL assay indicated that vaccinated mice were highly infiltrated with TNFα-producing T cells, which might be responsible for the synergistic effect. This was additionally confirmed by the fact that the addition of the TNFα inhibitor etanercept fully abolished this synergy [76]. Strikingly, the combination of two treatments did not result in any additional systemic side effects.

In additional experiments, a chemo-immunotherapeutic nanoparticle delivery system (TLNP) was prepared, co-encapsulating the PTX as the chemotherapeutic and a non toxic derivative of

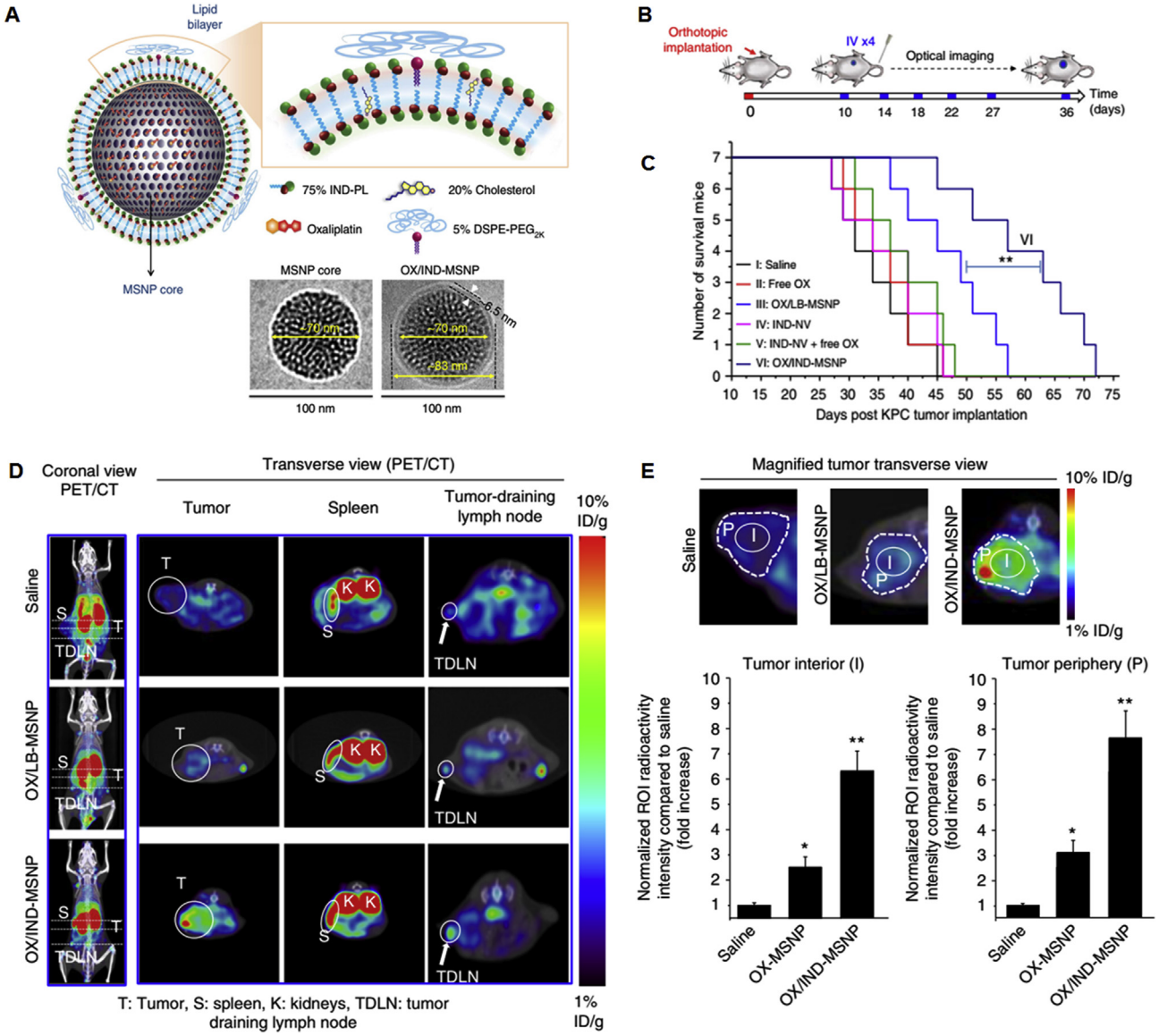

Fig. 5. Nano-enabled pancreas cancer immunotherapy using immunogenic cell death and reversing immunosuppression. (A) Schematic to show the structure of OX-laden MSNP, in which the drug is trapped by a lipid bilayer that contains the IND-PL. (B) Orthotopic tumor-bearing B6/129 mice were IV injected with the OX/IND-MSNP to deliver the equivalent 5 mg/kg OX and 50 mg/kg IND every 4 days, for a total of 4administrations. (C) Assessment of the survival effect of OX/IND-MSNP vs. the controls was conducted by repeating the experiment in (B). (D) Animals with established orthotopic tumors were different formulations on days 10, 14, 18, and 22 post KPC cell implantation into the pancreas. At day 26, 100 μL doses containing 1.07–2.33 MBq (29–63 μCi, 2.3–5.3 μCi/μg)^{89}Zr radiolabeled cDb in saline was IV injected to the same animals. 20 h later, microPET and CT scans wereacquired by a G8 PET/CT scanner (Sofie Biosciences). (E) To evaluate the CD8$^+$ signal at the tumor site, the operator-defined ROIs were used to demonstrate a 6.2- and 7.5-fold increase in the signal intensity in the tumor interior and periphery, respectively, during OX/IND-MSNP compared to saline treatment. Adapted from J. Lu et al. [72] MSNP, mesoporous silica nanoparticles; IND-PL, phospholipid-conjugated IND prodrug; IND-NV, IND-PL nanovesicles; OX/LB-MSNP, OX encapsulated in an IND-PL free carrier; OX/IND-MSNP, OX plus IND using LB coated MSNP; ROIs, regions of interest.

lipopolysaccharide (LPS) as the immunostimulant [77]. Compared to the PTX and LPS treatment alone, a significant higher percentage of activated CD8$^+$ CD4$^+$ T cells and DCs was found in TLNP treated animals, which is associated with enhanced tumor regression [77]. Detection of high amount of TNFα and IL-12, two major cytokines secreted by activated APCs, indicated the combination therapy could effectively induce infiltration of macrophages and DCs in the TME, and further activate these cells to an immune stimulatory state. By reverting the immunosuppressive TME into a functionally active state, 70% of tumor bearing mice treated with TLNP survived 30 days after treatment whereas only 50% of PTX treated and 30% of LPS treated mice survived that long [77]. Based on the above, PTX produced tumor antigens by ICD could assist a strong tumor specific immune response of LPS, which might in return strengthen the cytotoxic activity of PTX [77].

5.2. Anti-invasion and metastases

The process of tumor metastases is the major cause of cancer-related deaths, accounting for more than 90% of the mortality in tumor patients [78]. Tumor metastases is an extremely complex process, accompanied by high tumor heterogeneity. Several critical threats relevant to tumor heterogeneity give rise to the occurrence and development of tumor invasion and metastases, such as cancer-associated fibroblasts (CAFs), vascular endothelial growth factor A (VEGF-A), TGFβ, TAMs and matrix metalloproteinase (MMP) [79,80]. As the first approved anti-angiogenic mAb, bevacizumab increased patient's

OS when used in conjunction with chemotherapy for metastatic lung, colorectal and kidney cancers [81]. In a preclinical work, the simultaneous targeting of TAMs and tumor cells was suggested as a powerful means to decrease pulmonary metastatic nodules [68]. As indicated, the therapeutic efficacy of combination treatment led to a significant decrease in mRNA expression of MMP-9 and VEGF-A in comparison with its monotherapy counterparts. It was clearly demonstrated that the concurrent delivery system could efficiently inhibit tumor growth and metastases in multiple tumor models of murine breast and colon cancer [68]. Furthermore, an MMP-2 sensitive hyaluronic acid-PLGLAGG-DOX prodrug (HA-Psi-DOX) exhibited elevated tumor accumulation ability and reduced systemic side effects [82]. The blocking of PD-1/PD-L1 axis by PD-1 blockade during HA-Psi-DOX treatment induced robust tumor infiltrating lymphocytes (TILs) recruiting to tumor bed, and improved the anti-metastasis capability of the HA-Psi-DOX nanoprodrug. This enhanced anti-metastasis effect was mainly ascribed to the robust immune response elicited during the HA-Psi-DOX treatment and drug-mediated cytotoxicity to tumor cells [82].

5.3. Reversing drug-resistance

Chemotherapy alone may temporarily suppress the tumor progression, but gradually disappear the drug-sensitive subgroup. Tumor cells that develop resistance to chemodrugs often results in the ultimate failure of chemotherapy. For example, over-expression of protein wingless-type MMTV integration site (Wnt) family in CAFs is considered to impair apoptosis and infiltration of tumorigenic immune cells [83]. Off-target exposure to CIS NPs leads to increased secretion of Wnt16, giving rise to stroma reconstruction and drug resistance of tumor cells. In this case, Leaf Huang's research group synthesized quercetin phosphate prodrug and formulated it into lipid-calcium-phosphate (LCP) NPs. After intravenous administration of LCP NPs, Wnt16 expression was significantly downregulated and resulted in a 50% reduction in collagen compared to the untreated control group. When combined with CIS NPs, it acquired a synergistic anti-tumor effect in a stroma-rich bladder cancer model [84].

The ATP-binding cassette (ABC) transporters (e.g. P-gp and MRP-1) participate in the development of multiple drug resistance (MDR) in cancer treatment, playing a pivotal role in the chemodrug resistance [85]. A preclinical study provided new instructions that the orderly treatment of TLR3 agonist polyinosine-polycytidylic acid (PIC) and low dose CIS reduced the expression of P-gp and MRP-1, which consolidated the chemotherapy [86]. Meanwhile, the immunosuppressive networks dampened *in vivo*, such as MDSCs, TAMs and CAFs, were also positively related to the enhancement of the combination treatment as well as alleviated adverse effect [87].

High-level expression of glutathione (GSH) and cysteine released by fibroblasts also promotes drug resistance, as the electrophilic properties of glutathione react with cytotoxic agents and inactivate them [88]. On a related note, fibroblasts were identified to diminish CIS accumulation in ovarian cancer cells by releasing thiols and elevating intracellular GSH levels. Moreover, the work uncovered that $CD8^+$ T cells and IFNγ selectively targeted stromal fibroblasts and shaped their thiol metabolism, which had a profound impact on tumor chemotherapeutic response [88]. Therefore, capitalizing upon the interplay between chemotherapy and immunotherapy may subvert chemoresistance and revitalize tumor sensitivity to chemodrugs.

Moreover, the combination with ICBs brought out paramount importance to overcome drug resistance in patients with mesothelioma in a clinical practice. The correlated research illustrated that the treatment of GEM with ICBs dramatically prolonged the OS of patients who were previously resistant to GEM or anti-PD-1 monotherapy [89].

5.4. Preventing tumor recurrence

Tumor relapse after initial regression post-chemotherapy is a major challenge in cancer treatment, as it often accompanied by local recurrence or distant metastases. The metabolic imbalance between co-stimulatory and inhibitory signals closely correlates with tumor recurrence and indicates a poor prognosis. In mice with advanced lymphoma, adoptive transfer (AT) of tumor-specific $CD4^+$ T cells following CTX treatment provoked a robust initial anti-tumor immune response, but also resulted in the expansion of inflammatory monocytes ($CD11b^+Ly6C^{hi}CCR2^{hi}$) [90]. These immunosuppressive monocytes inhibited a long-term tumor control and allowed subsequent relapse by mediating functional tolerization of anti-tumor $CD4^+$ effector T cells through the PD-1/PD-L1 axis. Considering that the monocytes are highly proliferative, it was speculated that these cells might be sensitive to low-dose chemotherapy for tipping the balance toward unrestrained anti-tumor immunity. Consequently, the combination treatment abolished the suppressive function of inflammatory monocytes and prevented tumor relapse [90]. It was also reported that dacarbazine (DTIC) injected one day before peptide-vaccination plus IFNα improved the anti-tumor lytic activity and enlarged the repertoire of Melan-A-specific T-cell clones. The treatment possessed an improved anti-tumor polyfunctional effector profile due to the co-production of TNFα, IFNγ and GrzB, all of which helped inhibiting late tumor relapse after chemo-immunotherapy [91].

In addition, hypoxia inducible factor (HIF)-regulated gene network is also a leading cause of chemodrug resistance, tumor metastasis and recurrence [92]. Based on this, a biomimetic core-shell nanoplatform was developed to deliver catalase and DOX (mZCD) for relieving tumor hypoxic environment (Fig. 6) [93]. The core served as an oxygen generator and drug reservoir, while the shell provided tumor targeting ability and elicited immune response due to abundant antigens. It is demonstrated that oxygen generation is pivotal to downregulate the expression of HIF1α, which can further enhance the therapeutic effects of chemotherapy and reduce the expression of PD-L1. Combined with co-delivery of ICBs, the dual inhibition of the PD-1/PD-L1 axis elicited a significant immune response and presented a better effect on inhibiting tumor metastasis and lengthening tumor recurrent time.

6. Concluding remarks and future perspectives

Chemotherapy-induced immune activation may be an effective supplement to expand the population benefiting from immunotherapy. A number of cytotoxic agents have been shown to induce ICD, while others are capable of modulating the tumor microenvironment by reducing the number and function of immunosuppressive cells such as Tregs, MDSCs and M2-type TAMs, or sensitizing tumor cells to immune attack. The effective chemo-immunotherapy strategy embodies four key points of treatment: (i) induction of ICD; (ii) promotion of antigen presentation; (iii) elimination of immunosuppressive factors; and (iiii) improvement of the activity of immune cells. The ultimate goal of the strategy is to produce more effector T cells to bind to antigens expressed by tumor cells, or to mobilize macrophages and other myelocytes to attack tumor cells. It is tempting to speculate that the synergy derived from chemo-immunotheray kill tumor cells that are not sensitive to chemotherapy, elevate the overall anti-tumor efficacy, and effectively prevent tumor from recurrence and metastasis.

On the other hand, despite the comparative advantages of the combination therapy, there are several issues that are worthy of consideration. The main contradiction exists in the necessity for a deeper understanding in the interaction between chemotherapy and cancer immunotherapy, and the accompanied impact of the combination treatment on the body's immune system. This can help more effectively predict the benefit population of combination therapy at a practical level. In addition, the paradoxical role of the chemotherapy in cytotoxic effect and myelosuppressive effect requires a deeper understanding. How to balance the effectiveness and safety profile remains a critical issue. The accurate dose ratio and time node of chemo-immunotherapy need accurate data in order to correctly maximize the synergy effect.

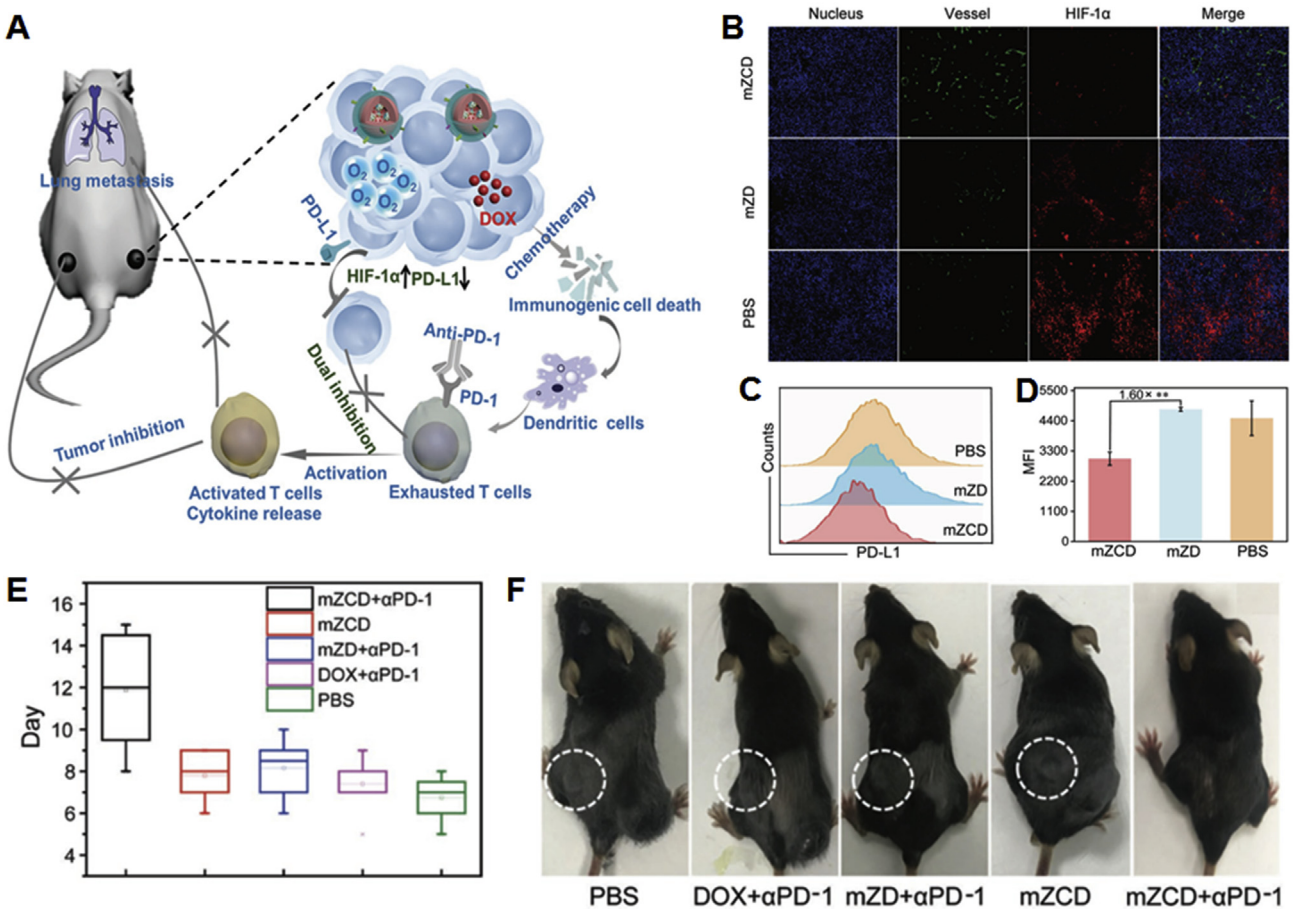

Fig. 6. The proposed mechanism of chemo-immunotherapy, *in vitro* and *in vivo* mechanism of anti-tumor immune responses induced by hypoxia in combination with chemotherapy and αPD-1. (A) The proposed mechanism of chemo-immunotherapy of mZCD and αPD-1. (B) Immuno fluorescence staining of nuclei, blood vessels, and HIF-1α in tumor tissues after treatment with mZCD, mZD and PBS. (C) Flow cytometry analysis of the PD-L1 expression in tumor tissues after treatment with mZCD, mZD and PBS and (D) their corresponding mean fluorescence intensity (MFI) analysis. (E) The day of challenge tumor recurrence and (F) their corresponding images after various treatments at Day 28 (circle: challenge tumor). Adapted from X. Zhang et al. [93] ZIF-8, zeolitic imidazolate framework 8; CAT, catalase; ZCD, ZIF-8@CAT@DOX; ZD, ZIF-8@DOX; mZCD, multifunctional nanoplatform membrane coated ZCD; mZD, membrane coated ZD.

Finally, it is necessary to pay more attention to the control of myelosuppression and immune-related adverse effects (irAEs), which might happen in the practical application of the chemo-immunotherapy.

Declaration of interest

The authors confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Conflicts of interest

The authors declare that they have no competing interests.

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