Dendritic Cell Vaccine for the Treatment of Cancer

By

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A thesis submitted to the Department of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I/We have acknowledged all main sources of help.

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Approval

The project titled "Dendritic cell vaccine for the treatment of Cancer" submitted by [Tanzila Haque (18146090)] of Spring, 2018 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on 24.02.2022

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Ethics Statement

The study does not involve any kind of animal or human trial.

Abstract

Dendritic cells (DCs) serve as sentinels for the immune system, antigen-specific immune responses are initiated and regulated. Cross-priming, a mechanism in which DCs activate CD8 T cells by presenting external antigens to their major histocompatibility complex is crucial for CD8 T cell immunity and tolerance. Immunosuppression caused by tumors and the functional restriction of routinely utilized dendritic cells generated from monocytes are two important obstacles to the effectiveness of DC-based vaccinations. Exosomes generated from DC have piqued interest as cell-free therapeutic agents due to being inert vesicles, they are resistant to tumor-mediated suppression. Another fascinating breakthrough is the utilization of DCs that circulate naturally rather than in vitro grown DCs, which has demonstrated encouraging effects in clinical trials with both human blood cyclin dependent kinase and plasmacytoid DCs.

Keywords:

DCexos; Plasmacytoid DC; Dendritic cell; Immunity.

Dedication

Dedicated to my respected supervisor.

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Firstly, I would like to express my gratefulness to my respected supervisor Ms. Marzia Alam for her continuous guidance and support during the project work as well as her patience, inspiration, excitement and knowledge which was invaluable during the review and drafting.

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DC	Dendritic Cell
APC	Antigen Presenting Cell
MLR	Mixed Lymphocyte Reaction

Chapter 1 Introduction

Vaccines are a sort of immunotherapy that aids the body's ability to fight disease. The immune system is stimulated when people receive immunizations. The immune system produces antibodies to recognize and attack the disease's harmless forms. Once the body has produced these antibodies, it will be able to recognize the sickness if it is exposed to it again. Vaccines are designed to recognize proteins on specific cancer cells in the same manner as they operate against illnesses. Antigens are substances that cause the immune system to react to them. Antigens on the surface of a virus, for example, cause the immune system to attack it. Antigens are found on both body cells and cancer cells. Antigens present in cancer cells are known as tumour associated antigens. Normal cells either do not carry these antigens or have a very minimal amount of them. Vaccines for cancer treatment seek to aid your immune system in recognizing these antigens. And to go for and kill the cancer cells that have them(E.J, 2021).

The leading cause of death is cancer metastases. With an estimated 10 million deaths worldwide due to cancer, it is the leading cause of deaths predicted by 2020.

In 1973 dendritic cells, distinguished by the late Ralph Steinman, are the body's fundamental antigen-introducing cells, antigens are presented to CD4+ and CD8+ T cells and trigger defensive T cell reactions once activated. When a cancer-specific antigen is given, an antitumor response can occur. Dendritic cells have long been considered as most develop vaccines based on cell due to the significance of T cell responses in triggering an immune response against malignancies. In 1990s, scientists discovered the concept of 'pulsing' antigens specific to tumors ex vivo into dendritic cells, which was critical cause dendritic cells are being developed as vaccinations. Despite the fact that the time to disease progression was not changed, vaccination based on dendritic cells sipuleucel-T was used to treat patients with metastatic hormone-refractory prostate cancer, in 2010 IMPACT trial, a multi-centre phase III reported and in 2006 two additional phase III trials reported showed a benefit in induction of a T cell response and in median survival. Based on this, sipuleucel-T was licensed in 2010, it was approved for the treatment of advanced prostate cancer the first dendritic cell cancer vaccine. Sipuleucel-T is a treatment that is tailored to the individual. Each patient's dendritic cell progenitors are removed and pulsed with a prostatic acid phosphatase fusion protein and the cytokine GM-CSF, which aids the maturation of antigen-presenting cells. The patient is then reinfused with pulsed dendritic cells numerous times. Despite the fact that sipuleucel-T isn't frequently used, combining hormonal therapy with sipuleucel-T boosted the survival of metastatic castration-resistant prostate cancer patients, according to new research.Other clinical

trials are being done that combine sipuleucel-T with radiation, targeted, hormonal, or other immunotherapies. Currently, sipuleucel-T is the only licensed vaccine based on immunotherapy for prostate cancer in the United States, as well as the only cell-based immunization(Stevens et al., 2021).

The immune system has a crucial function in of cancer development. Both acquired and innate immune systems are capable of recognizing altered cells of cancer– which are esteemed as nonself – and eliciting a certain immune response. The immune response's goal is to eliminate the altered cells in order to limit their multiplication and, as a result, tumor formation. Dendritic cell vaccines have had a poor clinical response rate overall, however as more information becomes available, novel and more sophisticated ways to increase the effectiveness of vaccines based on dendritic cells are being researched. Ex vivo approaches to produce dendritic cells that are more developed and 'effective', alternate antigen combinations, optimal dendritic cell loading, and dendritic cell transfection with RNA or DNA are among the ways being investigated. Dendritic cell subsets are being studied, as well as various drugs other than GMCSF that has the potential in in vivo mobilization of dendritic cells, such as FLT3L(Marte, 2020). The vast majority of DC vaccinations involve monocyte-derived DCs grown in vitro, are largely ineffective, with just 5–15 percent of patients achieving objective immune responses. The only FDA-approved "DC" cancer vaccine in over ten years is Sipuleucel-T, which is made up of concentrated cells of blood for antigen-presenting cells (APCs), including DCs. Despite a string of failed clinical trials, DC vaccines clinical trials using neoantigens have yielded promising outcomes to an intriguing for cancer immunotherapies a novel development in DC vaccines has been made. The relevance of developing and refining vaccines based on DC as monotherapy or combinational immunotherapies was recently underlined by the finding of the important role of cDC1s in cross-priming tumor specific antigen CD8 T cells and determining the cancer immunotherapies efficacy.(Gelao et al., 2014a). Immunosuppression caused by tumors and functional constraints of routinely employed DCs that have been differentiated in vitro are two main roadblocks to DC vaccination effectiveness. Exosomes generated from DC (DCexos) are immune to stimuli that are related to tumor because they are inert vesicles. As a result, vaccines containing DCexos could be a new form of DC-based vaccine capable of overcoming immunosuppression caused by tumor. The utilization of naturally circulating blood DCs and in vivo DC-targeted vaccinations are other alternatives to DCs that have been differentiated in vitro are promising, which are used in the majority of clinical trials. The positive clinical studies of pDCs, the potential of integrating pDCs with

cDCs, as well as a human pDC cell line is being used in recent clinical trial, promote the development of pDC-based cancer vaccine immunity. Previously unknown exosomes generated from pDC (pDCexos) are an intriguing new addition to the arsenal of vaccinations based on DC, as vaccines containing pDCexos have the ability to combine the benefits of both DCexo and pDC vaccines. (Fu et al., 2020). DC exist as immature DC prior to antigen contact. This is distinguished by strong intracellular MHC II expression in compartments of late endosome-lysosomal, low costimulatory molecule expression and chemokine receptor expression. Immature DC, on the other hand, are biologically prepared to catch and absorb antigen endocytosis, pinocytosis, and phagocytosis mediated by receptor. After antigen uptake and capture, chemokine receptors are upregulated by DC with antigen such CCR7, allowing DC-T cell interaction to occur, which is essential for the start of the responses of T cell. For the start of T cell responses to specific antigens, DC maturation from immature to mature is critical. Through allogeneic mixed lymphocyte reaction (MLR) tests, effective stimulation of the functional response of DC to T cells can be proven in vitro. Furthermore, DC require extremely little antigen to activate T cell proliferation, and they have been found to be more effective T cells stimulators, requiring macrophages more than 100-fold and B cells to trigger a MLR response that is proliferating. To govern the sort of T cell response evoked, increased expression of MHC I and MHC II molecules on the surface, expression of receptors of chemokine, escalated expression of costimulatory molecules, and cytokine release are all physiologic alterations that occur in DC. DC maturation also causes the endocytic vacuoles to drop pH, allowing for proteolysis is triggered, and peptide-MHC molecules are transported to the surface of the cell, but antigen capture is reduced. Because DC have a receptors range for microbial and viral pathogen detection, injured, apoptotic, stressed and necrotic cells, including autologous cells, also maturation can be induced by the stimuli of environment. (Steinman & Banchereau, 2007). Receptors for pattern recognition such as Toll-like receptors, lectin receptors which are c-type, receptors which are like NOD, and RIG-I and MDA5 which are and DNA/RNA receptors allow DC to recognize microbial and viral invaders. They use activating and inhibitory Fc receptors to detect immune complexes. IFN, TSLP, TNF, IL-10, CD40L and TSLP receptors from other immune cells send immunogenic and tolerogenic signals which DC respond .The release of chemicals ordinarily located intracellularly, including as heat shock proteins , ATP and HMGB proteins, allows DC to detect injured cells and tissues(Gallucci & Matzinger, 2001). Utilizing signals which are highly immunogenic produced by cell death, another technique currently being utilized to develop more potent vaccines based on DC against cancer is to use the mechanisms of recognition that DC utilize

to endogenous recognition and non-endogenous signals. The ability of specific types of DC to cross-present intracellular antigens is another essential trait. As a result, DC can take up foreign antigens, resulting in the presentation of MHC I complex which is an antigenic peptide that can activate CD8+ cytolytic to response against immune complexes, non-replicating microorganisms, and dying cells. While the mechanisms underlying cross-presentation are yet unknown, it is apparent- protein antigens which are endocytosed and most rapidly travel towards MHC class II presentation can reach the cytoplasm also, where processing of proteasome takes place prior to introduction of the peptide-MHC I (Santos & Butterfield, 2018). In this study, I will be discussing the different types of vaccines based on DC for treatment of cancer and advantages of vaccines based on DC over the conventional anti-cancer treatment methods.

1.2 Epidemiology of Cancer

In this chapter I Will give a concise overview of the most recent cancer epidemiology data. gathered from the World Health Organization (WHO) official databases and the American Cancer Society (ACS) in the attempt to provide current information on the mortality, frequency and survival expectancy in worldwide the 15 most common types of cancers in this brief report(Gallucci & Matzinger, 2001).

Table-1: Leading cause of mortality in the different regions of the World Health Organization (2016) (*Mortality and Global Health Estimates*, 2019)

Disease	Western Pacific	Africa	America	South East Asia	Europe	Eastern Mediterranean
Total	13.778	8.845	6.876	13.819	9.215	4.122
Ischemic heart disease	2.391	0.512	1.091	2.234	2.342	0.835
Cancers	3.141	0.524	1.348	1.361	2.121	0.410
Stroke	2.393	0.373	0.437	1.250	0.986	0.326
Lower respiratory infections	0.470	0.917	0.311	0.783	0.245	0.221

Preterm birth complications	0.055	0.344	0.045	00.364	0.024	0.181

Table-2: List of cancers with the highest mortality rate from the World Health Organization (WHO) Global Cancer Observatory(Bray et al., 2018).

Cancer	Mortality (million)				Risk 0-74 years (%)			Death Rate (%)
	Total	Men	Women	Ratio	Total	Men	Women	
All cancers	9.555	5.386	4.169	1.29	10.63	12.71	8.7	53
Lung (and trachea and bronchus)	1.761	1.185	0.576	2.06	2.22	3.19	1.32	84
Liver (and intrahepatic bile ducts)	0.781	0.548	0.233	2.35	0.98	1.46	0.53	93
Stomach	0.783	0.513	0.269	1.91	0.95	1.36	0.57	76
Breast	0.627		0.627				1.41	30
Colon	0.551	0.290	0.261	1.11	0.50	0.59	0.41	94
Esophagus	0.509	0.357	0.151	2.36	0.67	1.00	0.36	89
Pancreas	0.432	0.227	0.205	1.11	0.50	0.59	0.41	94
Thyroid	0.411	0.156	0.255	0.61	0.05	0.04	0.05	7
Prostate	0.359	0.359				0.60		28
Cervix uteri	0.311		0.311				0.77	55
Rectum	0.310	0.184	0.126	1.46	0.35	0.46	0.26	44
Leukemia	0.309	0.180	0.129	1.39	0.33	0.40	0.26	71
Non Hodgkin's lymphoma	0.249	0.146	0.103	1.42	0.27	0.35	0.21	49
Bladder	0.200	0.148	0.052	2.87	0.18	0.29	0.08	36
Kidney	0.175	0.114	0.061	1.86	0.20	0.28	0.12	43

Because the rate of death (measured as the ratio between mortality and frequency) for certain types of malignancies than for others is manifestly higher, rank in the mortality and rank in the frequency do not overlap completely. Lung, liver, and stomach cancers in general are most 3 lethal cancers. Still these 3 cancers rank first, second, and third in males, respectively, while prostate cancer is fifth, right below esophageal cancer. Breast cancer, unlike in men, is the main reason of women death, after that stomach and lung cancers. Specially, rectal and colon cancers are the second and fourth major reason of cancer death respectively in women and men.(Bray et al., 2018).

Between the ages of 0 and 74, the risk of dying overall from cancer is 10.6% (8.7% in women and 12.7 percent in men); the risk of malignancy is highest in men is lung (3.19 percent), liver (1.46 percent), and stomach (1.36 percent), while in women it is breast (1.41 percent), lung (1.32 percent), and cervix uteri (0.77 percent). Except for cancers that are specific to men and women, the mortality proportion of men to women is greater than one with the exception of thyroid cancer, for all cancer (i.e., 0.61). Cancers of the esophagus (2.36), bladder (2.87), and intrahepatic bile ducts and liver (2.35) have the greatest men/women ratios. Pancreatic cancer has the highest death rate (94 percent), followed by liver and intrahepatic bile ducts (93 percent), esophagus (89 percent), and bronchus, trachea, and lung cancers (84 percent), while thyroid cancer has the low death rate (7 percent), and prostate and bladder cancers have relatively low death rates (28 percent and 36 percent, respectively)(Bray et al., 2018).

1.3 Types of Vaccines in the treatment of cancer

Various forms of vaccines of cancer are being studied by scientists, as well as their different ways of action. Before a complete picture of their effectiveness in treatment and which vaccines can able to treat which cancers, more studies are required. Types of cancer vaccines are now being researched which are given below:

Protein or peptide vaccines

These vaccines are produced using unique proteins of cancer cells. Alternatively, tiny bits of protein might be used. These vaccines try to boost ability of immune system to fight cancer. Many cancer cell proteins' genetic codes have been deciphered, allowing scientists to massproduce them in the lab.

DNA and RNA vaccines

These vaccines are manufactured from fragments of DNA or RNA discovered in cancer cells. To improve the ability of immune system these vaccines can be injected into body to recognize and destroy cells of cancer.

Whole cell vaccines

The full cancer cell, not just a single cell antigen, is used to create a whole cell vaccination. In the lab, cells of cancers are modified to make easier to detect by the immune system. By using your own cancer cells scientists create the vaccine, cells of cancer from another individual, or cells of cancer generated in lab.

Dendritic cell vaccines

In attacking and identifying aberrant cells like cancer cells DCs assist immune system. In the lab, Dendritic cells are produced alongside cancer cells to create the vaccine. Your immune system is then stimulated to attack the tumour by the vaccine.

Virus vaccines

In the lab, scientists may modify viruses to utilize them as a form of carrier to carry antigens of cancer to your body. They alter the viruses so they cannot be capable of causing significant sickness. The modified virus is referred to as a viral vector.

To inject antigens of cancers into body, some vaccinations use a vector of virus. From immune system the vector of virus will elicits a response. As a result, immune system is capable to respond and recognize to antigens of cancers in a better way. T-VEC (Imlygic), is a therapy that works similarly to virus vaccinations. A cold sore viral strain is used to make it. Virus has been altered by changing the genes that control how it behaves. It instructs the virus to ignore healthy cells and to target the cells of cancer. This mechanism helps the immune system in locating and eliminating other cells of cancer. Now T-VEC is accessible as a therapeutic option for patients with melanoma skin cancer and those patients are unable to have their disease surgically removed. It's being tested also in neck and head cancer trials(*Vaccines to Treat Cancer | Cancer Research UK*, 2021).

Chapter 2

Methodology

This review paper has been performed based on topic based and relevant research papers which are from journals having high impact factor. A proper search has been done through articles and peer reviewed journals and research articles. To ensure the quality of the paper information's from books were taken. Several types of search engines such as- PubMed , Elsevier and google scholar have been used to collect the papers. Dendritic Cell Related journals were shortlisted according to their publication time and impact factor. Most recent and relevant ones were chosen to ensure the quality of the review paper and finally information's were taken , summarized and were settled according to their headings.

Chapter 3

Dendritic Cells in Anti-Cancer Vaccines

Heterogeneous DCs are, sparsely dispersed collection of APCs which are special and generated from CD34+ bone marrow stem cells. Human and mouse investigations in vitro and limited in vivo human investigations as well, reinforce our understanding of DC differentiation. Monocytes and DCs are thought to come from same shared progenitors known as DC and monocyte progenitors, according to the most recent recognized paradigm (MDPs). When MDPs transform into committed DC progenitors and monocyte progenitors in bone marrow, these two cell types diverge (CDPs). Pre-DCs are created by CDPs, which migrate away from bone marrow give rise to 2primary DC sub-populations. Although this paradigm has primarily been investigated in mice, human research has also validated these findings. granulocyte monocyte DC precursors in humans evolve into monocyte DC precursors that give birth CD1c+ DCs, CD141+ DCs, and CD142+ DCs and plasmacytoid DCs are three basic subsets of DCs produced by common DC progenitors. Bone marrow, cord blood, bone marrow, lymphoid organs and blood of humans all have a migratory phenotype (hpre-cDC) that maintains cDC pools through differentiation. Flt3L has also been demonstrated to augment the pre-cDC pool in people when given systemically(Breton et al., 2015).

The most common cells which presents antigen are LCs discovered in skin's epidermis. They contain huge granules known as Birkbeck granules and CD45, Langerin, MHC-II, epithelial cell adhesion molecule, and CD45 are all positive. Antigens are picked up to transferred to regional lymph nodes by LCs in the epidermis, where T cells are exposed to them in order to initiate the responses of immune system. PDCs and inflammatory DCs are both attracted to one’s dermis during inflammation. Inflammatory DCs are distinct from monocytes circulate in the bloodstream and exclusively penetrate the inflammatory/infectious site during inflammation/infection(McGovern et al., 2014).

CD1c+ and CD141+ DCs are seen in lymphoid tissues, and specialized resident DC subsets can be found in several secondary lymphoid tissues.CD103+ DCs, been detected in mesenteric lymph nodes (MLNs), for example. Where they play an important function in development of tolerance to commensal microorganisms and antigens in the diet. Furthermore, these cells may resemble CD103+ DCs found in mouse MLNs, which have a higher vitamin A metabolizing

capability to make retinoic acid that stimulates the development of gut-homing regulatory T cells(Agace & Persson, 2012).

3.1. Immunity Induction

The ability of strategically located DCs in lymph nodes to activate the responses of T cell substantially early and independently of migratory DCs has recently been proven in mice. These DCs are found in the endothelium of the lymphatic sinus, in there they can "scan" lymph for soluble antigens of lymph-borne and promptly elicit responses to immune system. Adjuvant platforms of certain antigen could design to gain access to subsets of these DC. T cells identify antigens attached on the surface of DCs to MHC molecules through their T cell receptors. CD8+ T lymphocytes recognizes peptides linked to MHC class I molecules are recognized and CD4+ T lymphocytes recognizes peptides linked to MHC class II molecules. T cell activation is influenced by the duration and intensity of DC-T cell contacts, which are mediated by the immunological synapse (IS). IS is formed when the cytoskeleton of T cell reorganizes, resulting in TCRs and signalling molecules are dynamically clustered into supramolecular activation clusters that gives an optimal environment for signalling molecules downstream of the TCR. During DC maturation, overexpression of costimulatory and MHC molecules is crucial for establishing long-lasting and stable connections with T cells via the IS. T cell growth and can differentiate into memory and effector T cells require this protein(Yamazaki et al., 2010).

DCs can activate memory and naive B cells also, mostly through stimulating CD4+ T cells that increase proliferation of B-cell and production of antibody. The synthesis of substances which activate and increase proliferation of B-cell influences antibody class switching. Follicular DCs that are found in the lymph nodes germinal centers, help to maintain memory of B-cell by forming numerous complexes of antigen-antibody and stimulating B cells on a regular basis. IL-12, IL-15, and type I IFNs are also used via DCs to activate NK cells. The cytolytic activity of NK cells is increased by IL-12 generated via DCs. Interactions with NK cells, on the other hand, can promote DC maturation. NK cells and CD8+ T cells have the ability to recruit and trigger XCR1-expressing DCs which have specific responses by producing XCL1 and XCL2(Fox et al., 2015).

Finally, NKT cells are activated via DCs expressing molecules of invariant CD1 and presenting molecules of glycolipid. As a result, in responses of both innate and adaptive immune system

played crucial role by DC's by mobilizing the immune systems several arms.(Roberts et al., 2016).

3.2. Current Approaches to DC Vaccination

There are several types of DC vaccines which have different approachable methods. DC vaccines are applied in different ways which depend on the disease and the patient. Differentiation from monocyte progenitors or CD34+ hematopoietic precursors, in vivo expansion of circulating DCs, most recently, separation and DC subsets enrichment in circulating blood are all current strategies for generating DCs employed in clinical trials. Despite the fact that no clinical trials have directly compared all of these techniques of DC synthesis, assessments of the DCs transcriptional profiles created ex vivo utilizing diverse processes have revealed basic dissimilarity from in vivo subsets of DC. DCs generated from monocytes are related to macrophages than CD34+-derived DCs. In addition, Ex vivogenerated DCs produce immune system-related transcripts that differ from those expressed by in vivo subsets of DC, signalling differences on functions. The different procedures of DC differentiation and advantages of them are given below:

3.2.1. Monocyte-derived DCs: The most frequent method for distinguishing DCs from monocytes in PBMCs acquired from blood or leukapheresis is DC and monocyte differentiation in PBMCs derived from blood or leukapheresis. Monocyte-derived DCs are DCs that are derived from monocytes (MDDCs). CD14+ monocytes are isolated from PBMCs utilizing positive selection with immunomagnetic beads or plastic adherence, and then IL-4 and GM-CSF were added to the culture for several days to develop into immature CD14CD83 DCs. To mature DCs, immature DCs are stimulated by being exposed to a stimulus of maturation and then antigen of tumors is loaded for another 1-2 days. After that, DCs that have been differentiated, developed, and loaded with antigen are extracted and cryopreserved in aliquots, which are subsequently thawed before each vaccine. This method is take less time and expensive to implement, but in many treatment clinics it is used. Some have turned the method into a programmed closed culture system. Despite the fact that autologous DC immunization is the recommended method, allogeneic DCs have been studied and shown to be immunogenic in the treatment of cancer of renal cell. This method was also explored in acute myeloid leukemia patients. CD11c+ DCs produced from umbilical cord blood can also be a source of allogeneic DCs. May be allogeneic DCs a promising source since they can be made from a healthy donor who is unrelated whose immune system has not been affected in the same way

that cancer patients' immune systems have been impaired. Finally, while DC manufacture would still be costly and time-consuming, allogeneic approach would allow for a more practical and appealing off-the-shelf treatment for pharmaceutical companies(Fabre, 2001).

Because (a) HLA-mismatched DCs can generate significant immune response and (b) using other DC donors can nevertheless boost the response of immune system to the interested antigen, Allogeneic DCs that are partially HLA-matched could be a good source for immunization. However, because to antigen competition, it's probable that this strategy won't work for a long time.(Wells et al., 2007).

3.2.2.CD34^{+} precursors Prior to leukapheresis, granulocyte-colony stimulating factor can be used to mobilize CD34+ precursors from the bone marrow. The cells are then grown in vitro in the presence of GM-CSF, TNF, Flt3L, TGF, and SCF for up to 12 days, yielding a mixture of MDDCs which look like epidermal LCs and a substantial percentage of myeloid cells at various phases of differentiation. Mature LCs generate responses of CD8+ T cell responses more effective than MDDCs and dermal-interstitial DCs, a process that requires IL15 generated by the LCs. MDDCs were less effective than LCs at stimulating responses of T cell which are specific to antigen in a clinical experiment comparing LCs loaded with peptide and MDDCs for melanoma, while MDDCs treated with IL-15 stimulated considerably more antigen which are specific to effector memory T cells. Although retroviral transduction of LCs, that allows for persistent antigen production, may improve their effectiveness in vivo, this strategy has not been well received. CD34+ cells grown alongside in the presence of Flt3L, SCF, and GM-CSF MS5 stromal cells have recently been demonstrated to differentiate into all 3 major subsets of DC. The development of three subsets from their progenitors has been clearly delineated using this innovative method. The researchers discovered that differentiation of granulocyte-monocyte-DC progenitors into monocyte-DC progenitors, monocytes and common progenitors of DC are then produced. The three DC subsets emerge from these shared DC progenitors. Furthermore, the researchers discovered a DC precursor population in the bloodstream and demonstrated that in response to Flt3L treatment, these cells develop from typical DC progenitor cells. Researchers have been able to obtain more of each DC subset by adapting this procedure; for example, results of CD141+ DCs and pDCs can be boosted by 9 and 1.5 times, respectively. In this culture system, the CD1c+ DCs are poorly defined, and they may be similar to MDDCs than the CD1c+ DCs more in the blood. Importantly, for good manufacturing practice (GMP) production to be adjusted these studies have allowed the methodology, permitting clinical comparisons of the three DC subsets for their relative

immunogenicity. This method can also be used to genetically manipulate subsets of DC in order to increase their capability of presenting antigen.(Wells et al., 2007).

3.2.3. Blood DCs Provenge (Sipuleucel-T), A CD54- peripheral blood enriched vaccine is FDA-approved first therapy based on cell for hormone-refractory prostate cancer. Provenge is a combination of DCs, monocytes, B cells, and NK cells which have been grown using a recombinant fusion protein expressing prostatic acid phosphatase (PAP) in vivo and before GM-CSF injected intravenously back into patients of leukapheresis collection within 48 hours. Three freshly processed products are given to patients; products two and three contain primed T cells as well as activated APCs that include DCs. In phase I and II clinical studies Provenge has shown to be safe, with patients developing immunological responses to fusion protein. Natural protein reactivity develops as well, but it is weaker. The CD54 expression increase after fusion protein culture was linked to activation of APC and was employed as a marker of surrogate for therapeutic potency. At 36 months, the phase III IMPACT trial demonstrated a median overall survival improvement of 4.1 months, with 31.7 percent of treated patients surviving compared to 23.0 percent of placebo patients(Chen et al., 2004).

Clinical trials using Flt3L in conjunction with fusion protein DEC205/NY-ESO-1 and polyICLC are presently underway in melanoma, with preliminary yields supporting the immunogenicity and the combined product safety. B-cell lymphoma in low grade, the treatment is also given intratumorally in combination of poly-ICLC and radiation, with a rate of response more than 30%. Flt3L is an intratumoral protein that not only mobilizes DCs into the TME, but it also mobilizes DCs throughout the body. Flt3L may significantly boost T cell priming antitumor by raising numbers of DC and tumor's location while also delivering a maturation stimulus. In these situations, the role of individual DC subsets in receiving and presenting antigens generated from tumors is unknown. Nonetheless, such research allows for the investigation individual cell level of DC's by assessing their phenotypic and transcriptome using tumor biopsies acquired purposefully. Acute myeloid leukemia patients and colorectal cancer have received Flt3L alone, and melanoma and renal cell cancer patients have received Flt3L in conjunction with HLA-A2-restricted TAAs emulsified in Montanide ISA-51 adjuvant(Roberts et al., 2016).

3.3. Maturation process of DCs

Mature DCs have increased expression of molecules of costimulatory, generate the cytokines and chemokines required for effective T cell activation, and can move to lymphoid organs. Immature DCs, on the other hand, do not elicit responses specific to antigen and can even

trigger the development of regulatory T cells. Only mature, peptide-loaded DCs were found to be capable of inducing responses of T cell which are specific to antigen in healthy persons and metastatic melanoma patients in clinical trials.

Maturation stimuli Different methods for maturing DCs exist. TLR agonists including poly IC (TLR3), LPS (TLR4), and resiquimod (TLR7) are widely employed in the laboratory to activate DCs. The gold standard for maturation in the clinic was previously established as a combination of proinflammatory cytokines TNF, IL-1, and IL-6 mixed with prostaglandin E2 (PGE2). Molecules of MHC class I and II, CD40, CD80, CD86, and CCR7 were all upregulated by this cocktail, while IL-12p70 was not effectively stimulated. When other DC maturation stimuli compared, in terms of activation of markers of DC maturation, cytokine cocktail caused uniform maturation, with highest production and recovery; it stimulated proliferation of allogeneic T cell also and production of cytokine the most, as well as priming the responses of Th1. Other research has found that PGE2 can cause regulatory T cell differentiation and Th2 responses, IDO expression and the lack of IL-12p70 production as well. PGE2, on the other hand, has been found to play essential roles in increasing DC migration into lymphoid tissues by upregulating CCR7 on DCs, as well as in enhancing proliferation of T cell by inducing OX40L, CD70, and 4-1BBL on DCs.(Krause et al., 2009).

Alternative DC maturation strategies have a been investigated also. CD40 ligand (CD40L) is largely expressed via activated T and B cells, and on DC's it interacts to the CD40 receptor. Upregulation molecules of costimulatory and release of cytokines like IL-12 occurs when CD40L interacts with CD40 on DCs. To develop DCs for immunization in melanoma patients, researchers used an irradiated CD40L-expressing K562 cell line in combination with IFN. The amount of IL-12p70 generated through the DC vaccination was positively linked with activation of CD8+ T cell which responses to HLA-A2-restricted gp100 in binding experiments of tetramer. To activate DCs, TLR agonists have been employed also. Activation of TLR on DCs can result in DC maturation, costimulatory molecule upregulation, and cytokine and chemokine secretion. Furthermore, stimulating several TLRs on DCs at the same time has synergistic effects, culminating in IL-12 "superinduction." As a result, TLR agonists have the ability to induce optimum DCs in order to promote responses of immune system effectively while also to favor immune response development by training the environment in vivo. To mature MDDCs, clinical trials have used poly-ICLC which is the agonist of TLR3 in combination with TNF, IL-1, IFN, and IFN. -type-1 polarized DCs (DC1) are DCs that have been developed with this mixture. They produce a lot of IL-12p70, move in response to ligand

CCR7, and activate cytotoxic T lymphocytes to attack tumor-associated antigens (TAA). DC1 treatment was proven to be safe and immunogenic in high-grade glioma patients, with 9/22 patients experiencing free progression status for 12 months at least. In ovarian cancer patients, a clinical grade TLR4 agonist, LPS, has employed for vaccination for maturation of DCs. In patients with melanoma, LPS-matured DCs generated IL-12p70 and triggered responses of Tcell specific to tumors. 3-O-deacylated mono phosphoryl lipid A (MPLA), less toxic variant of LPS and modified has been given permission for use in humans and is utilized in conjunction of Cervarix. MPLA develops DCs and has been shown to generate significant levels of IL-12 and in vitro CD8+ T cell responses stimulated when combined with IFN. In mouse research, glucopyranosyl lipid A, a novel synthetic TLR4 agonist, was utilized in combination of antiDEC205 to target HIV gag p24 to DCs. In vivo, it upregulates CD40 and CD86 and causes DCs to produce IL-12p70. It's presently being utilized in human research to modulate the TME in follicular non-lymphoma, Hodgkin's both with and without anti-PD-1(Andrei et al., 2015).

Alternatively, to develop DCs in vitro, a cocktail of routinely used prophylactic vaccinations, including BCG (Bacille Calmette-Guerin)-SSI, Influvac, and Typhim, which all contain TLR agonists, was utilized. The DCs that were developed with this cocktail had strong CD86, CD83 and CD80 expression, were capable to move, and generated IL-12p70. This combination of prophylactic vaccinations was evaluated on mature DCs in melanoma patients in a clinical experiment. The DC vaccination elicited responses of T cell which overall associated with longer survival; however, a number of patients experienced local and systemic adverse effects of grade 2 and 3, which were linked to BCG which is present in maturation of cocktail, preventing any further utilization of cocktail(Roberts et al., 2016).

The timing of the maturation process is another potential element which can alter the quality of Dc vaccines. In clinical trials, DC vaccines used are matured for 24-48 hours normally, at which time cytokine tests are done. The majority of cytokines produced following maturation of DC are created within 24 hours, and only they regain their ability to generate cytokines after T cell contact and ligation of CD40L. As a result, maturation regimens of less than 24 hours may be preferable so that DC vaccines keep their potential to generate cytokines after injection in vivo.

As a result, the one of the best maturation strategy is that produces the most potential APCs in order to produce most effective in vivo immune response.(Roberts et al., 2016).

3.4. Migration and Management of DCs

In order to trigger immunological responses, lymphocytes must migrate to lymph nodes. Vaccines of DC have given subcutaneously, intra-dermally, intranodally, intra-venously and intra-tumorally, but the best way to provide them is still unknown. A vast amount of the 111indium-labeled DC vaccination melanoma peptides are loaded that was delivered intradermally stayed at the site of injection, lost viability, and was removed within 48 hours, with just 5% lymph nodes that reach the draining. DC vaccines administered intratumorally retained at the site of injection with little identified lymph nodes in the draining, pointing that vaccinations failed to reach their targets(Lesterhuis et al., 2011).

Delivery of DC vaccines by multiple routes, such as intravenously and intradermally to generate a systemic response, and administer directly into lymph nodes, are newer techniques. Within 30 minutes of injection, DC vaccine which are administered intranodally, melanoma peptides are loadeded and distributed again to multiple lymph nodes; however, despite the fact that DCs were delivered directly to lymph nodes, responses of immunologic generated were better or comparable than those elicited by DC vaccinations given intradermally. This result was most likely influenced by a number of things. The technical difficulties of correctly injecting a vaccine into lymph nodes is one potential; incorrect administration could damage lymph node anatomy. Another theory is injection of intranodal sends all vaccinations to lymph nodes, whereas only injection of intradermal provides viable, mature, and fully functional DCs to elicit responses of T cell. The number of DCs doesn't matter to make it to the lymph nodes; one study found that lowering the number of injected cells boosted DC migration. It has been discovered that the injection site is pre-conditioned can help improve DC vaccine movement. Preconditioning with the activated DCs or cytokine TNF, which was first investigated in mice by Martin-Fontecha et al., enhanced homing lymph node and DC vaccination effectiveness. Topical administration of the TLR7 agonist imiquimod that attracts T cells and DCs to the dermis, also increased migration of vaccine of DC. Mitchell et al. Recently reported, preconditioning location of vaccine of DC injection with tetanus/diphtheria toxoid vaccination improved migration of DC in patients with glioblastoma multiforme through elevating CCL3 levels, which resulted in CCL21 overexpression and boosted DC vaccine lymph node migration. Increased DC vaccination migration was linked to a higher survival rate overall.

However, vaccine immunogenicity of DC may be due in part to antigen of virus targeted via the vaccine. As a result, inducing response of local inflammatory at the site of injection of the DC vaccination could help migration of DC. To better understand how these tactics could be included into DC vaccines to improve DC arrival in lymph nodes, more research is needed.

Other unknowns include whether directly these DCs induce responses of T cell, as proposed in animal research, or whether a component of maturing DCs is then cross-presented through resident DCs via uptake of trogocytosis, dying cells, or a process known as "cross-dressing." It is known that from tumor cells CD103+ DCs receive antigen and move to lymph nodes which are draining to activate CD8+ T lymphocytes in animal tumor models. The advantage of injecting DCs intravenously is that the injected cells can quickly reach secondary lymphoid tissue. In melanoma mouse trials, mature DCs injected intra-tumorally can alter the TME, turning an immune suppressive TME into one which promotes T cell recruitment and, eventually, control tumor. In this scenario, a lack of CCL4 caused by Wnt signaling hindered DC recruitment to the tumor bed that could overcome through increasing DC populations within the tumor. In such settings, administering Flt3L along with maturation cues may be able to overcome these hurdles in vivo(Salmon et al., 2016).

3.5. Most recent DCs

Human pluripotent stem cells, including induced pluripotent stem cells and embryonic stem cells, are differentiated into DC's, is another strategy being investigated. Currently, there are two ways to distinguish DCs from hPSCs: one uses embryoid bodies, an aggregate structure that mimics development embryo, and other relies on co-culture with stromal cell lines. Both entail a multi-step process that uses multiple growth factors to induce differentiation, such as BMP-4, VEGF, GM-CSF, SCF, Flt3L, and IL-4, at crucial intervals. Most importantly, unlike current approaches that are largely dependent on operational quality requirements, this unique source of DCs offers the essential for huge-scale production employing bioreactors. Recently, hPSCs were transduced with a lentiviral vector to express the tumor antigen MART-1. The transduced hPSCs developed into DCs which expressed normal markers of DC after being stimulated with TNF. Additionally, the DCs also were capable to prime $CD8^+$ T cell response which are specific to MART.(Zeng et al., 2015).

As a result, DC immunization could be improved using this this novel method by providing an endless supply of DCs, as well as the ability to directly transduce the antigen, assure MHC class I presentation, and trigger responses of CD8+ T cell. DC subsets isolation are radically immunogenic more, such as the CD141+ DC subset, will be made possible by the creation of

DC's generated stem cells for clinical use(Zeng et al., 2015). The clustered regulatory interspaced short palindromic repeat-associated 9 system is a potent and adaptable genetic engineering tool which allows for supreme genome editing control. In a nutshell, the guide

RNA attaches to the sequence which is targeted, and Cas9, an endonuclease, then targets the DNA specific sequence which matches the RNA guide. This technology can be utilized to regulate DCs to prevent molecules of inhibitory and cytokines from being expressed, hence improving their benefits in vivo, or to induce CD8+ T cell development preferentially. This method has been successfully used in the lab to remove target genes in DCs(White & Khalili, 2016).

3.6 Cancer related Immunity

Understanding the processes through which tumor cells evade immunity, as well as the interactions that occur between the immune system and cancer that include both eradication and detection of altered cells of cancer, is critical. Amazingly, the immune system may aid progression of tumor through favoring the survival of the fittest cells of cancer in an immunocompetent host or by modifying the microenvironment tumor to promote tumor growth. This is known as cancer immunoediting, in which immune system may simultaneously stimulate and repress tumor growth(Schreiber et al., 2011).

Immunoediting's anticancer effect can be summarized in three phases: elimination, equilibrium, and evasion, based on evidence from preclinical and clinical investigations. When intrinsic tumor suppressor pathways fail, this cancer immunoediting suppressive mechanism is efficient. The immune system defeats tumor cells in the elimination stage, which occurs before clinical identification. This task requires the cooperation of lot of immune cells and substances from both the innate and adaptive immune systems. The cascade is halted and the host is protected if it is successful. If some cancer cells manage to evade identification, the 2ndphase of equilibrium kicks in to avoid development of inflammatory environment which promotes growth of tumor. This is accomplished by using adaptive immunity, lymphocytic T scellspecifically, interferon- (IFN-) and interleukin-12 (IL-12), to keep cancer cells inactive. It's worth emphasizing that this phase includes tumor immunogenicity editing, which could spell the end for cancer cells(Yang, 2015).

Nonetheless, some genetically unstable tumor cells may emerge as a result of ongoing immune pressure on tumor cells selection, lead to 3rd escape phase. Cancer cells fall into one of 3 groups during this stage. The first is tumor cells that are unidentified via the adaptive immune system due to loss of antigen. The 2nd type of tumor cell is one which is resistant to the removal methods. Third, tumor cells that are surrounded by an immune-suppressive microenvironment.

The immune system could no longer control tumor cell development at this phase, resulting in a clinically identifiable tumor(Saadeldin et al., 2021).

Inflammation is a key factor in the progression of cancer. Chronic inflammation related to tumor influences both systemic and local immunological responses, promoting the formation of an immune-suppressive milieu and tumor growth. Acute inflammation, alternatively increases the activities of both effector T cells and dendritic cells, promoting antitumor activity.

A type of innate immune cell is plasmacytoid dendritic cells (pDCs) which helps the body fight cancers and viruses. In the case of cancer, pDCs play a role in both responses of pro- and antitumor. pDCs have a role in cancer immunosuppression by secreting inducible costimulatory ligand (ICOS-L), which activates CD4+ T cells that are pro-tumoral cells and increases infiltrating pDCs of intra-tumoral in many forms of cancer. While pDCs primarily fight cancer by stimulating the immune system, their antitumor functions include transporting tumor-associated antigens (TAAs) to lymph nodes, cross-priming CD8+ T cells, the tumor microenvironment controlling, and infiltration of intratumor and cytokines secretion which modulate immunosuppression associated to tumor. (Perez & De Palma, 2019).

Chapter 4

DCs in cancer therapy as vaccines

The field of cancer therapeutic vaccination is still undergoing clinical trials and research. All vaccines rely on DCs to behave as cells which presents antigens for T cells and are classed as either nontargeted, ex vivo loaded or, in vivo targeted based on their strategy. GVAX, which uses irradiation tumor cells engineered to express GM-CSF to recruit and mature DCs at immunization site to improve antigen uptake and delivery, was the first cancer vaccines among other cancer vaccines to make substantial progress in the clinic(Palucka & Banchereau, 2013).

The inclusion of stimulating chemicals, such to activate STING as cyclic dinucleotides, or such as oncolytic viruses, other vehicles for GM-CSF delivery, has been built on this notion. Recombinant proteins/peptides are combined with adjuvants which contains many formulations of TLR agonists in the more traditional and thoroughly established technique. These can be administered alone or in combination with antibodies which specifically target DCs in vivo, such as Clec9a, DEC205, or DC-SIGN. Peptide vaccines multiple trials are currently underway, despite the fact that the in vivo targeting strategy has not showed significant clinical progress(Palucka & Banchereau, 2013).

Ex vivo use of DC generated from allogeneic monocytes (moDCs) peptide-rich showed efficacy in humans, a technique initially approved with Sipuleucel-T for prostate cancer that has spread is resistant to castration. In this example, cells of mononuclear from the peripheral blood are separated and a fusion protein was pulsed containing prostatic acid phosphatase of human and GM-CSF. CD40 ligand, IFN-, and/or TLR agonists can also be used to develop GM-CSF-induced moDCs ex vivo(Carreno et al., 2013). However, because moDCs have little ability move to lymph nodes or to cross-present antigen, it is unclear to what extent cells of these are operating as cells that present antigen or vehicles to deliver antigen, with some studies showing which DCs of endogenous are essential for priming of T cell (Petersen et al., 2011). These cancer vaccines are single-agent vaccinations which are generally tolerated well and induce a response of systemic immune system against the antigen of tumor, but in clinical trials of late-stage, they have yet to show significant potency. A microenvironment of suppressive tumor, which can impede infiltration of T cell and effector activity, is one probable explanation for this failure. Vaccines against programmed death-1 in combination with antagonist antibodies are currently being tested to see if this route has been a substantial impediment. The

antigens which are targeted haven't been optimized because they've primarily been limited to non-tumor-specific antigens that are overexpressed or aberrantly expressed which is another option. The use of next-generation vaccines incorporating neoantigens that are specific to patients will aid in determining whether this has been a major impediment to effectiveness. Alternatively, it's possible that the lack of efficacy was owing to insufficient or incorrect immunological activation. In support of this, vaccination of DC made with tetanus toxoid has been shown to be effective in patients and mice both with glioblastoma(Mitchell et al., 2015), and a Listeria mesothelin-expressing attenuated strain increased pancreatic cancer patients survival overall when used to boost an injection of priming GVAX (Le et al., 2015). Approaches that cause pDCs to release IFN- may be an alternative to using complex vaccination formulations to exploit anti-pathogen responses(Gardner & Ruffell, 2016).

4.1 Activation of In vivo

Many same stimulatory pathways that have been employed to generate vaccines could also be exploited to boost activity of endogenous DC within tumors. This method offers the ability to target a wider range of antigens, allow for neoantigen targeting without the need for vaccine specific to patient production, and reduce difficulties connected with live cell techniques. Injection of TLR for intratumoral or agonists of STING has been found to inhibit the growth of tumor in mice through increasing the response of CD8+ T cell(Ohkuri et al., 2014). These agonists may stimulate lymph node movement, which might hypothetically increase antigen delivery, in addition to the expected rise in cDC maturation. Monotherapy with these agonists, like immunization, is unlikely to show significant therapeutic efficacy. Instead, it may be important to target numerous pathways in order to reverse immunological suppression while increasing cDC activation. One of the first examples was employing CpG and an anti-IL-10 receptor antibody to treat tumor-bearing animals, when cytotoxic therapy is used as agonist of surrogate immune system, the results could be comparable. (Vicari et al., 2002).

In addition, systemic injection of tyrosine kinase 3 ligand related to FMS causes a 4-fold increase in B16 melanomas in CD103+ cDCs, overcoming minimal infiltration of tumor and delaying tumor progression to the same extent as Polycystic kidney disease (I:C). necessarily, combining of Flt-3L and Poly(I:C) results in good control of tumor, which is boosted by inhibition of checkpoint or stimulation of immune system. Determining additional pathways which may altered to promote activation, infiltration, or effector function of cDC should help

improve the efficacy of any anti-tumor immune therapy modalities(Sánchez-Paulete et al., 2016).

4.2. Factors suppressing tumor DCs

Immunogenic tumors differ from non-immunogenic cancers in several ways. The importance of immunoediting based and link between immune checkpoint blockade and mutational burden response, neoantigens frequency appears to be crucial determinant(Dupage et al., 2012). A second component, as previously mentioned, could be the degree of maturation of DC as a result of the type and intensity of death of cell within malignancies. A 3rd element is most likely the tumor's amount of systemic and local suppression of immune system. Although direct inhibition of effector of T cells is widely understood, transition from immunogenic to immunosuppressive states which occurs during tumor growth is linked to a phenotypic shift in DCs also.(Scarlett et al., 2012).

The mix of stimulatory and repressive signals within the microenvironment of tumor is likely to be crucial in dictating cDCs ability to generate and maintain a response of T cell, and understanding this relationship will be crucial in therapies development to boost immunity of T cell. DC activation is inhibited in vitro by a number of substances identified in the microenvironment of tumor. Vessel endothelial growth factor (VEGF), and interleukin-10 (IL10) and prostaglandin E2 (PGE2) are examples. VEGF, IL-10, IL-6, and colony-stimulating factor 1 (CSF-1) have also been demonstrated to prevent progenitors of bone marrow or monocytes from maturing into DCs, instead pushing monocytes to a suppressive phenotype(Zong et al., 2016). Because within tumors, monocytes are not a source of cDCs, the importance of these inhibitory mechanisms in vivo is unknown. However, after blocking VEGF receptor 2, larger numbers of DCs of CD11c+CD83+ were observed in mouse tumors, and in tumors we found CD103+ cDC1 and CD11b+ cDC2 in higher percentage following neutralization of CSF-1 or IL-10 receptor blockade during paclitaxel chemotherapy. TLR2mediated activation of the receptor of IL-10 by tumor macrophages also decreases IL-12 expression through CD103+ cDC1 tumor that may be sensitized to respond(Tang et al., 2015). The metabolic inefficiency within tumors is another mechanism that is likely to decrease DC function. Lactic acid and hypoxia, for example, in tumors, control function of macrophage and decrease activation of DC in vitro(Gottfried et al., 2006).

As was previously revealed for activation of the ER stress response protein XBP1 and resultant buildup of lipids of intracellular, metabolic reprograming inside DCs is critical during

activation of TLR-mediated and dysfunction could mediate within malignancies. The tumor microenvironment is likely to influence cDC migration to the lymph node, but this has yet to be determined. Finally, in mouse and human malignancies, BDCA3+ cDC1 and CD103+ constitute least common myeloid populations. Due to a shortage of cell numbers, the functional importance of these cells may be limited. Either injection of cDCs for intratumoral or systemic expansion in melanoma has been shown to improve responsiveness to checkpoint blockade(Salmon et al., 2016). It will be critical to begin validating some of the putative suppressive mechanisms in vivo, in a therapeutic context examining their efficacy to modulate anti-tumor immunity as well, now that tumor cDCs can be distinguished from macrophages(Gardner & Ruffell, 2016).

Chapter 5

DC vaccines ongoing clinical and pre-clinical trials

In this chapter, the clinical trials based on DC vaccines and also some insights from the trials will be provided. Despite no association with outcome, BC-infiltrating DCs were found in >40 percent of patients with advanced and early BC in early and advanced investigations(Gelao et al., 2014).

In BC, DCs appear to be capable of providing a memory response to tumor antigens as well as inhibiting tumor growth. Gong et al. found that fusing BC cells with DCs resulted in autologous CTLs capable of killing cells of cancer(Gelao et al., 2014b). In addition, DCs containing allogeneic cells of BC induced tumor-reactive CTLs, which resulted in the death of target cells. HER2-positive BC mice were inoculated with DCs expressing the receptor of DEC205 to increase the immunogenicity of human EGFR2 (HER2), and despite the modest quantity of HER2 protein, B-cell and T-cell immunity were detected in high levels. Using an Exosomes produced by DCs that are specific to OVA (EXOOVA)-targeted CD4+ (OVA-TEXO) vaccine based on T cell against neu-expressing Tg1-1 BC in transgenic FVBneuN mice, some researchers have investigated the ability to overcome trastuzumab resistance(an antibody to HER2), resulting in the protective immunity development(Wang et al., 2013).

HER2-adenovirus-transduced DCs were used to test the usage of genetically engineered DCs, which reduced BC development in HER2-transgenic mice. In mice, efficacy of a whole-cell BC vaccination was assessed using an immunocytokine made comprised of IL-2 and directed an antibody against the factor phosphatidylserine which suppress the immune system. A total of 80% of mice were free of tumor, and their splenocytes had much higher specific cytotoxicity than control mice's splenocytes(Huang et al., 2011).

The role of immuno-modulatory factors in the development of an appropriate response of immune system is demonstrated in this study. Combination treatment was also tested in a preclinical setting. Utilizing adriamycin-induced apoptotic MCF-7 cells,(Zheng et al.)created new in vitro based on DC vaccination against BC(Zheng et al., 2012).

The human BC cell line MCF-7 was cocultured with iDCs generated from healthy donors after a 24-hour adriamycin treatment. The MCF-7 BC cell line’s immunogenicity is enhanced by adriamycin treatment, which results in the stimulation of maturation of iDC and activation of T-lymphocyte in vitro(Gelao *et al.*, 2014).

A list of currently ongoing trials is given below:

Table-3: List of DC vaccine based pre-clinical studies (Brossart et al., 2000)**.**

NCT number	Study Phase	Type of therapy (intervention)	Status
NCT01730118	I	Autologous adrenovirus HER2-transduced DC vaccine	Ongoing
NCT0088985	II	Trastuzumab and vinorelbine were given to autologous DCs that had been pulsed with E75 and E90 peptides.	Completed
NCT01042535	I/II	Adrenovirus p53transduced DCs with 1-methyl-Dtryptophan	Ongoing
NCT00266110	II	Trastuzumab and vinorelbine were given to autologous DCs that had been pulsed with E75 and E90 peptides.	Ongoing
NCT00978913	I	DC/s transfected with surviving, hTERT and p53	Ongoing
		mRNA with cyclophsphamide	

NCT00622401	I/II	DS/s tumor cell fusion vaccine ± IL-12	Ongoing
NCT00715832	I	DCs that have been loaded with oncofetal antigen and iLRP	Ongoing
NCT01522820	I	DCs/NY-ESO-1 fusion protein vaccine ± sirolimus	Ongoing
NCT00923143	I/II	HER-2/Neupulsed DC vaccine	Ongoing
NCT00197522	I	DCs infected with an adenovirus expressing Her-2	Completed
NCT00082641	I/II	Adenovirus p53-infected DC vaccine ± chemotherapy ± RT	Ongoing
NCT00128622	I	CEA-6D-expressing Fowlpox-Trico infected autologous DCs	Completed
NCT00004604	I	CEA RNA-pulsed vaccine of DC	Completed

These findings support the need to assess the function based on DC vaccinations in BC. The trials' goal is to show that this sort of immunotherapy is safe and effective in different subtypes and situations of BC patients. In 10 metastatic BC patients and severely pretreated advanced

ovarian cancer, Brossart et al. investigated the efficacy and feasibility of a vaccination method using MUC peptide-pulsed DCs or HLA-A2-restricted HER2 (Brossart et al., 2000).

All patients had positive immunologic responses and no side effects were present there, and, including those who had been highly pretreated, suggesting that peptide-pulsed DC vaccines could be utilized to remove residual illness after intense or even high-dose chemotherapy. Because vaccines of DC are limited by small number of antigens of tumor identified and low immunogenicity of them, one technique involves fusing autologous tumor cells with DCs. Metastatic breast and kidney cancer patients who were vaccinated with fusion cells made from tumor cells that are derived from patient and autologous DCs displayed immunological and clinical antitumor responses with low damage, according to Avigan et al(Avigan et al., 2004).

Patients with ER/PR-negative breast cancer had similar outcomes. As a result of immunological activation, approximately 58 percent of patients developed a delayed type IV hypersensitivity reaction, implying that tumor lyzate-pulsed DCs provide a diverse supply of antigens of BC which are active in eliciting anti-BC immune responses. The inclusion of cytokine adjuvants, such as IL-2 or IL-12, could improve the DCs vaccine's efficiency. In a Phase I/II clinical trial, six metastatic renal and four BC patients received a DC vaccination and IL-2(Baek et al., 2011). Patients were given mature DCs that had been pulsed with autologous tumor lyzate and low-dose IL-2 two times. Despite the fact that just one kidney cancer patient reached stable illness, the vaccine was well tolerated and generated specific immunity in all patients. In addition, a Phase I/II experiment is looking into the safety of DC/tumor cell fusion in combination with IL-12 to examine the work in treating women with stage IV BC. More research is being done to see if DC immunization can combine synergistically with other medical treatments like chemotherapeutic drugs (e.g., cyclophosphamide or vinorelbine) or targeted therapy(Gelao et al., 2014).

Targeting the adaptive and innate immune systems is another promising strategy for improving the outcomes of BC patients. Autologous cytokine-induced killer cells (CIKs) that have shown high cytotoxic effect in clinical investigations, could be one method. In 87 patients who had high-dose chemotherapy with docetaxel plus thiotepa, some researchers looked at the combination of DCs and CIKs. In comparison to 79 patients who received free progression survival, standard-dose chemotherapy and overall survival were improved in the high-dose chemotherapy group, demonstrating that the combination of high-dose chemotherapy with

DCs/CIKs can be an effective treatment option for selected patients with metastatic BC(Ren et al., 2013).

Chapter 6 Limitations of DC vaccines

Immunotherapy using DCs is a promising therapeutic method for the treating BC. Despite the fact that these cells appear to be successful in eliciting detectable tumor antigen specific for immunity and that DCs vaccinations are tolerated well and safe, therapeutic benefit remains unsatisfactory. The disappointing outcome of this therapeutic method, as well as the difficulties in generating DC-based medicines that are effective in treating BC, could be due to a number of factors(Gelao et al., 2014a).

6.1. Immunological and engineering issues

Technical difficulties during the DC generation technique, combined with poor antigen presentation due to the use of faulty DCs or inadequate TAAs, may have led to therapy's failure. Most frequent method for collecting DCs is to employ whole blood or leukapheresis to extract peripheral blood mononuclear cells. Other cells may contaminate the collection because this procedure does not permit for the selective harvesting of monocytes. Cells must also undergo further treatments after leukapheresis in order to be isolated, selected, and differentiated into DCs. All of these steps, while required, have the potential to have important impact on the amount and quality of DCs collected. Furthermore, it is still unknown that signals and combinations of stimuli for manipulation of ex vivo cause DCs to mature and become immunogenic. DC maturation is a key process, and if DCs are not sufficiently activated, they may remain immature, suppressing rather than triggering an immune response. As a result, while ex vivo modification of DCs is valid technique to use these cells in cancer immunotherapy, numerous obstacles must still be overcome in order to obtain appropriate cells to build a sufficient immune response. To improve the binding of tumor antigens to molecules of MHC or TLRs, it may be necessary to transfer genes producing cytokines or molecules of costimulatory into DCs. Furthermore, because numerous tumor-derived factors (e.g., VEGFR1 or PD-L1) can inhibit DC development and maturation, the combination of DCs with other molecules capable of boosting efficacy of antitumor could improve vaccine therapeutic effects. For example, using vaccination based on DC in combination with an anti-VEGFR antibodies or PD-L1 inhibitor, or with other drugs targeting molecules that suppress immune system (e.g.,

TGF-, IL-6 or IL-10) or signaling pathways such as MAPK, STAT3 and -catenin which negatively interfere with the responses of immune system, such as preventing the growth of tumor and stimulating an effective and adequate immune response to eradicate malignant cells, could be interesting(Kawakami et al., 2013).

Antigens could be delivered directly to DCs in vivo as an alternative. Specific monoclonal antibodies for certain DC surface molecules are used in this method to induce the natural DCs activation in vivo. This system appears to be a viable option. Despite diverse attempts, further research is needed to determine which strategies allow for the best functional DCs capable of improving immune response(Hawiger et al., 2001).

Several investigations conducted in BC have demonstrated the reduced activity of these immune cells, as previously mentioned. Another issue is that just a few antigens have been found that potentially trigger an immune response that leads to cancer eradication. There have been several breast tumor antigens identified, with HER-2, carbohydrate antigens, MUC-1, CEA, p53, and cancer-testis antigens receiving the most attention as vaccine antigens. The additional information gained from the proteomic and genomic classification of BC should aid in new specific antigens of tumor identification for successful immunotherapy and clarification of unique biologic categories of BC with varied amounts and patterns of tumor antigen expression. The use of DCs offers a way to get beyond BC's relative non immunogenicity and treat the underlying immunodeficiency. Even though vaccines of DC targeting single antigens have not always resulted in a significant immune response due to mechanisms of tumor escape, the ideal particular antigen should be overexpressed on cells of tumor and in normal tissue distribution have restricted. DCs transfection with amplified RNA or DNA which derived from tumor could be a viable approach, and advancements in this technology could lead to greater results(Tendeloo et al., 2007).

As a result, the success of future DC vaccines in BC will be determined by discovery of additional immunogenic antigens, development of the most effective delivery systems of antigen, and the discovery of the entire network of immune signaling pathways that control immune responses in the tumor microenvironment. Only by doing so would it be possible to establish a personalized immunological therapy based on the unique features of each patient's immune system as well as the profile of antigenic tumor.

Chapter 7

Conclusion and future prospect

For decades, DC has been renowned as environmental sensors and a powerful Antigen Presenting Cell. Vaccines of DC have shown in over 200 clinical trials to be safe, immunogenic, and able to elicit a persistent, tumor objective and response clinically in a previously treated, late-stage patient with cancer. Dendritic cell vaccines are giving us more options to treat cancer patients effectively. We can rely on this vaccine as it has already shown better efficacy than other vaccines and the safety data are also published. Because of DC vaccination's ability to activate the immune system and its lower toxicity, it's a great option for use in tandem with other anti-cancer therapies. The outcomes of combination studies of chemotherapy and/or radiotherapy, as well as targeted therapy with DC-CIK vaccination in NSCLC, demonstrate the clinical success of combination therapies. Similarly, studies in melanoma showed that DC-based treatment plus ICIs have a synergistic impact. Further studies can be performed to understand the mechanism clearly and it will open the way for to know more about this vaccine.

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