Immune System Regulation With Cancer Vaccines Based on Dendritic Cells

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Abstract

The interplay between host immunity and tumor cells has opened the possibility of targeting tumor cells by modulation of the human immune system. Dendritic cells initiate and regulate T-cell immunity and are thus the key to optimization of all types of vaccines. DC biology insights offer a variety of opportunities to improve immunogenicity. Cancer immunotherapy involves the treatment of a tumor by utilizing the recombinant human immune system components to target the pro-tumor micro-environment or by revitalizing the immune system with the ability to ill tumor antigens. In this review, current immunotherapy approaches to cancer with special forms on dendritic cells based cancer vaccines and some recent development and findings for the clinical development of cancer vaccines are discussed.

Keywords - *Dendritic Cells, Immunotherapy, Immunogenicity, T-cells.*

I. INTRODUCTION

The potential link between the immune system and tumor was reported by William B Coley after he observed tumor regression upon systemic bacterial infections.

In 2001-2002 the concept of cancer immune system a nd its three component phases-elimination, balance, a nd escape-were proposed to explain the complicated i nteractions between the endogenous immune system and the tumor in development leading to apparently c ontradictory results of suppression and promotion of cancer (Figure-1). Thereafter, extensive research in animal models has demonstrated the existence of tumor specific antigens that are recognized by our immune system. The was strengthened when mice vaccinated with killed tumor cells presented the relapse after being challenged with original tumor but not with another tumor [1]. The role of innate and adaptive immunity in cancer immunosurvrillance has been well established.

II. IMMUNOSURVEILLANCE OF CANCER

Impacts using RAG2 deficient mice with inability to produce peripheral mature lymphocytes and some other studies using TCR-alpha and TCR-delta knockout mice which point out the gamma-delta T-cells and alpha-beta T-cells as the important and possible RAG dependent lymphocytes playing a vital role in anti-tumor immunity [2, 3]. The consequences of anti-tumor cells involve IFN-gamma are as gamma-delta T-cells which may then regulate the effector functions of tumor-induced CD4/CD8 T-cells which leads into the formation of an important chain in cancer eradication [4].

The immune system utilizing some cytotoxic molecules like perforin to kill the cancer cells and also inducing expression of TNF related apoptosis-inducing ligand (TRAIL) on immune cells primarily NK cells, dendritic cells and monocytes [5, 6]. It is critical and also necessary to understand the mechanism used by our immune system to differentiation between tumor cells and normal cells for the successful production and development of the cancer therapeutic drugs. CD4+/CD8+ T-cells recognize tumor antigens processed and present to MHC-2/MHC-1 molecules molecules by antigen presenting cells of our immune system. Tumor antigens are highly specific to the tumor include viral antigens produced in tumor caused by viruses, antigen produced by point mutation in expressed genes and cancer germ-line antigens like MAGE, BAGE and GAGE that are especially expressed in ovarian cancer and in germ-line cells. While some peptides derived from proteins like wild-type p53 that are over expressed in tumor cells and less specific to the tumor cells [7, 8].

Apart of there some stress signals like NKG2D and uric acid also play an important role in recognition targets [9, 10]. At the stage of tumor cell eradication by the immune system, some tumor cell resist the immune attack, renovates further to form new variants and enter in equilibrium with our immune system. Therefore, the tumor cells either endure in this phase or escape from all the immune attacks by restricting the antigen processing and presentation pathways along with enhancing pro tumor immunosuppressive environment dominated by IL-10, TGF-beta cytokines,

inhibitors of T-cells responses like indole amine 2,3-dioxygenase galectin-1, immunosuppressive co-stimulatory ligands B7-H3, B7-H4 and non-classical HLAs [11, 12, 13, 14, 15, 16].

During the escape phase, tumor cells secrete soluble forms of stress ligands like NKG2D which block the NKG2D receptors on immune effector cells thereby presenting the recognition of tumor cells [17]. Observations indicates the up-regulation of immunosuppressive T-cell population like Treg cells and IL-13 producing NKT cells which further augments the proliferation of tumor cells [18]. Tumor cells have also been recognized to dysregulate the expression of immune system inhibitors like CTLA-4, PD-1 to achieve immune resistance [19]. Immunotherapy targets the transformed malignant cells with the ability of our immune system and utilize it to mount anti-tumor immunity.

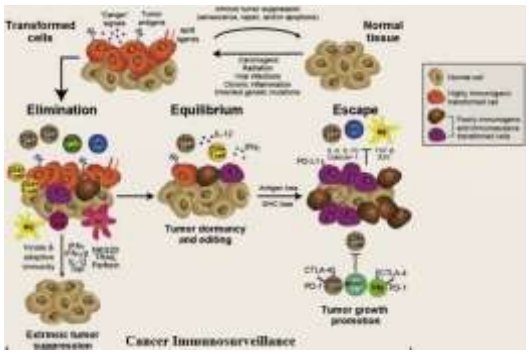

Figure 1. Cancer Immunoediting [20]

III. IMMUNOBIOLOGY OF DENDRITIC CELLS

Dendritic cells are special type of leukocytes able to alert the immune system to the presence of infections. The ingenious and adaptive immune responses play an important role. The DC function is regulated by activating a certain number of microbe products collectively referred to as a micro-subject associated molecular pattern (MAMP) of certain receptors at the cell surface, called toll-like receptors (TLR). TLRs initiate an event cascade that together define the DC maturation process.This phenomenon allows DCs to progressively acquire varying specific functions. DC maturation depends on the nature of the perturbation and permits unique and efficient immune response for each pathogen [21]. Conventional dendritic cells type (CDCs) are necessary for inducing anti-tumor T-cell responses. This appears to trace the ability of migratory CDC1 to deliver tumor antigen and cross-present to CD8+ T-cells. spontaneous anti-tumor immunity is dependent on activation of CDCs by type-1 interferon expression of cytosolic DNA and activation of the stimulator of IFN gene complex pathway.

Intratumoral CDC1s are capable of re-stimulating CD+ T-cells and may be important within tumor for antigen presentation and cytokine expression. the next generation of vaccines consisting of patient specific neoantigens or attenuated pathogens may demonstrate single agent efficacy or find utility in combination with checkpoint blockade.

Internalization of exogenous antigens by various pathogens result in loading of tumor peptides to MHC-2 molecules, it can lead to cross-presentation of peptides to MHC-1 molecules by loading in endocytic compartments or TAP (transport associated with antigen processing) mediated transfer to ER. On the other hand endogenous antigens are loaded on both MHC-1 &2 molecules [22]. Mature DCs express high level of antigen presentation molecules along with co-stimulatory molecules like B7-1/CD58, B7-2/CD86 leucocyte functional antigens (ICAM-1/CD54) which facilitates interaction with lymphocytes and their stimulation. Maturation of DCs results in decreased capacity to uptake antigens accompanied by increased expression of MHC and co-stimulatory molecules [23]. Activation pf DCs is followed by their migration to the lymphoid tissue where they interact with T-cells by virtue of high level of surface MHC and co-stimulatory molecules. The outcome of the T-cell priming by DC-subtype, plasmacytoid-derived DC2 activates TH1 cells when cultured in IL3 supplemented media [24]. The differentiated CD8 + T cells to CTLs generate secretory vesicles which cause neighboring cell lysis when released. The CD4+/CD8+ T-cells also differentiate into central memory and effector memory function respectively [25]. Upon activation of DCs, some chemokines are released that attract new DC precursor and also activate NK cells. Activated NK cells are shown to kill immature DCs and help in inducing protective CD8+ T-cell response [26]. DCs have also reported to induce B-cell proliferation and plasma cell differentiation through a B-cell activating factors

[27].DCs therefore offer the ideal candidate for cance r immunotherapy with the many ways that protecting immunity can be induced. (Figure-2)

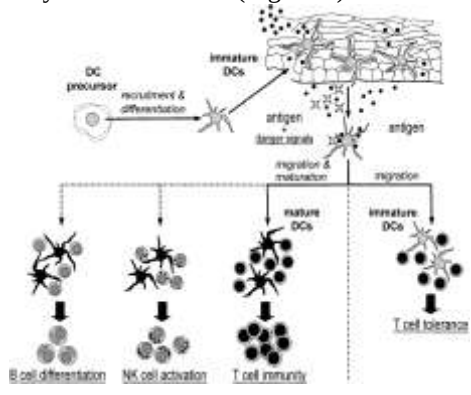

Figure 2. DC Immunobiology[28]

IV. MOLECULAR MECHANISM OF ACTION OF DC-BASED CANCER VACCINES

DC based vaccines aim to load DCs with tumor antigens ex-vivo or in-vivo followed by maturation of DCs that leads to their activation. Upon infusion into the patient, the ex-vivo mature DCs generate anti-tumor T-cells responses resulting from CD8+ effector T-cells. Exogenous antigens, prime CD8+ T-cells in addition to CD4+ T-cells by cross presentation to MHC-2 CTL differentiation programme is initiated by naive CD8+ cells on encountering tumor derived peptides presented by DCs (Figure-3). This is followed by expansion of T-cells, differentiation into memory CD8+ T-cells for generation of long term T-cell responses and tumor antigen specific effector cells that secrete cytokines and mediate tumor lysis. CD4 + T cells regulate CTL expansion and memory response induction. They also activate macrophages, further accentrating the overall anti-tumor response. Another important mechanism employed by T-cells for tumor cell lysis is, adherence of CD103 expressing CTLs to E-cadherin which leads to tumor rejection [29].

Monocytes (CD14) and stem cells (CD34) are the cell types that can be used for generating and expanding DC population ex-vivo. Generation of monocyte derived DCs is a 7 day process which involves culturing adherent population of PMBCs (peripheral blood mononuclear cells) in the presence of GM-CSF (granulocyte macrophage-colony stimulating factor) and IL-4, both of which are reported to differentiate CD14+ cells to a pure population of DCs [23]. The non-adherent fraction of PMBCs (CD34+) is cultured for 12 days in the presence of TNF-α and GM-CSF to yield DCs. We can also use antibody based separation technique for isolation of CD34+ cells [30].

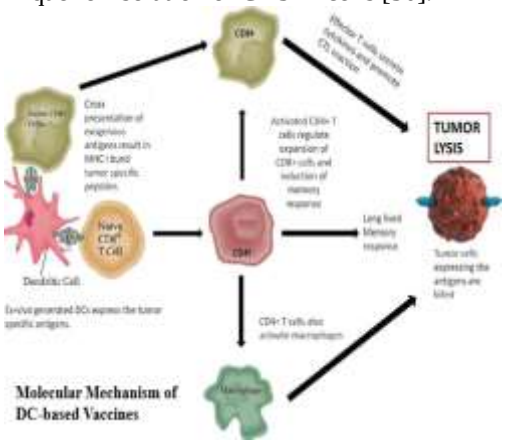

Figure 3. Molecular Mechanism of DC-based vaccines [31]

V. IMMUOTHERAPY TO TREAT CANCER

Immunotherapy or biologic therapy which leads to the cancer treatment that boosts the body's natural defenses to fight cancer. It includes targeted antibodies, cancer vaccines, adoptive cell transfer, tumor infecting viruses, checkpoint inhibitors, cytokines and adjuvants. Cancer immunobiology provides the chance to develop our immune system to eradicate the tumor cells. The main role of immunotherapy is to induce a memory immune response to prevent relapse by target the specific cancer cells. Passive immunotherapeutic approaches like immune checkpoint inhibitors target the mechanisms used by the tumor cells to escape the tumor attack, thereby reducing the protumor immunosuppressive environment. **Ipilimumab**,an antibody against cytotoxic T-lymphocytes-associated protein (TLA-4) which interferes with the co-stimulation required for T-cell activation was the first immune checkpoint inhibitor to be approved for use in cancer treatment [32]. Dendreon has recently confirmed in its pivotal phase-3 study (IMPACT trial n=5) that its first generation cellular vaccine product sipuleucel-T significantly improved survival asymptomatic, hormone refractory prostate cancer even though classical regressions did not occur and time to progression was not prolonged.

Some other immunotherapies that have been approved by the FDA include recombinant cytokines like Proleukin (IL-2), monoclonal antibodies targeting cancer-associated proteins like Her2, EGFR, VEGF and CD20 [33]. Due to the low specificity for tumor cells, immune checkpoint inhibitors results in development of autoimmune reaction.

Tumor specific therapies like enriching tumor-infiltrating lymphocytes from patients with melanoma and adoptive transfer of the TILs after in-vitro selection have shown objective response in 6 patients out of 13 [34]. Some other similar trials along with results with HPV-specific T-cells persistent in peripheral blood of cervical cancer patients for months [35]. T-cells immunoglobulin and ITIM domain (TIGIT) is a T-cell co-inhibitory receptor of the immunoglobulin superfamily member. TIGIT is expressed by subsets of CD4+ cells (memory and regulatory), CD8+ cells and NK cells. The ligands for TIGIT are Ig like trans-membrane cell adhesion molecules called nectin. CD155 (polio-virus receptor PVR), CD12 (PVRL2) and lower affinity CD113 (PVRL3, NECTIN-3) [36]. OX40 is a member of TNFRSF is expressed by both CD4+ and CD8+ T-cells during the antigen-priming phase, in response to TCR/CD3 cross linking and in the presence of pro-inflammatory cytokines in the tumor micro-environment, OX40 is not expressed in the resting or non activated T-cells [36].

A study reported rare skin reactions, tumor mass liquification with fatal outcomes, gastritis, aseptic meningitis and CNS inflammation as the immune related adverse events (irAEs) in melanoma patients who recieved **Ipilimumab** [37]. Cytokine therapy such as human recombinant IL-2 ha s also been associated with side effects such as capillary leak syndrome[38]. The adoptive

transmission of tumor-infiltrating lymphocytes (TIL) has increased in IL-2 by 50 to 100 fold more effectively than lymphokine-activated killer (LAK) cells to mice carrying micro-metastases from different type of tumor. Therefore the use of TIL was explored for the treatment of mice with large pulmonary and hepatic metastatic tumors that do not respond to LAK cell therapy [39]. **Bevacizumab,** is a humanized monoclonal antibody (mAb) that binds to all isoforms of the vascular endothelial growth factor (VEGF) receptor ligand, VEGF is a key mediator of developmental angiogenesis and has been shown to regulate the vascularization of tumor anti-VEGF antibody therapy has proven effective in multiple cancer sub-types including colorectal cancer, renal cancer, glioblastoma cancer etc. Ovarian cancer is a promising candidate for VEGF therapy [40].

Talimogene laherparepvec (T-VEC), a newly-approved FDA treatment for advanced melanoma, is the most advanced agent in clinical development. T-VEC is a modified oncolytic herpes simplex virus type-1 in which two ICP34.5 genes are deleted to prevent neuronal involvement. these genes have been replaced by the coding sequence for the cytokine GM-CSF. Enhanced local expression and GM-CSF secretion supports APC micro-environment recruitment and thus encourages the induction of immunity against tumors. An early study utilizing a (TLA-4) antagonist mAb, demonstrated the possibility of activity of immune checkpoint inhibitor in colorectal cancer [41]. Difficulty in obtaining autologous immunogenic tumor-specific lymphocytes in appropriate amount limits the use of this strategy. Hence autologous T-cell expressing (TCR) or chimeric antigen receptor (CAR) are being exploited to improved efficacy of T-cell based vaccines in clinical settings [42]. Employing allogenic T-cells in another approach but it poses a life threatning risk for graft-versus-host-disease (GVHD). Among allogenic patients with hematological malign ancies, engineered T-cells expressing HSV thymidine kinase are shown to regulate and overcome GVHD[43]. Allogenic anti-CD19 CART-cells have been accounted to induce remission in 8 out of 20 patients with B-cells malignancies and no case of acute GVHD was reported [44]. Another immune system component that seems to be promising since the first published clinical report in 1995, are the dendritic cells [45]. DC based vaccines aim to load DCs with tumor antigens ex-vivo or in-vivo followed by maturation of DCs that leads to their activation. Upon infusion into the patient, the activated DCs generate anti-tumor T-cell responses resulting from Cd8+ effector T-cell.

VI. VACCINATION WITH EX-VIVO GENERATED DCS

DCs can be generated ex-vivo, loaded with different forms of antigens, activated and injected in affected individuals [46]. Clinical studies from the past 15 years have analyzed (1) different DC vaccine preparations (2) different DC activators (3) different forms of antigen preparations from peptides to complex whole tumor cell hybrids and (4) different routes of DC injection. These studies were initially performed as single treatments but combination studies are now being assessed with agents such as systemic adjuvant eg. poly I:C [47, 48, 46, 49]. These studies concluded that DC based vaccines are safe and can induce the expansion of circulating CD4+ T-cells and CD8+ T-cell specific to tumor antigens. Immature DCs internalize agents by phagocytosis, macropinocytosis and endocytosis. The taking of foreign antigens and signals of TLR can result in DC ripening, followed by lymphoid migration.

Several chemokine receptors like CXCR-1, CCR-1, CCR-2, CCR-5 and CCr-6 are shown be expressed by immature DCs, whereas maturation of DCs is characterized by altered expression levels of CCR-6 and CCR-7 [50, 51, 52]. Maturation process further acquaints DC with the properties essential to present peptide loaded MHC complexes to the cell surface and increased expression of co-stimulatory molecules which amplify T-cell receptor (TCR) signaling and support T-cell activation [53]. Thus maturation status of DCs in the vaccine is an important parameter to determine the migratory and T-cell stimulation properties of the DCs. To make use of the best anti-tumor potential of DCs. Immature DCs are cultured with maturation stimuli following antigen uptake a variety of factors can trigger maturation including double stranded viral RNA, poly (I:C), bacterial-derived antigens (LPS, peptidoglycan), ligation of certain cell surface receptors (CD400 and inflammatory cytokines (TNF-alpha, TNF-beta, TNF-gamma) [54, 55, 56].

DCs matured using poly (I:C) retain their ability to secrete IL-12 in lymph nodes suggesting poly (I:P) as a low-cost and appropriate maturation stimuli for DC based vaccines. While several clinical trials are ongoing in several institutions around the globe to use DC-based vaccines to induce anti-tumor immunity against various cancer types including ovarian cancer, prostate cancer, renal cell cancer, melanoma and glioma, it is important to understand which antigens or peptides will be most useful. Summary of the first 100 patients who received dendritic cell vaccines by Ridgway in 2002 highlighted maximum number of trials to use peptide pulsed DCs. Major challenges in developing peptide vaccines is the identification and selection of appropriate T-cell epitopes that are unique to the tumor to prevent development of immune tolerance and in a few cases autoimmunity. The peptide vaccines face a major limitation on the population coverage due to MHC restriction. Therefore, there is need to shift to personalized multiepitope vaccines where the source of tumor antigens can be whole tumor lysate, whole tumor RNA and apoptotic tumor cells.

Tumor cell vaccines can either use tumor antigens derived from patients tumor sample or from established tumor cell lines. Using tissue biopsy sample from patients as a source of antigens offers advantage of having unique patients specific tumor associated antigens but faces the limitation of availability of sufficient tumor sample from the patient.

VII. IN-VIVO DC TARGETING

Developing studies from Ralph Steinman and Michel Nusseuzweig demonstrated the principle of targeting antigens to DCs in-vivo through the coupling of antigens to antibodies specific to DC surface receptor such as DEC205 or DCIR [57, 58, 59]. Importantly in the absence of adjuvants, targeting antigens to DEC205+ DCs in-vivo induces antigen-specific tolerance [59], which can be used as treatment against autoimmune diseases such as type-1 diabetes. Administration of these complex vaccines with DC activators such as TLR-3, TLR-7, TLR-8 or CD40 agonists enables the maturation of DCs and thus the establishment of immunity rather than tolerance [61]. The induced immunity was shown to be protective in a number of diseases including various infections (eg. malaria, HIV) and cancer [60, 61]. DC-targeting based vaccination studies in non human primates demonstrated robust T-cell immunity in prime boost design with HIV gag DEC205-targeting vaccine [62].

Currently, numerous *In vitro* and *In vivo* studies in human and mice are focused on developing DC-targeting vaccines. For example, targeting antigens through the DC surface lectins DCIR [63, 64], DC-SIGN [65], dectin 1 [66], CLEC9A [67] and langerin [68] results in humoral and cellular responses including those of both CD4+ and CD8+ T-cells. As observed in the original studies which DEC205, the presence or absence of adjuvants has a profound impact on immune responses. Thus, in the absence of adjuvants, injection of antigens coupled to antibodies against CLEC9A results in strong antibody responses which are linked to the generation of Tfh cells [69]. It also results in priming of Treg cell immunity [70] but not CD8+ T-cell immunity, despite the capture.

VIII. EX-VIVO DC-BASED VACCINE FOR DIFFERENT CANCER TYPES

There are many types cancer and these cancer types have different modalities which are clinically examined. These clinical testings ex-vivo DC-based vaccination for different types of cancers like multiple myeloma, melanoma, colon cancer, renal cell cancer, glioma [46]. In one of 10 patients with liver cancer and with KLH common, late type-hypersensitivity in 7 patients, a phase 1 analysis with autologous DCs pulsed with tumor lysate in combination with tumor necrosis factor and keyhole limpet-hemocyanine (KLH) showed tumor regression in 1 [71].A variety of funding organizations are designing DC-based vaccinations and conducting trials in order to check their medical response.

A. Renal Cell Carcinoma

Argos Therapeutics drug AGS-003 is prepared with synthetic Cd40L RNA, for ex- vivo loading of DCs with RNA amplified from the tumor. Combined with sunitinib, AGS-003 has resulted in clinical gain in 62% of the patients and OS in 33% of patients with at least 4.5 years [72].

B. Glioblastoma

DCVax-L is an active autologous treatment for DC in glioblastoma patients using whole tumor lysate, which is the source of tissue biopsy of the patient. DCVax-L is manufactured by Northwest Bio-therapeutics.The new diagnosis found that OS was increased to 48 months for 33 per cent of patients with DCVax-L, in multiform glioblastomas. In addition, different subgroups of GBM patients can have different benefits from DC therapy. Phase III clinical trial of 312 subjects was performed at DCVax-L [73].

C. Ovarian Cancer

CVacTM developed from Prima Biomed consists of dendritic monocyte derived cells loaded with the protein mucin 1, the abnormally expressed protein of various epithelial tumors, including ovarian cancer.MUC1, the recombinant protein VNTR is combined with mannan oxidized. CVac has demonstrated its survival in patients with ovarian cancer by 10.3 months and demonstrated increased progression-free survival of patients with second-hand clinical remission in maintenance therapy [74, 75]. Vaccell by Tella is another DC-based vaccine used in the identify antigenic system where MHC-I-restricted Wilms tumourgen 1 peptide antigens are incubated with the PBMDs of patients (WT1). The average survival time was 14.5 month after vaccination of 56 patients with recurring ovarian cancer [76]. Sotio is also assessed for ovarian cancer treatment for whole tumor lysate pulsed DCs. The DCVAC / OvCa product is currently being studied in phase I / II (J. Clin. Oncol. 32:5s 2014).

D. Non-small Cell Lung Cancer

Vaccell and MelCancerVac, developed respectively by Tella and Dandrit Biotech, are the products that entered the Phase II clinical trial for non-small cell lung cancer (NSCLC). Wilms tumorgen 1 peptide antigen includes DCs from patients with pulsated MHC-I (http://www.sotio.com/clinical-trials/lung-cancer).

The preparation of the MelCancerVac contains DCs filled with antigen-containing allogenic melanoma cell lysate, known to be expressed in approximately 40

percent of NSCLC's lines. PhaseI/II tests showed positive reactions among MelCancerVac vaccinated patients and phase IIb testing of this product [77].

E. Prostate Cancer

Sipuleucel-T was FDA approved to treat metastatic (hormone refractory) prostate cancer with asymptomatic or at least symptomatic metastatic symptoms. Ex-vivo DC precursors have been loaded with GM-CSF-fused recombinant PAP (prostatic acido phosphatase) [78, 79]. Sotio's DCVAC / PCa also is a phase III clinical trial using the killed PSA-positive prostate cancer cell line (LNCaP). DCVAC / PCa has shown that PSA-specific T-cell replies and cell down-regulation are being induced to enhance OS in patients [80].

F. Other Types of Cancer

Eltrapuldencel-T is used as a source of tumor antigen for patients with melanoma by the irradiated cells from autologous cancer cell lines. The findings of Phase I / II analysis showed a mean follow-up of 13.8 months to 95% OS [81]. In pancreatic cancer patients with non-liver metastasis, the Vaccell by Tella was induced by a pilot Phase I study with tumor immunity [82]. The MelCancerVac Phase II analysis resulted in 40% clinical response in MAGE-positive patients with MelCancerVac [83]. APCEDEN®, with a survival benefit of 200 days and an objectives response rate of 29 percent in advanced phased refractory solid malignancies, is the Autologous DC loaded with whole tumor-lysate and then poly(I:C) ripening [84].

IX. CANCER TREATMENT COMBINATORIAL APPROACH

Tumor growth and progression are prevented from oncosignaling and host immune reactions through immunotherapy with monoclonal antibodies, immune control point inhibitors and small molecules such as TKIs. Active cancer immunotherapy with specified or unknown tumor antigens, including ex-vivo loading of immature DCs, improves the ability in the immune system to fight cancer. Autologous DCs are safer than standard chemotherapy as vaccine candidates and are better suited to cancer that has advanced following chemotherapy than previously Autologous DCs. The use of strengths of different types of immunotherapy approaches and traditional methods can therefore help to limit one's weaknesses and improve the clinical result. (Figure-4)

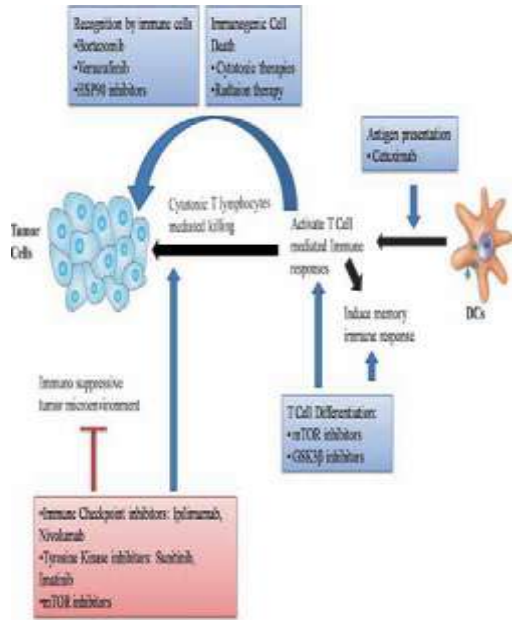

Figure 4. Combined cancer cure. In other therapies, which support different pathways (shown with blue arrows) leading to anti-tumor immunity, the immune function of the effector activated through DC-based vaccines can be enhanced[31]

Therefore it is necessary for the optimum choice of the combination with the correct biomarker-based selection of the patient, dosage of individual treatments and the management of adverse events with the availability of comprehensive anticancer therapies.

X. DISCUSSION

A modified tool to kill tumor cells directly without toxicity would be the most effective therapeutic vaccine for cancer. DC-based vaccines offer positive clinical results for both personalized loads of antigen such as whole tumor lysate, tumour-specific RNA and nonpatient sources of antigen such as recombinant proteins and lysate from tumour-cell lines. The various methods of producing DCs, mating impulses, antigen origins and vaccine routes throughout the world have shown variable benefits in clinical settings. DCs can be formed either from GM-CSF, IL-4/IL-13 or GM-CSF-incubated, Flt3-ligand and TNF-α monocytes CD14 +. Antibodies ex vivo are loaded and followed by DC maturation, which allows better DC movements to the lymph nodes. The pseudo-progressive tumor concept is an important consideration in the evaluation of the effectiveness of DC vaccine. Activated DCs increase the immune system's tumor-fighting potential, which also leads to a tumor site infiltration of active immune cells which is viewed by the RECIST criteria, as a growth in the tumor mass, and categorized as progression of disease. Immune related response criteria (irRC) therefore define better conditions to characterize illness status when active DCs are administered. The immune response induction and stability of DC vaccines must

be tested before administration and the memory T-cell response follow up should be followed up for long enough period of time to validate memory development. Many of the DC-based vaccine products have to be standardized for better efficacy and uniformity. With cancer cells capable of inducing immune suppressant cells and escaping immune detection, immunological conditions can increase the success rate before or parallel to DC-based vaccines. Modern cancer treatment such as few drugs for anti-cancer and radiation therapy that increase tumor cell recognition through the active immune cell. In T-cell activation, memory response and down-regulated immune suppressive molecules may help small molecules that are immunomodulatory including tyrosine kinase inhibitors. For better clinical and symptomatic patient treatment, DCs in combination with other anti-cancer therapies need to be explored.

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