

Dendritic Cell-Based Immunotherapies and their Potential Use in Colorectal Cancer Immunotherapy

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Abstract

Dendritic cells (DCs) are professional antigen-presenting cells, which are resident or proliferating in organs. Major histocompatibility complex (MHC) Class I and II on DCs in normal steady conditions process and present antigens including cancer antigens. Many approaches are used to enhance antigen presentation process of DCs and capture cancer cells. DCs are harvested from cancer patients and manipulated *ex vivo* in DC-based cancer immunotherapy. In addition, DCs' vaccines and other anticancer therapy combinations were discussed to optimize DCs' efficiency for cancer immunotherapy. This review addressed the use of the human conventional type-1 DCs, OX40⁺ plasmacytoid DCs, and DCs-derived exosomes. In addition, different combinations with DCs therapy such as combination with the monoclonal antibody, cytokine-induced killer cells, adjuvants, chemotherapy (DCs-based chemoimmunotherapy), and nanoparticles were listed and explored for their effectiveness against cancer, and mainly against colorectal cancer.

Keywords: Adjuvants, colorectal cancer, cytokine-induced killer cells, dendritic cells, dendritic cells-based chemoimmunotherapy, exosomes, nanoparticles-dendritic cells immunotherapy, OX40⁺ plasmacytoid dendritic cells

INTRODUCTION

The second cause of death worldwide and the third most common type of cancer is colorectal cancer (CRC). In developed countries, the early detection of CRC enhances the survival rate,^[1,2] whereas the main problem of CRC is the late discovery of the disease. In most of the cases, CRC was discovered as metastatic CRC (mCRC), which has been infiltrated to a near (i.e., liver) or far (i.e., lung and brain) organs. The CRC metastatic nature increases the need for treatments that have an affinity for multiple types of cancers. Chemotherapeutic and immunotherapeutic agents' development is encouraged for mCRC because of the CRC metastatic nature. Current protocols for the mCRC (Stage III and IV of CRC) included immunotherapy or chemotherapy after surgery or radiotherapy.^[2,3]

Chemotherapy has taken an interest in the last two decades. However, the un-selective chemotherapeutic nature leads to

the need for more targeted treatments.^[3,4] As a result, targeted monoclonal antibodies (as a type of immunotherapy) have increased the survival rate, which had become the standard anticancer approach for many malignancies. In general, accepted efficacies, good tolerance, and long duration of effectiveness in gastrointestinal-related cancers such as CRC were achieved by immunotherapies.^[3,4] In 2017, immune checkpoint inhibitor (ICI) therapy was approved for mCRC with heavy mutation and good immune cell infiltration.^[5] On the other hand, ICIs are ineffective in the tumor of low tumor mutation and the lack of immune cell infiltration. As a result, the low level of mutations and lack of immune infiltration were considered cancer immune resistance mechanisms.^[6]

Instead, the use of the immune cells for cancer targeting and removal approved its efficacy and uniqueness and could

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Received: 15-02-2021

Revised: 14-04-2021

Accepted: 17-04-2021

Published: 09-07-2021

Access this article online

Quick Response Code:



Website:
<http://www.jmau.org/>

DOI:
10.4103/jmau.jmau_20_21

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How to cite this article: Aldahlawi AM, Abdullah ST. Dendritic cell-based immunotherapies and their potential use in colorectal cancer immunotherapy. *J Microsc Ultrastruct* 2022;10:107-13.

be achieved by chimeric antigen receptor T-cell therapy and dendritic cell (DC) therapy.^[7-13] DCs are proliferated cells group, as bone marrow-derived leukocytes. Moreover, DCs are the essential key players in the message delivered between the innate and adaptive immune systems, as the most potent antigen-presenting cells (APCs). In addition, DCs are enhancers of the immune response and capable of T-cell activation.^[7,8,10-12,14-24] In this review, the mechanism and efficiency of the human conventional type-1 DCs (cDC-1), OX40⁺ plasmacytoid DCs (pDCs), and DCs-derived exosomes were discussed. In addition, different combinations with DCs therapy such as combination with the monoclonal antibody (mAb), cytokine-induced killer (CIK) cells, adjuvants, chemotherapy (DC-based chemoimmunotherapy), and nanoparticles (NPs) were listed and explored for their effectiveness against cancer, especially against CRC.

CANCER RESISTANCE AND IMMUNOSURVEILLANCE

Cancer cells have multiple mechanisms for escaping immune system recognition and surveillance. One of the immune suppression techniques, as cancer resistance mechanisms, is regulatory T-cell-mediated immunosuppression. As a result, regulatory T-cell suppression is a target for cancer immunotherapy. Cancer cell secretion for immune-inhibitory cytokines will promote T-regulatory cells to inhibit cytotoxic T-cell activation and increase the expression of inhibitory surface molecules on DCs that are responsible for T-cell inactivation. Then, T-regulatory cells can suppress T-helper cells (CD4⁺). Afterward, they will not be able to recognize the cancer-associated or specific antigens. In addition, the major histocompatibility complex (MHC) Class I absence is another cancer resistance mechanism that will not be recognized by the cytotoxic T-lymphocytes. Moreover, immune checkpoint molecules such as programmed death ligand (PD-L1) overexpression on cancer cells will result in peripheral T-cell inhibition.^[5,25]

DCs' functions are to engulf, process, and present antigens to T-cells to produce cytokines that will occur in the antigen, pathogen-associated molecular patterns, or danger-associated molecular patterns bindings.^[15,26-28] Moreover, DCs can elicit natural killer (NK) cells and NK T-cells.^[11,17,29,30] All of the DCs' capabilities directed the research interests to develop their vaccines conjugated with tumor antigens, which will initiate the antitumor immune responses, overcome cancer resistance mechanisms, and activate naïve and memory T-cells. As a thumb role, it is essential that DC vaccine efficiently present a tumor antigen to initiate long-life antitumor responses. DC vaccines and therapy were found to be safe and well tolerated in the past two decades. Moreover, DCs combined with other therapeutic approaches can result in more effective cancer immunosurveillance approaches.^[7,8,10,13,27] At the start, CRC was found to be not immunogenic cancer, and immunotherapy would not be efficient against CRC. In many studies, the lymphocytic reaction was approved to be an essential prognostic factor for CRC. DNA mismatch repair (MMR) gene

mutations are found in Lynch syndrome, the hereditary form of CRC. MMR proteins function to correct single-base nucleotide instability in the replication process. Deficient-MMR (dMMR) genes have been associated with about 15% of sporadic CRC. Moreover, dMMR cancers overexpress genes specific to cytotoxic lymphocytes. As a result of the MMR mechanism deficiency and the antigens generated from the mutations, the cell-mediated immunity can recognize the foreign antigens. Sporadic CRC of dMMR can be targeted by immunotherapy, especially DC therapy.^[5,7]

DENDRITIC CELL-BASED IMMUNOTHERAPY FOR CANCER

To eradicate the tumor cells, cytotoxic T-cells and NK cells are potent against tumor cells. The use of DCs, as APCs, helps to potentiate the cell-mediated immunity of the cytotoxic T-cells and NK cells.^[29-34] The immature DCs engulf antigens in the peripheral tissues and present them to fulfill the antigen presentation process and migrate to the secondary lymph node to activate T-cells. DC maturation and expression of co-stimulatory molecules (CD80 and CD86) were occurred during the migration. Afterward, the mature DCs secretes interleukin-12 (IL-12), IL-15, and IL-18 in lymph nodes for T-cell activation. For eliciting adequate immune responses, DCs carry processed antigens on the MHC Class I or II to the naïve cytotoxic T-cells (CD8⁺), or to the naïve T-helper cells (CD4⁺), respectively. On the other hand, the immune tolerance and a decrease in the infiltrated cytotoxic T-cells can result from the cancer cell secretion of transforming growth factor- β and IL-1.^[7,10,13,21,27] As a result, the specific antitumor immune response development is the goal of cancer treatment and is referred to as immunotherapy. Cancer immunotherapy includes active and passive immunotherapy. Antibodies or immune cell (T-cells and NK cells) therapy are passive or active immunotherapy, respectively. These methods increase the immune cell activation but cause adverse autoimmunity and toxicity due to the low antigen specificity.^[27] As a result of the DC-specific activation of NK cells and T-cells, the DC immunotherapy provides a good standalone alternative or better combined with other therapeutic approaches, as discussed in the following sections.

Human conventional type-1 dendritic cell therapy

There are currently four approaches to study DCs in CRC immunotherapy. The endogenous DC-antigens and *ex vivo*-generated DCs-tumor antigens conjugation are examples of DC-based immunotherapy.^[7,13] For the *ex vivo*-generated DCs, differentiated DCs were obtained from leukapheresis-isolated CD14⁺ monocytes (MoDCs) and cultivated with granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL 4 [Figure 1].^[10] *In vitro*, MoDCs are modulated with the migration ability to lymph nodes and their production capability of strong cytotoxic T-lymphocyte reactions.^[7,35] On the other hand, the natural circulating DCs, which have meager existence in the blood, are an essential cancer immunotherapy tool with many advantages. Moreover, the DC vaccines have a strong safety profile.^[10,26] Interestingly, cDC1-deficient animal models have reflected that these cells are of a central

role in cancer immunotherapy.^[8] The DC secretion of IL-12 enhances T-helper (Th1) cells' polarization and the secretion of interferon-gamma (IFN- γ) to establish the antitumor immunity. Afterward, Th1 lymphocytes kill tumor cells and elicit the antigen-specific cytotoxic T-cells' development and proliferation due to IL-21 and IL-2 secretions.^[36] In addition, Th1 lymphocytes activate the tumor-infiltrated macrophages. In conclusion, Th1 lymphocytes establish long-term cytotoxic T-memory cells. On the other hand, NK cell proliferation, cytolytic capacity, CD69 expression, and IFN- γ production are stimulated by mature cDCs. Finally, the DC requirements to be fulfilled for delivering a potent antitumor vaccine comprise an efficient cross-presentation, IL-12 production, co-stimulatory molecules expression, and efficient networking with NK cells for activation.^[7]

OX40⁺ plasmacytoid dendritic cell therapy

pDCs have roles in infection, autoimmunity, inflammation, and cancer, which are known to produce IFN- α in high levels after the activation of the toll-like receptors (TLRs) 7 and 9. Moreover, TLR activation is due to the viral and endogenous nucleic acid antigen binding. However, intertumoral pDCs were found to promote some tumors with decreased IFN- α and co-stimulatory cell surface molecules. Intertumoral pDC use in these tumors mediates immunotolerance through indoleamine 2,3-dioxygenase 1 and inducible T-cell co-stimulatory ligand expressions.^[9,14,19,37,38] On the other hand, pDCs can enhance the cytotoxic T-lymphocytes to elicit immune responses, which reflect their role in antiviral immunity.^[39] Similar to the tumor necrosis factor (TNF)-receptor superfamily members, OX40⁺ is a T-cell expressed co-stimulatory molecule after antigenic T-cell receptor excitation and T-cell stimulation.^[40] Interestingly, OX40⁺ pDCs were immunophenotypically and functionally distinct from their OX40-low equivalents based on increased expression of cell surface maturation markers. Besides, pDCs can elicit strong tumor-associated

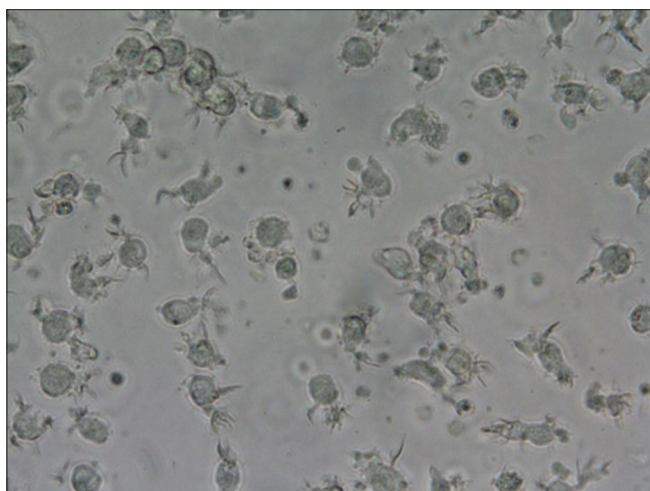


Figure 1: Peripheral blood monocyte derived dendritic cells morphology after 7 days of culture with interleukin-4 and granulocyte-macrophage colony-stimulating factor, Immunology Unit, King Fahad Medical Research Center (KFMRC)

antigen-specific (TAA-specific) cytotoxic T-cell responses after synergism with cDCs.^[18,40] In addition, TLRs 7 and 9 activations in response to TAA-specific can induce the pDC immunostimulatory and tumor-killing functions to accelerate the cancer cells apoptosis.^[39,41]

Dendritic cell-derived exosome therapy

Exosomes are nanosized (30–120 nm) vesicles with membrane-bound phospholipids, which are released by cells to enable intercellular communication. In 1987, exosomes' early discovery was in the immature red blood cells. Moreover, the exosomal contents can be proteins, lipids, and nucleic acids of DNA, mRNA, siRNA, miRNAs, and lncRNAs. Exosomes are carried in the same tissue of neighboring cells or in the blood to be delivered for distant target sites to modulate the cells' physiological functions. In addition, the immune responses, gene expression regulation, signal transductions, and carcinogenesis can be controlled by the exosomes.^[42–44]

Moreover, B-cells release antigen-specific MHC Class II inducing exosomes for T-cell response activation. On the other hand, DC-derived exosomes (dexosomes or Dex) were explored as a cancer vaccine alternative approach and released by the immature and mature DCs.^[43,45] Dex contains antigen presentation molecules, including MHC Class I, MHC Class II, co-stimulatory, and adhesion molecules. For Dex preparation, the monocyte differentiation into immature DCs with GM-CSF and IL-4 was followed by the mature DCs induction in the presence of IFN- γ and tumor-associated antigenic loading peptides. Specifically, IFN- γ induces co-stimulatory molecules' expression in DCs.^[7,42] Moreover, Dex associated with tumor peptide can control tumor growth in an MHC and cytotoxic T-lymphocytes cell-dependent manner by activating antigen-specific cytotoxic T-cells *in vivo*. As a result, exosome-based cell-free vaccines showed to be an important approach for suppressing tumor growth.^[44,45] The use of Dex was found to present maintenance immunotherapy after chemotherapy cessation.

In addition, it was reported that Dex boosted the antitumor activity of NK cells. On the other hand, Dex-TAAs conjugation may broaden the human tumor's coverage as proteins conjugated to the Dex. Moreover, mutated TAAs could improve T- and B-cell responses. However, Dex was engineered to overexpress co-stimulatory molecules, carrying TAAs or activation molecules coding mRNAs. In the same context, the expression of ICIs on Dex must be quantified and downregulated. Moreover, immune inhibitors of ICIs, as a booster of antitumor T- and B-cell response, could be used in combination with Dex. In addition, Dex with GM-CSF combination therapy can induce antitumor cytotoxic T-cells response. All the discussed approaches approve the Dex efficiency and how combination therapy could alleviate future clinical trials with Dex.^[29,34,46]

Monoclonal antibody and dendritic cell therapy

Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and

programmed cell death protein 1 (PD-1) are ICI molecules that lead to the negative regulatory activity of cytotoxic T-cells.^[26,27,47] As previously discussed, tumors can escape immunosurveillance, which induce T-cell inhibition and immune tolerance. As a result, mAbs with DC therapy against ICIs are among the most successful cancer immunotherapies. Regarding the PD-1/PD-L1 inhibitors, pembrolizumab is an interesting Ab example of this strategy. In this context, stimulation of PD-1 in T-cells leads to the T-cells anergy or apoptosis. In addition, many tumor types and mature DCs are expressing PD-L1.^[21] After the blockade of PD-L1 by mAbs, the cytotoxic T-cell activation can be induced by DCs and shifting the cytokine profile from IL-10 secretion by Th1 2 cells to an IL-12 by Th1 cells. Synergistic effects can result from the combination of mAbs against PD-1 with DC therapy.^[21,23] On the other hand, the PD-L1 or PD-L2 inhibition on DCs leads to the tumor-specific cytotoxic T-cells and Th1 cell activations, the secretion of IFN- γ enhancement, tumor TNF- α , IL-2, IL-5, and IL-12 secretions, and the tumor cells cytotoxicity facilitation.^[23,47,48]

In this context, ipilimumab and tremelimumab are anti-CTLA-4. CTLA-4 can inhibit CD28 and prevent the activation of naive T-cells. However, the combination of DC therapy and mAbs is more effective than ipilimumab and tremelimumab as monotherapies.^[49] Additionally, DCs co-stimulatory molecules and MHC class I down regulations, and the NK cells inhibition were results of IL-10 secretions from the T-regulatory cells or cancer cells. The combination between anti-IL-10 mAb, such as rituximab, with cDC vaccine enhances the NK responses and decreases the tumor growth.^[20]

Dendritic cell and cytokine-induced killer cell combination

CIK cells are one subset of NK-T lymphocytes, proliferated *ex vivo* after adding IL-2, OKT-3 antibody, and IFN- γ to enhance their antitumor activities, proliferation, and cytotoxic function, also prevent the generation of T-regulatory cells. The CIK cells were recognized as safe and not restricted to the MHC molecule for their activation. CIK cells act as an effective antitumor cell, making them good therapeutic agents for cancer immunotherapy.^[11,29] Immunological or genetic engineering techniques can improve the cytotoxicity of CIK cells and their treatment efficacy against cancer. The T-cells (CD3⁺ CD56⁻), NK-T cells (CD3⁺ CD56⁺), and NK cells (CD3⁻ CD56⁺) are of the CIK cell members. The CIK cells of CD3⁺ CD56⁺-phenotype present higher granzyme concentrations, which will lead to better cytotoxicity and antitumor activities than the other CIK cell members.^[11,29,30,32] Furthermore, CIK cells can detect and eliminate cancer targets without first priming, which can proliferate extensively. In addition, CIK cells are approved to be more effective than lymphokine-activated killer cells as cancer cytotoxic cells by apoptosis induction. Due to the high proliferation of the CD3⁺ CD56⁺-CIK phenotype and lower toxicity, the CIK cells received attention in the cancer immunotherapeutic.^[12,17,50] On the other hand, the DCs and CIK cells combined with no specificity against a tumor antigen results in better targeting of antigens and cancer removal because of

DCs' capability to present tumor antigen. Thus, DCs compensate CIK cells' lack of tumor antigen specificity.^[10-13,16,17,50] Besides, NK or CIK cell-mediated tumor death's cell debris gives DCs the tumor antigens and improves the DCs' presentation to Th1 and cytotoxic T-cells.^[17] As a result, the NK or CIK cells and DCs inter-relation are unique, essential to cell signaling. In conclusion, when NK or CIK cells are activated, they trigger the DC maturation and eliminate the immature DCs to reduce the tolerogenic reactions.^[50] Furthermore, DC-CIK co-culture was able to ameliorate the lymphopenia and eliminate cancer to trigger apoptosis without inflammation. Finally, the DC-CIK co-culture had improved safety and was able to minimize the adverse events associated with chemotherapy.^[11,23,30,32,51]

Dendritic cell-adjuvant combination

Adjuvants are the immune reaction enhancer molecules against a vaccine antigen, which elicit the humoral or cellular immunity modulators toward antigens. The use of adjuvants results in intensification of the immune response to stimulate DC function when antigens are not immunogenic enough. Some polysaccharides, such as alginate and chitosan, are considered adjuvant-like polymers.^[52-54] As a result of adjuvant activation for TLR signaling, DC maturation has occurred, enhancing MHC Class I and II molecule expression. Moreover, the use of adjuvants as TLR ligands on DCs was resulting in Th1 cells, cytotoxic T-cells, NK cell activations, IFNs production enhancement, immune cells migration augmentation in response to chemokine receptor 7 ligand, IL-2, pro-inflammatory cytokine secretions, and stimulation of humoral response.^[54] Moreover, TLR-adjuvant examples are imiquimod, CpG oligodeoxynucleotides, and polyinosinic-polycytidylic acid. Imiquimod is a ligand for TLR7 and TLR8. On the other hand, CpG oligodeoxynucleotides are ligands for TLR9, and polyinosinic-polycytidylic acid can adjoin to TLR3 or helicases.^[10,13,21,23,37,39]

Dendritic cells-based chemoimmunotherapy

Chemotherapy is a well-known approach to eradicate and eliminate cancer cells. However, chemotherapy has multiple immunological effects that are in-selective, such as immunogenic cell death of cancer cells. Thus, chemotherapy leads to antitumor immunity enhancement. Unfortunately, chemotherapy reduces immune responses for long-term treatment and leads to lower immunotherapy response.^[25,55] On the other hand, immunotherapy and chemotherapy combination leads to a synergism. Moreover, the relapse of tumor-induced immunosuppression, which results in lymphopenia, can be compensated using the chemotherapy and immunotherapy combination to enhance the antitumor effector cells and inhibit T-regulatory cells.^[5] Cyclophosphamide, temozolomide, and gemcitabine, as chemotherapeutics, cause cancer cells to be prone to death by antitumor immunity. However, they lead to transient lymphopenia in high doses. Compared to the cyclophosphamide monotherapy, the drug administration before DC therapy or simultaneous to its use resulted in a decrease of the T-regulatory cells and increased T-cells in the previous studies. Thus, T-regulatory cell depletion upon

using lower doses of cyclophosphamide enhances DC-therapy effectiveness, depending on the tumor type and DC used. In addition, this combination can decrease the dose needed for the cyclophosphamide and enhance the DCs' efficacy. Finally, DC-based chemoimmunotherapy is a vital management model.^[7,8,10,26,55]

Nanoparticles-dendritic cells carrier for immunotherapy

Macrophage and immature DCs residing in peripheral tissues captured NPs in human blood and lymph, which can change these cells' functions. The NP use to control DC functions in cancer immunotherapy was achieved because of the NPs physicochemical properties that encourage their use for both *ex vivo* and *in vivo* DC manipulations. DC maturation, homing capability, antigen processing, and presentation which induce T-cell differentiation are gained with the NP use as a novel DC targeting tool. In some NP-mediated targeting approaches, NPs can activate cytotoxic T-cells and cancer-killing molecules, which can be used as an efficient vaccine adjuvant for cancer.^[56,57] Moreover, the modulation of NPs' particular properties (specific size, shape, and composition) is needed to optimize the DC targeting for cancer. For instance, NP-antigen conjugates, which are identified by specific receptors, are formulated of antigens encapsulated within NPs. In addition, NPs can be used to protect the antigen or can be used to be recognized by specific receptors for selective binding and action. Moreover, NP conjugation with polysaccharides, peptides, antibodies, and drugs, which bind to a protein on the target cell, can cause-specific DC recognition. Thus, this NP-DC combination leads to enhanced localization of DC action. Furthermore, targeting DCs by cellular uptake can be achieved by the NP endocytosis or pinocytosis. As a result, this approach can activate safe, effective, and specific antitumor responses. In conclusion, NPs, such as alginate or chitosan NPs, can be used to encapsulate antigens, ligands, and DCs to increase the immunotherapy selectivity to a specific type of cancer.^[58,59]

COLORECTAL CANCER-DENDRITIC CELLS IMMUNOTHERAPY

In sporadic CRC patients, immune cells' levels are considered a strong prognostic tool for the disease progress. Thus, the use of cDCs, Dex, DC-adjuvant combination, DC-based chemoimmunotherapy, and DC-CIK has been encouraged for CRC management to decrease the adverse events for CIK or chemotherapy alone therapies and increase the cancer cells specificity. In addition, cDCs, Dex, DC-adjuvant combination, DC-based chemoimmunotherapy, and DC-CIK are known for recognizing multiple types of cancers, especially in the late stages of CRC when the cancer is metastasized to many organs. However, NP and DC combination is a promising approach for CRC management.^[11,21-23,25,27]

CONCLUSIONS

In DC-based immunotherapy, rational combination therapies,

as previously discussed, can be inducted to mask the DCs or the other therapy disadvantages and improve overall efficiency. The use of DC-cancer immunotherapy stimulates effector T-cells, which is with the matured and active DCs. Thus, the use of dex, mAb, CIK cells, chemotherapy, and NP targeting were combined with DC therapy and found to enhance DC maturation and activity and trigger a broad antitumor activity. In addition, several challenges must be encountered to improve DC-based cancer immunotherapy efficiency and clinical outcome. The synergistic immunotherapies choice, the selection of adjuvants, route of administration, the NP type, and formulation method and the different treatment times are examples of these challenges. The cancer immunotherapy future depends on achieving two goals, which are inhibiting tumor-induced immunosuppression and activating the antitumor immunity.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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