

## Synergistic Effect of Dendritic Cell Vaccine with Immune Modulating Chemo Drugs

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### Abstract

Dendritic cells (DCs) have been tested for cancer immunotherapy over the past two decades in different clinical trials. During this period, efforts were put forth to optimize different parameters influencing the anti-tumor efficacy of *ex-vivo* generated DCs including maturation stimuli, antigen source, route of vaccine administration and adjuvant usage. In a recent paradigm shift, combinatorial therapy has emerged as possible answer to improve the efficacy of DC vaccines. Specifically, chemotherapy is reported to be associated with synergistic effects with DCs by altering the innate and adaptive arms of immune system. Chemotherapeutic drugs promote the molecular rearrangement on apoptotic tumor cells rendering them to be recognized by phagocytic DCs. The phagocytosis of immunogenic tumor cells results in maturation of DCs leading to an effective antitumor response. While the tumor suppressive microenvironment is subverted, the actions of chemo drugs also stimulate the immune effector cells either directly or indirectly by causing the release of cytokines. Here, we reviewed the assessment of the clinical development in DC vaccine trials and focused on combinatorial approaches using chemo drugs while understanding molecular mechanism underlying the interactions between anti-neoplastic drugs and immune cells.

**Keywords:** Immunotherapy, dendritic cells, chemotherapy, vaccines, synergistic effect.

### Introduction

The dendritic cells (DCs) cancer vaccine based immunotherapeutic approach has emerged as one of alternative treatment options owing to its low toxicity in comparison to other standard methods. The clinical efficacy has been demonstrated with improvement in overall survival rate and low toxicity. Studies in the last two decades has unfolded the mechanism underlying the triggering of anti-tumor immune response, regulatory mechanism, check points and co-stimulatory molecules involved in immune signaling (Jeffrey, 2014). Among the immune cells, DCs are the antigen presenting cells (APCs) which play major role in the antigen presentation and activating the immune system against the cancer cells. DCs activate different cell types that mediate immune resistance to tumors. DCs activate the T cells with their ability to cross present the exogenous antigens. Two pathways are utilized for cross presentation including cytosolic (proteasome dependent) and vacuolar (proteasome independent). DC's potency to induce T cell proliferation is 10-100 times more compared to that of B cells or monocytes (Inaba *et al.*, 1989; Amigorena and Savina, 2010; Cintolo *et al.*, 2012). T cells specifically recognize the peptide molecules bound to MHC molecules on DCs surfaces. Peptides on MHC class I molecule are recognized by CD8+ T cells whereas, peptides bound to MHC class II molecules are recognized CD4+ T cells. CD8+ cytolytic T cells (CTLs) directly kill tumor cells (Sabado and Bhardwaj, 2010).

CD4<sup>+</sup> helper T cells assist in inducing and maintaining CTL responses. Furthermore, CD4<sup>+</sup> T cells can recruit inflammatory cells with tumoricidal activity, such as macrophages and granulocytes at the tumor site and also demonstrate anti-tumoral activity directly (Wimmers *et al.*, 2014; Kim and Cantor, 2014). Natural killer (NK) and natural killer T (NKT) cells mediate antitumor activity by direct cytotoxic activity on tumor cells (Sabado and Bhardwaj, 2010). Regulatory immune cells primarily include T regulatory (Tregs) cells and MDSCs (myeloid derived suppressor cells) (Alessandra *et al.*, 2014). Under the progressive tumor conditions, the tumor microenvironment becomes highly immunosuppressive and immune cells functions are altered leading to low antigen presentation by DCs, suppressed expression of co-stimulatory molecules and secretion of tumorigenic cytokines (Adil *et al.*, 2014).

The application of *ex-vivo*-generated DCs emerged in an effort to improve the therapeutic efficacy in cancer patients in whom the dysfunction of endogenous DCs is commonly observed. The most commonly used approach is the differentiation of DCs from peripheral blood mononuclear cells (PBMCs) obtained from peripheral whole blood or leukapheresis procedures. These DCs are called monocyte-derived DCs (moDCs). CD14<sup>+</sup> monocytes are first selected from PBMCs either by plastic adherence or positive selection using immunomagnetic beads.

The monocytes are induced to differentiate into immature CD14<sup>+</sup>, CD83<sup>+</sup> DCs by culturing for several days in the presence of Interleukin-4 (IL-4) and granulocyte macrophage colony stimulating factor (GM-CSF). The immature DCs are activated to become mature by culturing for an additional 1-2 days in the presence of a maturation stimulus. Mature DCs are characterized by presence of CD80 and CD86, CD40, CD70, or inducible T-cell co stimulator ligand (ICOS-L) molecules. CD86, CD40, CD70 and ICOS-L expressed on DCs interact with their counter- parts CD28, CD40L, CD27 and ICOS, respectively, expressed on T cells *in vivo*. In addition, DCs have elevated level antigen-presenting molecules, i.e., major histocompatibility complex (MHC) class I, MHC class II, and CD1 molecules (O'Neill and Bhardwaj, 2005; Gabriela and María, 2015).

The other way of generating *ex-vivo* DCs is to derive it from progenitors CD34<sup>+</sup> cells which are mobilized from bone marrow. The harvested cells are cultured over a period of one week in presence of TNF- $\alpha$ , GM-CSF and Flt3L to generate immature dendritic cells. These cells are matured in same way as monocyte derived DCs (Banchereau *et al.*, 2001). In another method, dendritic cells can also be isolated from the blood of patients after treatment with growth factors like Flt3L that helps increase DC population. Later circulating *in-vivo*-expanded DCs can be harvested and exposed to tumor antigens under *ex-vivo* conditions (Pulendran *et al.*, 2000; Marroquin *et al.*, 2002).

#### Clinical developments

In one of the initial reports demonstrating the clinical use, DC vaccine was administered to four patients with low-grade follicular B-cell lymphoma resistant to chemotherapy. The patients were immunized with DCs pulsed with target antigens of clonal immunoglobulin (idiotype) expressed by the non-Hodgkin's lymphoma. As the booster dose they received injections of keyhole limpet hemocyanin (KLH) and idiotype proteins. All four patients demonstrated measurable anti-tumor immune response but clinical improvement was observed in three only. The clinical response indicated a complete tumor regression in one, partial regression in second patient and resolved all evidences of disease in third patient (Hsu *et al.*, 1996). In another report, where monocyte derived DC were used first time for clinical trial, 16 melanoma patients were immunized with DC pulsed with a cocktail of gp100, MART-1, tyrosinase, MAGE-1 or MAGE-3 peptides to suit class I HLA molecule. Out of 16 patients 5 demonstrated the tumor regression including 2 complete responses lasting for over a period of 15 months (Nestle *et al.*, 1998). The recent renewed interest in the DC based cancer immunotherapy is the result of first successful commercialization of vaccine for treatment of castration resistant prostate cancer. Commercially named as Sipuleucel-T, the vaccine was approved by FDA in 2010.

It is the first approved autologous cellular immunotherapy to improve OS in patients with advanced cancer (Higano *et al.*, 2009). The approval was based on the phase III results of IMPACT (Immunotherapy Prostate Adenocarcinoma Treatment) trials in which patients prolonged median overall survival by 4.1 months (25.8 months overall survival) compared to the control group (21.7 months overall survival) in 22% of population involved in the trial. The preparation methodology of Sipuleucel-T involves several steps including the leukapheresis (separation of peripheral blood mononuclear cells) followed by *ex-vivo* culture of separated cells. The cells are treated with PA2024 molecule, which is combination of prostatic acid phosphatase (PAP) and recombinant GM-CSF. GM-CSF drives the differentiation and maturation of DC whereas PAP serves as source of tumor antigen peptides for human HLA type 1 and type 2 which in turn stimulates CD4<sup>+</sup> and CD8<sup>+</sup> cells for antitumor activity against PAP positive prostate cancer cells (Small *et al.*, 2006; Kantoff *et al.*, 2010; Hall *et al.*, 2011; Sims, 2012; Geary and Salem, 2013).

Head and neck squamous cell carcinoma patients immunized with dendritic cell vaccine pulsed with p53 antigen resulted in increased T cell frequency in 11 out of 16 and IFN- $\gamma$  secretion in 4 out of 16 patients (Schuler *et al.*, 2014) suggesting the beneficial effects of DC vaccine. In a separate study, 6 uterine cancer patients received Wilm's tumor gene1, electroporated into *ex-vivo* developed DCs which demonstrated HLA-A2 restricted T cell responses (Coosemans *et al.*, 2013). Patients suffering from in situ ductal carcinoma received lipo-polysaccharide and IFN- $\gamma$  matured DC loaded with Her-2/neu peptides demonstrated T cell immunity for over a period of 1 year post immunization (Koski *et al.*, 2012). Similarly, vaccination with IFN- $\gamma$  and DC pulsed with HLA-A2 $\beta$  prostate cancer antigen peptides provided stable disease in 4 out of 12 patients (Hildenbrand *et al.*, 2007; Apostolopoulos *et al.*, 2014). In phase I trial of invasive breast cancer and Her-2 positive ductal carcinoma *in-situ* (DICS), patients were treated with autologous DC. The result demonstrated complete response in 5 out of 27 patients and substantial loss of target antigen in remaining patients (Sharma *et al.*, 2012). Wilms tumor-1 mRNA electroporated DC was used for immunization of acute myeloid leukemia patients. The vaccine was effective in patients, with minimum residual disease but not with relapsed or progressive disease (Tendeloo *et al.*, 2010). Genetically modified DCs were used in combination with cytokine induced killer cells (CIK) for treating 28 patients of advanced renal cell carcinoma (RCC). The treatment provided objective response rate (ORR) of 39% and disease control rate (DCR) of 75% (Wang *et al.*, 2014). Okada *et al.* (2011) used  $\alpha$  type 1 polarized DC cells for treatment of 22 patients of recurrent malignant glioma.

DCs were loaded with synthetic peptides for EphA2, IL13R $\alpha$ 2, YKL-40, and gp100 HLA-A2 restricted epitopes. Out of 19 patients evaluated, 2 showed objective clinical tumor regression and 9 demonstrated progression free disease for at least 12 months.

#### *Efficacy of DC vaccine*

With more than over 200 DC based clinical trials done so far, melanoma was the most common cancer treated. Most of the earlier studies established the feasibility and safety of DC vaccines. However, there is still a lot of research to be done to improve the clinical efficacy. The main challenge in DC research for improving the efficacy is attributed to the reason that DC vaccines do not follow the linear dose-response effect unlike chemotherapeutic drugs. Lack of linear dose-response is attributed to a large number of factors. These include (a) antigen source (b) timing of antigen delivery and activation of DC (c) DC maturation method (d) administration route (e) vaccination schedule (f) Immunogenic dose (g) adjuvant type (h) Migration of DC to lymphoidal tissue and (i) immunosuppressive environment. Optimization of these factors is required to improve the efficacy (Sabado and Bhardwaj, 2010; Butterfield, 2013).

Another major reason behind the unimproved efficacy of DC vaccine can be attributed to the disease stage at which the patients receive the treatment. In most studies patients received the immunotherapy at late metastatic stage when tumor burden is high. Under such circumstances, immunosuppressive factors dominate and become difficult for DCs to overcome the immune regulatory mechanism. Further, late stage patients are heavily treated with conventional chemotherapeutics drugs prior to immunization. The chemo drugs are known to deplete the leukocyte count in blood non-specifically turning patient more immune-compromised. The highly metastasized disease in patients also develop immune resistance due to infiltration of T regulatory cells, release of immunosuppressive cytokines like IL-10 and TGF- $\beta$ , alterations in antigen-presenting cell subsets, reduced expression of MHC molecules, myeloid derived suppressor cells (MDSCs) and heterogeneity of tumor sub-clones at the genetic level (Butterfield, 2013; Ghirelli and Hagemann, 2013). T regulatory cells (Treg) are characterized by presence of CD4+, CD25+ and FoxP3 markers (Whiteside, 2014). Studies have shown that expansion of Treg cells is associated with poor prognosis and reduced survival and their depletion result into enhanced antitumor activity of vaccine (Dannull *et al.*, 2005; Griffiths *et al.*, 2007). Myeloid derived suppressor cells (MDSCs) are a heterogeneous population of immune cells from the myeloid lineage which possess strong immune inhibitory properties. Abnormal accumulation of MDSCs is correlated with tumor evasion mechanism. Study on MDSCs demonstrated that circulating MDSC increased in cancer patient's relative to the healthy one.

A significant correlation between circulating MDSC and clinical cancer stage was also observed. Moreover, among stage IV patients, those with extensive metastatic tumor burden had the highest percent and absolute number of MDSC (Diaz-Montero *et al.*, 2009). Despite the fact that traditional *ex-vivo* generated DCs based trial produced inconsistent results, the therapy was adopted for wide range of tumors. The several advantages it holds include: low toxicity (grade 3 and 4 level toxicities are rare), less possibilities of immunotherapy induced autoimmunity, improvement in quality of life of patients and induce immunogenicity in even advanced malignancies (Leonhartsberger *et al.*, 2012; Anguille *et al.*, 2014). The increasing clinical evidence suggests that inconsistent outcome of the therapy can be related to traditional method of evaluating response criteria. The method is not sufficient to characterize the new era therapies. To understand the clinical inconsistency of trials with patients having discrepancies between strong immunogenicity and survival benefit but poor overall response rate (ORR), immune related response criteria (IRRC) was proposed. This criterion includes index as well as new lesion(s) in measuring tumor burden and emphasizes the need for longitudinal surveillance to confirm progression which cannot be covered under response evaluation criteria in solid tumors (RECIST) (Wolchok *et al.*, 2009). Developments of new approaches are required to combat the immune suppression or the tolerance induced by tumor microenvironment to enhance the immune stimulant effect of DC based vaccines. In this direction, several studies have been done where combinatorial treatment regime was used to develop a synergistic effect of two or more approaches. Here we have focused the discussion on the combination use of chemotherapy and DC vaccine.

#### *Combinatorial use of DC with chemotherapy treatment for generating synergistic effect*

Chemotherapy is the first line of treatment used for cancer patients along with surgery. But as the tumor progresses it acquires drug resistance and limits its efficacy. The major reasons for resistance development include drug-targeted gene amplification (e.g. *BRAF* gene) and substitution mutation in some cancer cells leading to the escape of drug cytotoxic effect (e.g. T790M mutation in *EGFR*) (Engelman *et al.*, 2006; Corcoran *et al.*, 2010). Similarly, tumor also escapes the immune check as discussed above in section efficacy of DC vaccines. Several strategies were investigated to overcome the drug resistance and immune escape of tumor by combining both chemotherapy and immunotherapy treatment methods. Earlier studies in Balb/c mice demonstrated that direct intra-tumoral injection of dendritic cells partially eradicated the colon adenocarcinoma. However, the pre-treatment with low dose of cyclophosphamide before intra-tumoral DC injections led to the complete tumor eradication in treated animals.

Infact, the DC vaccine treated mice developed the resistance against tumor when challenged with same cancer cells suggesting that mice developed long term immunity (Tong *et al.*, 2001). In a similar study, C57BL/6 mice were treated with DC after low dose treatment with chemo drug cisplatin and 5-FU against colorectal adenocarcinoma. The intra-tumoral injection of DC led to the complete tumor remission. This activity was enhanced in comparison to DC or chemotherapy alone (Tanaka *et al.*, 2002). The study performed by Yu *et al.* (2003), demonstrated the synergistic effect of chemotherapy and DC vaccine. Mice bearing mammary adenocarcinoma were treated with paclitaxel drug followed by i.v. or intra-tumoral injection of DC vaccine after 36 hrs. The treatment resulted into CD8+ specific and CD4+ specific response against tumor in comparison to DC or drug alone. Further, the response was demonstrated only in the mice infused with intra-tumoral injections but not i.v. In another experiment, tumor was induced in C57BL/6 mice with fibrosarcoma cells. These mice were treated with paclitaxel followed by intra-tumoral injections of DC vaccines. The combinatorial treatment resulted in complete remission of tumor in contrast to partial regression with drug or DC vaccine alone (Choi *et al.*, 2005).

The clinical studies with 29 small cell lung cancer patients used dendritic cell transduced with wild type p53 gene and second line chemotherapy showed objective clinical response of 61.9%. This response rate was much higher than the vaccine alone treated patients in which only one patient showed the response. Clinical response to subsequent chemotherapy was closely associated with induction of immunologic response to vaccination (Antonia *et al.*, 2006). In another clinical study patients with glioblastoma multiforme were treated with DC vaccine alone or DC vaccine with subsequent chemotherapy or chemotherapy alone. The group of patients who received DC vaccine with subsequent chemotherapy exhibited significantly longer recurrence survival relative to their own previous recurrence times, as well as other groups of patients who received either only DC vaccine or chemotherapy alone (Wheeler *et al.*, 2004). The clinical study with patients suffering from non small cell lung cancer treated with gemcitabine plus platinum (GP) in combination with DC and CIK (cytokine induced killer cells) in observation group and only GP in the control group. The median disease free survival time in the observation group (28 months) was significantly longer than the control group (22 months). Further, the 3 years cumulative survival rate was higher in observation group (58%) in comparison to control group (37%). Overall, the GP regimen along with DC-CIK immunotherapy significantly improved the immune cell function in the postoperative NSCLC patients, in addition to reducing postoperative tumor recurrence and prolonging the survival time of patients (Zhao *et al.*, 2014).

Similar studies demonstrated that effective combination of DC activated CIKs along with chemotherapy enhanced anti-tumor activity (Yang *et al.*, 2013) via up regulation of anti-tumor cytokines including IFN- $\gamma$ , TNF- $\alpha$ , TNF- $\beta$  and MIG (Li *et al.*, 2009).

In a recent phase III randomized controlled trial, the efficacy of post surgical immunotherapy was evaluated in combination with standard chemotherapy. 103 non-small cell lung carcinoma patients were treated with either combination of dendritic cells along with activated killer T cells and chemotherapy (group A) or chemotherapy alone (group B). The two years overall survival rate were 93.4% and 66.0% in group A and group B respectively. The 2 and 5-year recurrence-free survival rates were 68.5, 41.4 and 56.8, 26.2% in groups A and B, respectively (Kimura *et al.*, 2015).

#### *Mechanism underlying the synergistic effect of DC and chemotherapy*

The antineoplastic activity of chemotherapeutic agents is accompanied with immunomodulation (Fig. 1). Immunomodulation is mediated through several mechanisms including (a) immunogenic tumor cell death facilitating the phagocytosis by dendritic cells (cyclophosphamide, cisplatin, doxorubicin, irinotecan, 5-FU) (b) inhibition of tumor induced immune suppression by Tregs and MDSCs (Gemcitabine, cyclophosphamide, paclitaxel, 5-FU) and (c) activation of effector immune cells like NK cells, DCs, macrophages and tumor specific cytotoxic T lymphocytes (cyclophosphamide, paclitaxel, cisplatin, gemcitabine) (Bergmann-Leitner and Abrams, 2001; Ghiringhelli *et al.*, 2007; Liu *et al.*, 2010; Ramakrishnan *et al.*, 2010; Weir *et al.*, 2011; Doloff and Waxman, 2012; Galluzzi *et al.*, 2012; Schiavoni *et al.*, 2013).

In a study, low dose paclitaxel was used along with intra-tumoral DC vaccine for murine lung carcinoma. The therapeutic effect was enhanced in comparison to vaccine or paclitaxel alone treatment groups in terms of tumor growth inhibition, CD8+ and CD4+ T cell tumor infiltration and induction of antitumor immune response in regional lymph nodes. The effect was examined by intra-tumoral microdialysis and attributed to increase in MCP-1 and IP-10 chemokines and decrease in IL1 $\alpha$  level resulting into increased tumor specific IFN- $\gamma$  production. Thus, paclitaxel altered the cytokine level at tumor site (Zhong *et al.*, 2007). Shurin *et al.* (2008) demonstrated that different chemotherapeutic drugs in low dose alter the level of Rho GTPases in DC. The expression of endogenous Rac, RhoA and RhoE was regulated by non-toxic level of chemo drugs. The alteration of DC activity was correlated to alteration in GTPases level. Another study showed that paclitaxel, doxorubicin, mitomycin C, and methotrexate up-regulated the ability of DCs to present antigens to Ag-specific T cells. The stimulated DC activity was associated with increased secretion of IL-12p70 expression.

Fig. 1. Schematic representation of mechanism underlying the synergistic effect of DC and chemotherapy: Tumor induced immune suppression is caused by Treg cells making dendritic cells tolerogenic. Designated chemotherapeutic drugs inhibit Treg induced suppression by blocking activity of Treg cells (a). Chemotherapeutic drugs indicated here can cause direct stimulation of T effector and NK cells that can lead to anti-tumor activity (b). Chemodrugs by their virtue of cytotoxic function leads to immunogenic cancer cell death thereby enhancing immunovisibility of tumor debris favoring phagocytosis by dendritic cells (c).

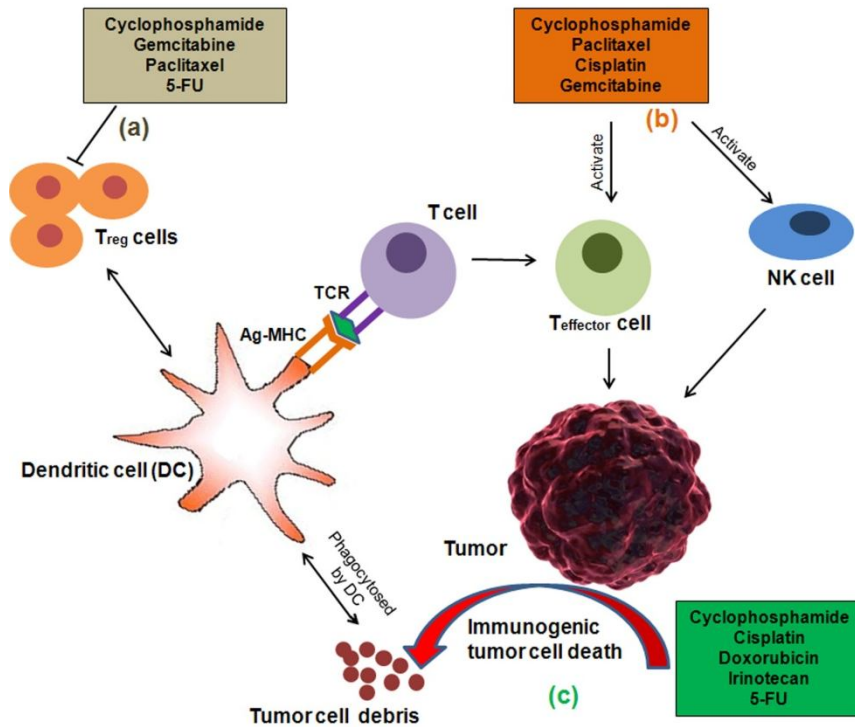


Table 1. Chemotherapeutic agents and their impact on the immune system.

Drugs	Type	Impact on immune system
Cyclophosphamide	Alkylating agent	Remove Treg induced immune suppression; enhance CD8 <sup>+</sup> T cell function (Weir <i>et al.</i> , 2011).
Doxorubicin	Anthracyclines	Enhance tumor immunogenicity (Weir <i>et al.</i> , 2011)
Paclitaxel	Taxanes	Enhance DC activation; increase expression of mannose-6-phosphate receptor on tumor cell required for granzyme B mediated killing (John <i>et al.</i> , 2010; Michels <i>et al.</i> , 2012)
Gemcitabine	Anti-metabolites	Decrease tumor induced immunosuppression; reduces Tregs and enhances CD8 <sup>+</sup> activation (Ko <i>et al.</i> , 2007; Rettig <i>et al.</i> , 2010)
Cisplatin	Platinum compound	Enhance tumor immunogenicity (Weir <i>et al.</i> , 2011)
5-FU	Antimetabolites	Enhance tumor immunogenicity (Weir <i>et al.</i> , 2011)
Vinblastine	Anti-microtubule agent	Upregulation of DC maturation factors like CD40, CD80, CD86, MHC-II, secretion of IL-6, IL-12 (Tanaka <i>et al.</i> , 2009)
Camptothecin –Sodium salt	Topoisomerase inhibitor	Upregulation of CD40, IL-1 $\beta$ , IL-6 and MLR (Tanaka <i>et al.</i> , 2009)
Mitoxantrone	Topoisomerase inhibitor	Secretion of IL-1 $\beta$ , IL-6, IL-12 and TNF- $\alpha$ (Tanaka <i>et al.</i> , 2009).
Etoposide	Topoisomerase inhibitor	Upregulation of CD40, CD80, CD86, MHC-II, secretion of IL-1 $\beta$ and TNF- $\alpha$ (Tanaka <i>et al.</i> , 2009).

The results demonstrated that doxorubicin, methotrexate, vinblastine and paclitaxel used IL-12 dependent pathway whereas mitomycin C and vincristine used IL-12 independent pathway for up regulation of DC activity to present antigen to Ag-specific T cells (Shurin *et al.*, 2009). Michels *et al.* (2012), proposed another mechanism for immunomodulation by intermolecular interaction between chemo drug paclitaxel and myeloid derived suppressor cells (MDSC). In this study, paclitaxel in ultra low concentration reduced the tumor suppression by inducing the differentiation of MDSC to dendritic cells and this transformation was TLR4 independent. In an attempt to study the pharmacological effects of drugs on DC 54 anti-cancer drugs were screened for DC stimulatory characters, 15 drugs were found to be inducing at least one of the maturational changes in mouse bone marrow derived DC. The drugs delivering DC maturation signals at concentrations caused only marginal DC death included topoisomerase inhibitors (for example, etoposide, mitoxantrone, doxorubicin), antimicrotubule agents (for example, vinblastine, paclitaxel, docetaxel) and the two alkylating agents mechlorethamine and diaziquone. Vinblastine emerged as most prominent inducer of the DC with induced production of IL-1 $\beta$ , IL-6, and IL-12, elevated surface expression of CD40, CD80, CD86, and MHC class II, and an augmented T cell-stimulatory capacity of DCs (Tanaka *et al.*, 2009). List of some chemo drugs and their influence on the immune system is mentioned in Table 1.

## Conclusion

With an immense background studies in both preclinical and clinical setup, the data obtained is evident that tangible benefits from DC vaccine can be realized by employing multifaceted approach including chemotherapy. Chemotherapy remains the backbone of current treatment regimen though its efficacy is limited by narrow therapeutic index, associated toxicities and acquired resistance. It is evident that chemotherapy deeply impacts not only tumor but also the immune system. Further, the traditional view of chemotherapy as immunosuppressive agent has also been challenged. The advances in chemo-modulation studies have unfolded the key mechanism which forms the basis for synergism or antagonism with immunotherapeutic approaches. Preclinical data have provided the suggestions that combination of chemo and immunotherapy may provide significant benefits. The data discussed here contributes to the understanding of mechanism-based rationale for combining specific chemotherapeutic agents with selective immunotherapeutic interventions, opening novel horizons for more effective treatment approaches for cancer.

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