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Conditioning neoadjuvant therapies for improved immunotherapy of cancer

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Abstract

Recent advances in the treatment of melanoma and non-small cell lung cancer (NSCLC) by combining conventional therapies with anti-PD1/PD-L1 immunotherapies, have renewed interests in immunotherapy of cancer. The emerging concept of conventional cancer therapies combined with immunotherapy differs from the classical concept in that it is not simply taking advantage of their additive anti-tumor effects, but it is to use certain therapeutic regimens to condition the tumor microenvironment for optimal response to immunotherapy. To this end, low dose immunogenic chemotherapies, epigenetic modulators and inhibitors of cell cycle progression are potential candidates for rendering tumors highly responsive to immunotherapy. Next generation immunotherapeutics are therefore predicted to be highly effective against cancer, when they are used following appropriate immune modulatory compounds or targeted delivery of tumor cell cycle inhibitors using nanotechnology.

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Graphical Abstract

Keywords

targeted drug delivery; nanoparticles; cancer immunotherapy; neoadjuvant therapy

1. Introduction

Combinatorial cancer therapies such as chemo-immunotherapy, radio-immunotherapy, or targeted therapies combined with immunotherapy have been rationally designed to impinge on different pathways of tumor growth in order to achieve additive or synergistic anti-tumor effects. For instance, patients with HER2/neu overexpressing breast cancer receive chemotherapy and anti-HER2/neu antibody therapy using Trastuzumab and Pertuzumab. Chemotherapeutics such as doxorubicin increase free radicals that cause DNA damage, as well as intercalate into DNA and disrupt the DNA repairing function of topoisomerase-II [1]. Trastuzumab induces antibody dependent cellular cytotoxicity (ADCC), increases endocytotic destruction of the receptor, and inhibits shedding of the extracellular domain of HER2/neu [2] while Pertuzumab inhibits homo- and hetero-dimerization of HER2/neu, thereby blocking signalling pathways of tumor cell proliferation [3]. The caveat for such traditional chemo-immunotherapies is that standard dose chemotherapies are highly toxic to the host immune system and thus less effective for being simultaneously combined with immunotherapy (Table 1). Recent advances in our understanding of the mechanisms of action of low dose versus high dose chemotherapies are changing the concept of and approaches to chemo-immunotherapeutic design. Many studies demonstrated that certain chemotherapeutics at low doses induce immunogenic tumor cell death (ICD) and confer immune stimulatory effects. Therefore, the rationale for low dose chemotherapies is to condition tumor cells to become highly responsive to immunotherapies. A similar concept applies to the combined use of other cancer therapies, particularly those that induce cell cycle arrest, as conditioning regimens for an effective immunotherapy of cancer. The new chemo-immunotherapeutic approaches are predicted to make immunotherapies highly effective against cancer (Table 1).

2. Low dose metronomic (LDM) chemotherapy for an effective immunotherapy of cancer

Standard chemotherapy dosing regimens have traditionally used the maximum tolerated dose (MTD) of a drug administered with acceptable side effects as determined through clinical trials. In addition to targeting the malignant cells, the nonspecific cytotoxic drugs damage healthy cells with a high proliferation rate such as gastrointestinal mucosal and immune cells. Consequently, an extended time period is required between treatments in order to allow for tissue recovery. LDM chemotherapy is an alternative dosing regimen that

is characterized by administering a cytotoxic drug at a low dose scheduled at a regular interval in order to minimize the drug-free time periods. Metronomic dosing schedules aim to achieve adequate disease control with less toxicity than MTD chemotherapy. The rationale for LDM is to not only inhibit tumor growth but also induce ICD and anti-tumor immune responses [4–7] to make patients highly responsive to immunotherapies. A LDM chemotherapy can control tumor progression in patients with early stage as well as those with advanced-stage cancers [8].

2.1. Non-immunogenic mechanisms of LDM chemotherapy

Proliferating malignant cells' oxygen requirements are met by forming inappropriate vascularization to the tumor. Tumor hypoxia results in the production and release of angiogenic cytokines, which leads to resistance to both antiangiogenic and chemotherapeutic regimens [9,10]. One of the earliest studies using low dose chemotherapy at regular intervals referred to the dosing regimen as antiangiogenic scheduling [11]. The study found that low dose cyclophosphamide given at regular schedule was able to kill cells that were resistant to a standard dose chemotherapy. The results have been reproducible [12,13], though the efficacy of low dose chemotherapy as a first line treatment for untreated cancers is yet to be determined. The tumor regression was attributed to sustained endothelial cell apoptosis that occurred due to the higher frequency dosing, which did not occur during the drug-free periods used in MTD chemotherapy. In fact, circulating endothelial cells are released from the bone marrow as an adaptive response to marrow suppression induced by MTD chemotherapy, allowing for damaged tumor cells to regenerate. In this aspect LDM chemotherapy has a unique mechanism in suppressing vasculogenesis by suppressing the source of vascular growth factors [14]. Promotion and maintenance of angiogenesis involves a balance of proangiogenic and antiangiogenic molecules acting within the tumor microenvironment. One of the earliest growth factors released from the tumor site in response to hypoxia is the transcriptional regulator, HIF-1 α . Doxorubicin at a LDM regimen has been reported to block this transcription factor, the inhibition of which has been shown to overcome resistance to antiangiogenic therapies and promote tumor regression [15,16]. LDM chemotherapy has been shown to decrease expression of proangiogenic molecules VEGF and VEGF receptor 2 [17] and increase the expression of the antiangiogenic thrombospondin 1 [18]. Taken together this data indicates that LDM chemotherapies suppress the tumor microenvironment's response to hypoxia by suppressing angiogenesis.

2.2. Immunogenic mechanisms of LDM chemotherapy

Certain chemotherapies at the MTD have been associated with immune stimulation through the induction of ICD. The term ICD was first introduced over a decade ago by Dr. Kroemer's group to indicate a functionally peculiar type of cell death induced by certain chemotherapeutics that can elicit an immune response against damage associated molecular patterns (DAMPs) in the absence of any adjuvant [19]. Inducers of ICD include doxorubicin, cyclophosphamide, epirubicin, idarubicin, mitoxantrone, bleomycin, bortezomib, 5-fluorouracil, paclitaxel and oxaliplatin [20,21]. On the other hand, some other chemotherapeutics such as cisplatin fail to induce ICD [22]. Animals challenged with doxorubicin-sensitized tumor cells were able to mount anti-tumor immune responses that

protected them from re-challenge with tumor cells of the same type [19]. Recent studies demonstrated that the lack of ICD is correlated with poor prognosis for breast cancer patients [23], and ongoing clinical studies have identified some standard-of-care chemotherapeutics that induce ICD [20,24].

Molecular components of DAMPs that induce ICD following chemotherapy have been identified as: i) cell surface expression of endoplasmic reticulum (ER) chaperones such as calreticulin (CRT), ii) release of ATP, iii) release of non-histone chromatin-binding protein high mobility group box 1 (HMGB1), and iv) secretion of immunostimulatory cytokines such as type I interferons [25–27]. ICD is induced even prior to cell death such as during autophagy or senescence [26]. Such chemotherapy-induced ICD recruited dendritic cells (DCs) to the tumor site and activated them to take up dead-cell associated antigens. The activated DCs undergo maturation and present tumor antigens to T cells, resulting in the induction of tumor-specific immune responses [26]. CRT, HMGB1, and ATP interact with CD91, TLR-4, and purinergic P2RX7 receptors on DCs, respectively. These interactions, in turn, result in antigen uptake, antigen presentation and production of IL-1 β by DCs [22,28,29]. Any defects in the DAMP-sensing machinery, such as type I interferon receptor alpha and beta, CD91, TLR4 or P2RX7 could alter the immune response to chemotherapy-induced ICD. Other chaperones such as HSP70 and HSP90, as well as uric acid are also considered as markers of ICD that interact with CD91 or TLRs [30]. Unfortunately, standard chemotherapies are also known to compromise immune surveillance by killing proliferating effector T cells, and contribute to treatment resistance [31]. On the other hand, LDM chemotherapies can induce ICD and also confer additional immune stimulatory effects without the significant killing of anti-tumor T cells. This immune stimulatory function of LDM chemotherapies is important because tumor cells are able to unleash an immunosuppressive network of cells composed of M2-polarized macrophages, regulatory T (Treg) cells and myeloid-derived suppressor cells (MDSCs) [32], which leads to tumor cell evasion by dampening anti-tumor immune responses. Breast cancer patients who were treated with low dose cyclophosphamide showed decreased Tregs and increased effector T cells as well as NK-cell-dependent anti-tumor immunity [4,5]. Cyclophosphamide also enhances Th17 and Th1 immune responses, and expands NK-cell and DCs in multiple mouse tumor models [33,34]. Other immune stimulatory chemotherapies that deplete circulating or tumor-infiltrating Tregs and/or circulating MDSCs include 5-fluorouracil [35,36], gemcitabine [37,38], oxaliplatin [39], paclitaxel [40], and docetaxel [41], and decitabine [42]. In addition, oxaliplatin promotes anti-tumor function of macrophages and neutrophils [43]; paclitaxel induces maturation of DCs [44] and tumor infiltration of NK cells in breast cancer patients [45]. In fact, the anti-tumor efficacy of doxorubicin has been suggested to depend on the host immune system [46] such that depletion of T cells compromises anti-tumor efficacy of doxorubicin [47]. LDM chemotherapy also has been shown to be a suitable preparative regimen for vaccination approach in order to boost anti-tumor immune responses against dormant cells [7]. Similarly, whereas fractionated radiation therapy (RT) is immunogenic and generates abscopal responses in mice, single high-dose RT fails to do so [48]. This failure is because of the upregulation of three prime repair exonuclease 1 (TREX1) which in turn inhibits type I interferone secretion, an ICD signal, by irradiated tumor cells [49].

3. Epigenetic targeting of tumor cells for immune modulation against cancer

Spontaneous cancers arise in immunocompetent individuals with active immunoediting mechanisms that make tumor cells weakly immunogenic [50]. Therefore, improving the immunogenicity of cancer is essential to improving cancer immunotherapy. Epigenetic modulators such as azacytidine (Aza) and decitabine (Dec) function as cytosine analogs, which lead to their incorporation into newly synthesized DNA strands during S phase of the cell cycle; these agents have been shown to enhance immunogenicity of tumor cells by inducing the expression of a panel of highly immunogenic cancer testis antigens (CTAs), and result in improved immunotherapy of cancer [51,52]. Both Aza and Dec also induce the expression of tumor suppressor gene p53 [53] and the death receptor Fas [54] on tumor cells. These functions are attributed to the capacity of these agents to mechanistically operate as potent DNA methyltransferase (DNMT) inhibitors through the formation of a covalent complex with a cysteine residue at the active site of DNMT1. This results in CpG island demethylation during cellular proliferation, which, in turn, results in hypomethylation within the promoter of tumor suppressor genes as well as highly immunogenic CTAs [55,56], leading to their enhanced transcription. Ultimately, the use of such epigenetic modulating agents renders tumor cells susceptible to CTA-reactive immune responses while potentially reducing the proliferative capacity of tumor cells by restoring p53 expression. In fact, aberrant CTA expression has been shown to elicit CTA-specific cytotoxic T cell responses in melanoma; treatment of CTA-expressing metastatic melanoma with autologous CTA-specific T cells has elicited long-term complete remission [57,58]. Dec in particular is an attractive therapeutic because it requires activation by deoxycytidine kinase (DCK), an enzyme preferentially expressed in tumor cells and myeloid cells. Therefore, it is expected to specifically kill tumor cells and MDSCs while leaving T and B cells unharmed. In addition, DCK has been found to be overexpressed in poor outcome breast cancer [59], suggesting that epigenetic therapy to induce CTA expression may prove to be an efficacious approach in breast cancer patients with poor prognosis. A low dose regimen of Dec was shown to render mouse mammary carcinoma highly susceptible to immunotherapy [52]. In colorectal cancer, Aza at a low dose increased type I interferon production within the tumor through the reactivation of endogenous retroviruses, and as a result enhanced anti-tumor immune responses [60]. In patients with solid tumors, low dose Dec increased TcR diversity, which is important for T cells to respond to antigenic diversity of tumor cells [61]. A low dose regimen of Dec was also reported to induce the expression of CD80 co-stimulatory molecule on tumor cells associated with enhanced anti-tumor immune responses [62]. Aza therapy in patients with relapsed or refractory Hodgkin lymphoma resulted in a complete response to immune checkpoint inhibitors [63]. We have also reported that the use of Aza combined with the immune modulatory lenalidomide induced the expression of CTAs within tumor cells, and generated CTA-specific immune responses in patients with multiple myeloma [64]. Similar results were observed in a mouse model of experimental metastatic breast cancer [65]. Therefore, Dec is an attractive candidate as a neoadjuvant immune modulator when combined with immunotherapy.

4. Control of cell cycle progression prior to immunotherapy

The dysregulation of the cell cycle is a classic hallmark of cancer growth and metastasis. Cyclin-dependent kinases (CDKs) are a family of multifunctional enzymes that can modify various protein substrates involved in cell cycle progression. All eukaryotic cells have multiple cyclins, which act during a specific stage of the cell cycle. Common cyclins include G0/G1-phase cyclins, G1/S-phase cyclins, S-phase cyclins, and M-phase cyclins. CDK4 and CDK6 are important for progression during the G₁ cell cycle phase [66], CDK2 is important for transition from G₁ into S-phase [67], and CDK1 is important during G₂ and M progression [68]. The inhibition of tumor cell cycle progression through CDK inhibitors has emerged as an attractive option for targeted cancer therapy. Three specific CDK4/6 inhibitors palbociclib, abemaciclib and ribociclib have been successfully tested in patients with hormone receptor-positive HER2-negative breast cancer [69]. Palbociclib inhibits cell growth and DNA replication in a number of retinoblastoma (Rb) proficient human cancer cells, including breast cancer because over 70% of breast cancers are Rb proficient [70]. Palbociclib is a well-tolerated cancer therapeutic [71] that induces G0/G1 arrest in HER2/neu- and HER2/neu+ breast tumor cells [69,72] as well as in neu positive murine mammary tumor cells [73]. Palbociclib can be administered at a concentration of 150 mg/kg through oral gavage and given daily for 3–4 weeks either alone or after the completion of chemotherapy in order to further reduce tumor cell burden. Palbociclib and chemotherapies may not be used simultaneously, because G0/G1 arrest by Palbociclib could render tumor cells resistant to cytotoxic function of chemotherapy [74]. Palbociclib does not induce apoptosis in bone marrow hematopoietic cells, and its function as a cell cycle arresting agent is reversible upon its removal [75]. Unlike pan-CDK inhibitors, palbociclib is not toxic to T cells [76]; thus, it can be used with immunotherapeutics. Roscovitine is a selective CDK5 inhibitor that is able to induce the apoptosis of drug-resistant breast cancer cells [77]. A sequential use of chemotherapy and roscovitine can induce G2/M arrest and apoptosis in highly invasive triple negative breast cancers [78]. Roscovitine is not toxic for tumor-reactive T cells [79] and could sensitize breast cancer cells to immunotherapy by TRAIL-induced apoptosis [80]. Very recently, it was reported that IFN- γ -induced immunoediting via PDL-1 expression is a CDK5-dependent event; thus, roscovitine can suppress IFN- γ -induced expression of PD-L1 [81].

The rationale for use of CDK inhibitor is to push tumor cells towards dormancy so that the immune system can control tumor growth by inducing tumor cell death. It has been reported that dormant tumor cells, while become refractory to chemotherapy, remain susceptible to immunotherapy [65]. Therefore, the next generation immunotherapeutics are expected to be highly effective against cancer, when combined immune modulatory compounds.

5. Targeted delivery of tumor cell inhibitors: nanotechnology

Although LDM chemotherapies were found to be immunogenic, they could still affect the normal cells because of their administration over an extended period of time. Therefore, tumor immune modulatory chemotherapies that induce ICD and increase the expression of MHC or other immune modulatory receptors such as Fas or CD80 on tumor cells would be more effective through targeted delivery than systemic administration of the drug. To this

end, targeted delivery of cell cycle inhibitors prior to immunotherapy would be desirable. Folate (vitamin B9) receptor (FR) is an attractive target because of high level of expression on tumors of epithelial origin compared to normal tissue, including ovarian, breast, brain, lung and colorectal cancers [82]. Folate-conjugated nanoparticles that carry desirable drugs are internalized upon binding to FR and the acidic microenvironment inside tumor cells dissociates FR from the drug-carrying nanoparticles resulting in drug-induced apoptosis or inhibition of tumor cell proliferation. Cytotoxic drugs have been successfully delivered to tumor cells via targeting the FR and using nanoparticles as a potent drug carrier. In a breast tumor model, paclitaxel-loaded folate modified lipid-shell and polymer-core nanoparticles (FLPNPs) showed similar antitumor efficacy but lower toxicity compared to paclitaxel. The paclitaxel-loaded FLPNPs confer a higher tumor inhibitory effect than the nontargeted paclitaxel-loaded LPNPs [83]. Cases of successful oral delivery of Dec to abdominal tumors by means of nanostructured lipid carrier have been reported [84]. It was demonstrated that nanoconjugated delivery of Dec to human glioblastoma cells could overcome chemo-resistance by rendering tumor cells susceptible to alkylating chemotherapy [85]. In humans, carbon nanoparticles have been successfully used to protect parathyroid glands in patients with thyroid cancer. Such a nanotechnology approach reduced incidence of hypoparathyroidism and hypocalcemia after surgical removal of thyroid tumor [86]. Safety and efficacy of SGT-53 liposomal nanoparticle delivery of p53 gene into refractory solid tumors in combination with chemotherapy have also been established [87]. Very recently, therapeutic nanoparticles plus trastuzumab with and without cyclophosphamide were successfully tested through enhanced permeability and retention in patients with HER2 positive metastatic breast cancer [88]. These trials suggest the feasibility of tumor-targeted nanoparticle drug delivery using cell cycle inhibitors as a conditioning regimen for immunotherapy of cancer. However, the feasibility and efficacy of such targeted delivery of tumor immune modulators in combination with immunotherapy remain to be investigated.

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Table 1

Current concepts on combinatorial cancer immunotherapies

Concept	Objective	Approach	Weakness	Strength
Traditional	To impinge on different pathways of tumor growth in order to achieve additive or synergistic anti-tumor effects	Adjuvant therapies at maximum tolerated doses	Toxicity Immune suppression	Tackle multiple drug resistant mechanisms
New	To condition the tumor microenvironment and make tumor cells highly responsive to immunotherapy	Low dose neoadjuvant conventional therapies and standard dose adjuvant immunotherapy	Tumor immunoediting and escape	Immune stimulatory Safe