REVIEW ARTICLE

Dendritic Cell as Potential Immunotherapy for Nasopharyngeal Cancer: A Review

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Abstract

BACKGROUND: Dendritic cell (DC)-based cancer therapy is a promising adjuvant therapy for nasopharyngeal cancer (NPC) after chemoradiation. Owing to low immunity after chemoradiation, DC therapy activates immune responses. Moreover, DC-based cancer therapy can decrease tumor progression, prolong lifespan, and increase the quality of life of patients. Various studies regarding the use of DC therapy for NPC have been reported, however there are limited reviews on the implementation and foundation of DC immunotherapy to expand this technology.

METHODS: A literature search was performed on EMBASE, ScienceDirect, PubMed (MEDLINE), and Cochrane Library, with the term dendritic cells therapy for nasopharyngeal cancer, dendritic cell immunotherapy in nasopharyngeal cancer patients, and DC therapy in NPC, as the search keywords.

RESULTS: A total of 199 literatures were reviewed, and four clinical trials were identified as relevant for this review. DC vaccines can be processed with various maturation and activation processes. Selected literatures reported antigens used when incubating the DC are latent membrane protein (LMP) 1, LMP2, and Epstein–Barr virus nuclear antigen 1 (EBNA1). Although DC therapy was produced from different pathways, it has been reported that there are increases of cluster of differentiation (CD)8+ T cells, CD4+ T cells, and the progression free survival (PFS) rate in DC immunotherapy patients than the radiochemotherapy patients.

CONCLUSION: It can be concluded that DC could be used as an adjuvant therapy alongside the standard therapy of NPC, which prolongs NPC patient survival.

KEYWORDS: adjuvant cell therapy, nasopharyngeal cancer therapy, dendritic cells

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Introduction

The current standard treatment for nasopharyngeal cancer (NPC) is radiation or chemotherapy or its combination, chemoradiotherapy. Several studies have reported that 10-20% of NPC patients who have undergone primary chemoradiotherapy suffer from cancer relapse, indicating that chemoradiotherapy alone is insufficient to kill cancer cells, especially in the case of advanced NPC. (1,2) Nowadays, dendritic cells (DC)-based therapy for cancer is a promising adjuvant cell therapy for NPC after chemoradiation. DC therapy works by activating the immune response such as T cells to recognize tumor cells and destroy the tumor cells. DC can activate and increase immune response due to their ability as antigen presenting

cells (APC) and potent activators of naive T cells become cytotoxic T cells.(3-5) In the tumor microenvironment, DC can present tumor-associated antigen (TAA) on major histocompatibility complex (MHC) class I and MHC class II molecules.(6-8)

Accordingly, if an autologous DC is isolated and trained *in vitro* to recognize TAA of NPC, then DC will be able to recognize and present TAA to naive T cells for activation upon re-injection to the patient.(9) Three proteins that maintain the infection in NPC, latent membrane protein (LMP) 1, LMP2, and Epstein–Barr virus nuclear antigen (EBNA-1), are commonly targeted for DC immunotherapy.(10) Upon DC activation, naive T cells will be differentiated into cytotoxic T cells and will have a major role in killing the NPC tumor cells. Upon tumor cell destruction, TAA will be released from the tumor debris, which the resting DC in the body will uptake, and subsequent presentation to naive T cells will occur continuously until the tumor has been managed.(9)

Immunotherapies are known to have generally low response rates, but they often prolong patient survival. DC vaccines are reported to improve overall median survival.(10) The first cell-based cancer therapy approved by the Food and Drug Administration (FDA) is the Sipuleucel-T DC vaccine (Provenge) with median survival by 4 months in patients with metastatic castration-resistant prostate cancer in 2010.(11) Another DC product approved by the Indian government agency (Central Drugs Standard Control Organization) is a DC-based vaccine (APCEDEN®) for prostate, ovarian, colorectal, and non-small cell lung carcinoma.(12) The vaccine modified with LMP2 and EBNA1 succeeded in increasing the response of cluster of differentiation (CD)8^+ and $CD4^+$ cells after injection with low toxicity in Hong Kong and UK patients.(13) As an alternative approach, DC of patients transduced with an adenovirus expressing a truncated LMP1 peptide and LMP2 protein was also used.(14) Only one of 12 patients had a detectable T cell response to LMP1, LMP2, or EBNA1 in the *ex vivo*. Therefore, it is important to produce DC with a high potential to activate cytotoxic T cells ($CD8^+$) and helper T cells ($CD4^+$) to prolong the immune system. Similar results by using genetically modified DCs to express cancer-specific antigens have been reported.(15-19) TriMixDC is one type of mRNA-engineered DC, which gained interest owing to their enhanced antitumor activity and feasibility compared to other mRNA-based vaccines.(20,21) Synthetic mRNA-based vaccines produce fewer side effects and show a higher possibility for optimization and large-scale generation than whole-tumor mRNA-based DC vaccines.(22-24) Co-electroporation of TriMixDC with mRNA encoding a fusion of melanoma antigen and DC-lysosomal associated membrane protein (DC-LAMP), an approach named TriMixDC-MEL, stimulated antigen-specific $CD8^+$ T and Th1 cells in vaccinated patients.(25) In a phase II trial, TriMixDC-MEL combined with ipilimumab, a cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibitor, showed a 20% complete response and ~18% partial response in 39 patients with advanced melanoma.(26) TriMixDC-MEL plus ipilimumab also resulted in 28% overall survival (OS) after 390 weeks of median follow-up and 18% progression-free survival (PFS) after 5 or more years in patients with stage III or IV melanoma.(27) Another research using engineered DC expressing chimeric receptors that are able to take up and process TAAs in situ, called as human epidermal growth factor receptor-2 (HER2)-specific extracellular vesicle-internalizing receptor (EVIR)-expressing DC, with a significant increase of antigen-specific cytotoxic T cells.(28)

Rather than using specific antigens or mRNA for DC-pulsed, other TAA-loading strategies include whole-tumor-cell lysate-pulsed DC (29,30), which express a broader range of tumor antigens suitable for personalized treatments, tumor cells fused with DC vaccines (31,32). In addition, the method is simpler and does not require complex instrumentation.

The various studies regarding the use of DC therapy for NPC lay the foundation to expand this technology for future use and to better understand DC therapy efficacy for NPC. DC was believed to decrease tumor progression, prolong the lifespan, and increase the quality of life of NPC patients. Therefore, this review aims to address the use of DC to determine its safety and efficacy for future adjuvant therapy of NPC.

Methods

Literature Screening

The available literature was organized according to the Preferred Reporting Items for Reviews and Meta-Analysis (PRISMA) for this review. Article screening was performed with the keywords of "dendritic cell therapy" and "nasopharyngeal cancer" using PubMed, EMBASE, the Cochrane library, and ScienceDirect. The titles and abstracts of all research were examined, and the full-text versions of the publications were reviewed. For this review, the publication date was set from 2012 to June 2023. Flow chart of the selection process was shown in Figure 1.

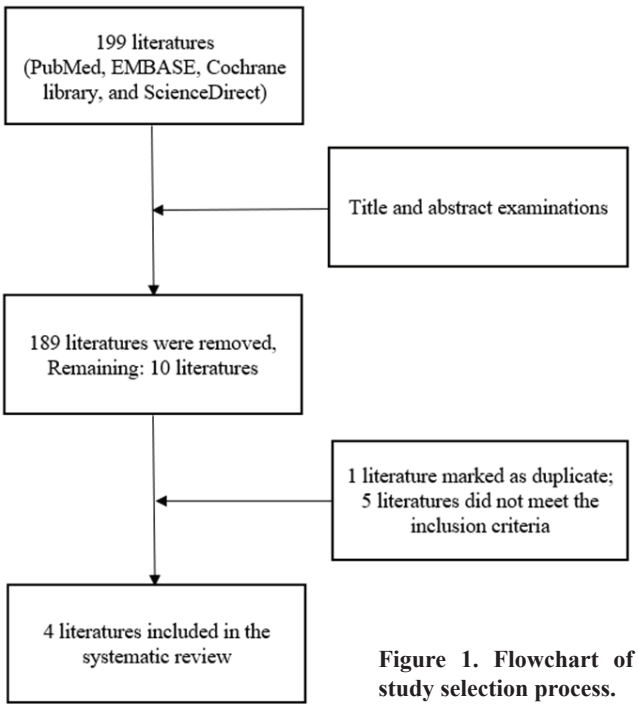

Figure 1. Flowchart of study selection process.

Table 1. PICO study design.

Component	Description
Problem (P)	Nasopharyngeal cancer
Intervention (I)	Dendritic cells
Comparison (C)	Standard therapy
Outcome (O)	Improved lifespan of patient
Study design (S)	Clinical trials

Literature Selection and Research Question

Literature selection was performed based on the following inclusion criteria: articles with controlled human models towards nasopharyngeal cancer patients with Epstein-Barr virus (EBV). The articles were in English with interventions of any application of DC to the study group. We formed a Participants, Intervention, Comparison, Outcome, and Study Design (PICOS) question to establish the eligibility criteria (Table 1). Following that, the focus question was "How is the efficacy of DC as an adjuvant therapy for nasopharyngeal cancer?". Highlighted outcomes from the articles were increased lifespan of NPC patients with DC adjuvant therapy compared to the standard one. Duplicates and review articles were excluded from the study.

Literature Analysis, Data Extraction and Synthesis, Degree of Evidence Analysis

Authors independently analyzed selected literatures and tracked the data. All data related to cell preparation methods; interventions; follow-up duration; main outcome; significant differences between groups and other outcomes were extracted and discussed among all authors. Selected literatures were characterized based on levels of medical evidence diagram in evidence-based practice (Figure 2).(33)

Results

From the literature screening, 199 literatures were identified. After title and abstract examinations, 189 literatures were removed. Ten literatures were further reviewed using full-text versions of the publications. One literature was marked as duplicate and five literatures did not meet the inclusion criteria. Therefore, a total of four literatures were included in this review. All four literatures were clinical studies with limited population and evidence, thus categorized as level III based on the Degree of Evidence Analysis.

All literature studies were reported in analyzing the effect of DC therapy induced by various antigens, such as LMP1 and LMP2. Despite LMP1 and LMP2, CD137L was used also as a mediator to induce a more robust immune response to fight against NPC progression. In all studies, autologous peripheral blood mononuclear cells (PBMC) were used as sample sources. Monocytes were isolated from PBMC and cultured for 5 to 7 days in culture media. Media used in four studies were RPMI-1640 and serum-free medium, while others did not state clearly. These processes

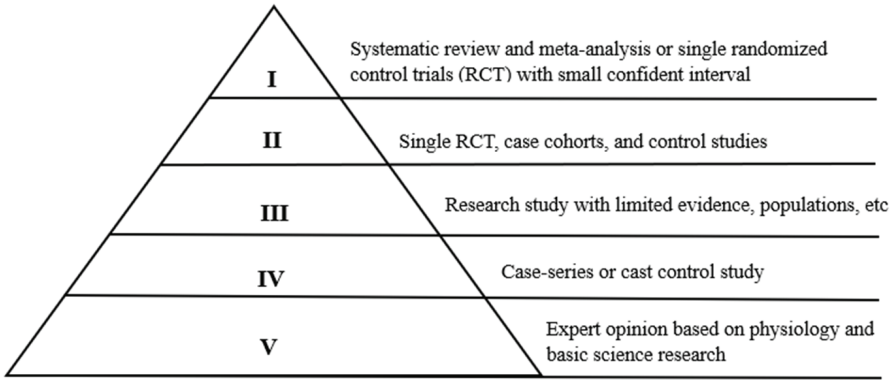

Figure 2. Diagram degree of evidence.(33) (Adapted from Slack Incorporated).

generated immature DC that must be transduced by a vector to create a vaccine candidate. The DC number injected into patients and intervention plan are stated in Table 2. Overall, DC in the number of 2 x 10^5 to 2 x 10^{11} was injected 3-7 times biweekly.

Clinical outcomes of the four studies used were summarized in Table 3, including the assessment of main outcome measures, score and result of the main groups, statements of statistical significance, other parameters, and a list of different outcome measures. The PFS rate was 1.92 - 4 months (16.5 weeks), and the cytotoxic T lymphocyte (CTL) response increased to 62.1%. Based on a selected study, a five-year survival rate of 94.4% in responders and 45.5% in non-responders was shown by follow-up data of 29 vaccinated patients in studies on the long-term effects of DC injection in NPC patients.

Regarding safety, no serious adverse events (SAE) in all selected studies. Some studies reported that patients experienced tenderness, local rigor, or swelling in the injection site, myalgia, fatigue, and positive reactions to delayed-type hypersensitivity (DTH) but in smaller magnitude. In all four studies, functional outcomes were achieved despite the different antigens or ligands that induce an immune response, as shown by anti-EBV and anti-NPC immune responses induced by DC, which contribute to the prevention of metastasis of NPC.

Discussion

The use of immunotherapy such as DC as an adjuvant therapy in NPC is still rarely conducted, as can be seen from the limited articles available. Difficulty in finding the best method to ensure efficacy and repeatable isolation of patient blood limits the use of DC as immunotherapy. Even though DC has great potential, it is very limitedly studied because of the difficulty in producing highly potential DC that can activate cytotoxic T cells to eliminate tumor cells. Moreover, DC works by directing T cells and not directly affecting the cancer cells.(34) According to the selected studies, DC can work synergistically with chemoradiotherapy to control cancer progression, thereby increasing the survival rate by inducing NPC cells into apoptosis, activating $CD8^+$ T cells, and reducing the radioresistance of some cancer stem cells (CSC).(10,14,35,36)

To date, *ex vivo* expansion of DC is the most common approach to develop vaccine.(10) The number of antigens used when incubating the DC varies in each study, some used LMP1 only, but another study used all three antigens (LMP1, LMP2, and EBNA1). Besides pulsed DC with Epstein-Barr virus (EBV) antigens, mature DC can be induced by ligands expressed on APC, such as CD137L. It resulted in stronger adhesion, higher secretion of proinflammatory cytokines, and improved survival and proliferation.(10) No patients showed any clinical or biochemical symptoms of an autoimmune illness in any of the selected studies. A dose escalation study can be implemented to ensure the safety of the patients.(10,36) However, a separate study must be conducted to determine the maximum dose that can cause moderate effects on patients. Based on selected studies, no SAE was reported, indicating DC therapy was well tolerated.

Prior to DC injection, it was found that cytotoxic T cell count was low, and naive T cell count was high. Conversely, in post-DC injection, the progressors group in the selected studies experienced an increase in cytotoxic T cells.(10,14,35,36) In addition, several researchers found that cytotoxic T cells can be increased up to three months after DC injection. As multiple injections of DCs are employed, a decreasing number of naive T cells and increment of $CD4^+$ and $CD8^+$ memory T cells were present. The rise in $CD4^+$ T cells was assumed as an after-effect of immune stimulation by MHC Class II-restricted tumor antigen.(36) These conditions showed the ability of DCs to suppress tumor activity.(10) Similar results also showed an increasing level of CTL after DC multiple injections.(14,35,36) The PFS rate in DC immunotherapy patients was longer than the radiochemotherapy patients in all selected studies.(1,37,38) This longer PFS rate may be caused by radioresistance reduction of CSC, which normally, these cells still survive even after radiotherapy.(39) Thus, multiple injections are required to prevent cancer remission.

Antigens used for DC have different impacts on NPC treatment. EBNA1 directly induces oncogenesis. The ectopic expression of EBNA1 induces tumor growth and metastasis of NPC xenograft. EBNA1 directly modulates signal transducer, transcription activator, and NF-kB, causing increased growth, survivability, metastasis, and angiogenesis of NPC.(40) Meanwhile, LMP1 can increase the proliferation and survivability of NPC cells by inducing the expression of mitogenic receptors. It can also activate the mediator of cell growth and apoptosis, such as nuclear factor kB (NF-kB). In addition, LMP1 can increase expression and release matrix metalloproteinase.(41) LMP1 can induce DNA methyltransferase 1, 3a, and 3b, which causes hypermethylation of suppressor gene promoters such as E-cadherin.(42) In addition, B-cell lymphoma (Bcl-2) is expressed excessively in NPC. LMP1 and Bcl-2 work

Table 2. Overview of the studies.

DC Source	Test Subjects	Cells Preparation	Intervention To the Main Group	Control(s)	References
Autologous PBMC	Patients were treated with systemic chemotherapy such as cisplatin and gemcitabine at least 3 weeks before DC injection.	- *Ex vivo* CD137 ligand-DC vaccine was generated by plating autologous PBMC onto 145 x 20 mm dishes precoated with 5 g/mL anti-CD137L antibody for seven days. - PBMC were isolated from leukapheresis and suspended in RPMI-1640 medium supplemented with 10% gamma irradiated fetal bovine serum. - The adhered cells were matured with 1 g/mL R848, 50 ng/mL IFN-γ.	Injections were placed near the inguinal region on alternate sides, and up to 7 vaccines were administered at biweekly intervals. For each CD137L-DC-EBV-VAX, approximately 5–50 × 10^6 cells were administered. Vaccines were thawed and immediately used at the point of administration. Dose delays were allowed for a week to allow recovery from adverse events.	The control data was the historical control.	(10)
Autologous PBMC	Patients underwent chemotherapy and radiotherapy ≥3 months.	- PBMC were obtained from patients through venesection. Monocytes were isolated through adherence and were transduced into immature DC with 100 ng/ml GM-CSF. - Immature DC transduced with Ad- LMP1-LMP2 vector and cytokine cocktail was grown and harvested after 7 days.	Subjects with metastatic NPC received Ad- LMP1-LMP2 vaccines biweekly for up to five doses. The dose for DC injection was 3.69-5.06 x 10^6 cells.	The control data was the historical control.	(14)
Autologous PBMC	Patient already received at least one line of polychemotherapy and no therapy or experimental agents for at least 4 weeks before vaccine administration.	NPC patients are immunized with 2 x 105 LMP2-DC by intradermal injection at week 0, week 2, and week 4.	NPC patients are immunized with 2 x 10^5 LMP2-DC by intradermal injection at week 0, week 2, and week 4.	The control data was the historical control.	(35)
Autologous PBMC	Patients with concurrent chemotherapy more than 12 weeks prior to vaccinations.	rAD5-EBV-LMP2 vaccines were made by transfecting dendritic cells (DC) with EBV-LMP2 recombinant serotype 5 adenoviruses (rAD)5.	Patients were enrolled into three dose-level groups ($2x10^9$, $2x10^{10}$, $2x10^{11}$).	The control group was the untreated group.	(36)

DC: dendritic cells; GM-CSF: granulocyte macrophage colony stimulating factor; LMP1: latent membrane protein 1; LMP2: latent membrane protein 2; NPC: nasopharyngeal cancer; PBMC: peripheral blood mononuclear cells; rAd-LMP2: recombinant adenovirus vectors expressing LMP2; rAD5: recombinant adenoviruses serotype 5.

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Table 3. Clinical studies results.

Assessment of Main Outcome	Main Outcome Measure(s)	Scores/Results	Significant Difference between Groups	Other Evaluation	Other Outcome Measure(s)	References
12 weeks after 7th vaccination, every 3-6 months thereafter	CB rate	2-3 years	In comparison between the clinical benefit group and the progressors group: - EBNA1 DNA levels in plasma were reduced after receiving 7 vaccinations, - Naïve T cell decrease were differentiated to CD8+ and CD4+ memory T cells after receiving 7 vaccinations.	-	-	(10)
	PFS	16.5 weeks				
	T cell frequency	Low naïve T cell level High CD8+ memory T cells and CD4+ memory T cells				
14 weeks	Toxicity	62.5% of patients responded to the immunodominant CMV antigen	In comparison between the DC therapy group and the control group: - There was a modest efficacy of the therapy, but the potency of the current vaccine is too low for significant benefits, - Patients with a higher EBV-DNA load had a statistically longer OS than those with high EBV-DNA.	DC taken from the patient is cocultured with autologous PBMCs to measure whether DCs can activate LMP1 and LMP2 specific T cells.	It is possible to generate DC vaccine with Ad-ΔLMP1-LMP2, even in heavily pre-treated patients.	(14)
	DTH	Positive reaction decreases along with the vaccination rate				
	PFS	Median of 1.92 months				
	OS	Median of 6 months				
5th and 8th weeks	CTL response to LMP2	CTL response increased in 62.1% of patients compared with pre-immunized subjects	DC immunotherapy via intradermal injection shows a great result for cancer treatment.	- DC therapy efficacy in early-stage vs. advanced-stage NPC patients. - Plasma EBV genome copy before and after therapy.	- Intradermal injection in early-stage NPC patients improves CTL response specific to LMP2 than in the advanced stage of the disease. - Plasma EBV genome copies before and after DC immunotherapy were negative in all NPC patients.	(35)
	CD83, CD86, and DC-DR expression of DC surface	Expression of CD83, CD86, and DC-DR was lower in non-responders than in responders, causing no CTL responses to LMP2				
	IgA antibody titers	No changes in IgA antibody titer to EBV-VCA				
	five-year survival rate	94.4% in responders and 45.5% in non-responders				
D_0, D_7, D_{14}, D_{28}, D_{35}, D_{58}	$CD3^+$, $CD4^+$	Significant increase of $CD3^+$ and $CD4^+$ in high dosage patients	In comparison between the DC therapy group and the control group: - The use of rAD5-EBV-LMP2 vaccine was significant in the high dose group in increasing CD3+ and CD4+ cells, - There were no SAE in all patients, the most common adverse events were tenderness in the inoculation site.	Disease-progression events	4/24 patients (16.6%)	(36)
	$CD8^+$	No significant increase in any dosage		Failure-free rate	20/24 patients (83.3%)	

CB: clinical benefit; CMV: cytomegalovirus; CTL: cytotoxic T lymphocytes; DC: dendritic cells; DTH: delayed-type hypersensitivity; EBNA1: Epstein–Barr virus nuclear antigen 1; EBV: Epstein-Barr virus; LMP1: latent membrane protein 1; LMP2: latent membrane protein 2; OS: overall survival; PBMC: peripheral blood mononuclear cells; PFS: progression-free survival; SAE: serious adverse events.

synergistically to induce fast and uncontrolled cell growth. (43) There is also evidence that LMP1 can induce an angiogenesis process in an NPC environment.

LMP2 can be found in NPC cells since it has a role in activating the oncogenic pathway. LMP2A can induce the transformation of epithelial cells through the phosphatidylinositol 3-kinase (PI3K)/Akt pathway. The transformation of the P13K gene at the genomic level, such as gene mutation and amplification, is correlated with metastasis in the lymph node, advanced carcinoma progression, and a worse prognosis.(44) Epithelial cells that LMP2A transfects show increased proliferation, epithelial-mesenchymal cell transition, invasion, and migration. Although the function of LMP2B is still unclear, LMP2B is supposed to activate PI3K/Akt signal and promote cell motility through cellular adhesion disruption. LMP2 contains a lot of $CD8^+$ and $CD4^+$ cell epitopes, therefore, the sensitization of DC with LMP2 will further induce T-cell activation when DC is re-administered into the body.(43)

Some phase I and II clinical trials have shown the safety of DC induced with RNA tumors or DC genetically modified to express specific endogenous antigens. TriMixDC-Mel vaccine is known as autologous DC for melanoma patients that are pulsed with mRNAs (tyrosinase, MAGE-A3, MAGE-C2, gp100) responsible for CD40L, CD70, TLR-4 ligand.(42) Aside from that, viruses are applied to deliver specific antigens to dendritic cells. However, some research showed the persistence of virus material in the bloodstream of the patient. The suitability of a virus vector for a certain application depends on factors, such as cell or target tissues, packing capacity of the virus to bring specific antigen, interaction potential of viral capsid against other immune cells, and the tendency to conduct immunotoxicity.(3) Capsin protein or envelope endowed virally is a foreign protein that can be an adaptive immune response target. This mechanism is possibly caused by the amount of virus that is injected, making the reaction must be monitored periodically.

There are some limitations to this study such as these studies utilize different assessments and parameters to measure the efficacy of the trials. Thus, causing various outcomes that are impossible to analyze quantitatively. The use of historical controls may also cause biases in this study. Although it may reduce the number of patients involved in clinical studies using a placebo, historical controls do not have the same eligibility, treatment and evaluation as randomized control trials.(33) Further randomized control trials are required to understand the DC mechanism as immunotherapy in nasopharyngeal cancer. Therefore, it can be concluded that DC therapy successfully initiated T cell response. However, it is hard to determine the optimal dose of DC to elicit T cell response, due to the variation in doses among the selected studies. Besides, the number of required doses also depends on cytokines and DC antigen maturation during culture.

Conclusion

DC immunotherapy could increase $CD8^+$ and $CD4^+$ T cells and PFS rate in NPC patients, which prolongs NPC patient survival time. However, more clinical trials with big sample sizes are needed to confirm the benefit and to determine optimal DC therapy for NPC for future clinical application.

Authors Contribution

ABS, YAD, CRS, AW and KL were involved in concepting and design the research. ABS and KTM performed the data tracking or collection. ABS, RH and GF calculated the experimental data and performed the analysis. ABS and KTM drafted the manuscript, while KTM and MNK designed the figures. ABS, RH, GF, and MNK aided in interpreting the results. ABS, RH, GF, and MNK took parts in giving critical revision of the manuscript. All authors have agreed with the revised version of the manuscript. YAD, CRS, AW and KL supervised the whole project. Lastly, AW gratefully acknowledges the funding support.

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