

## REVIEW ARTICLE

**Potency of Peripheral Blood- and Umbilical Cord Blood-derived Dendritic Cells and Their Secretomes as Vaccines for Cancer**Rima Haifa<sup>1,2</sup>, Cynthia Retna Sartika<sup>2</sup>, Ahmad Faried<sup>3</sup>, Yuni Elsa Hadisaputri<sup>1</sup>, Angliana Choww<sup>2</sup>, Andi Wijaya<sup>2,4</sup>, Melisa Intan Barliana<sup>1,5</sup><sup>1</sup>Department of Biological Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Bandung, Indonesia<sup>2</sup>Prodia StemCell Indonesia, Jakarta, Indonesia<sup>3</sup>Department of Neurosurgery, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia<sup>4</sup>Prodia Clinical Laboratory, Bandung, Indonesia<sup>5</sup>Center of Excellence in Pharmaceutical Care Innovation, Universitas Padjadjaran, Bandung, Indonesia

Dendritic cell (DC) vaccines, as immunotherapy agents, can gather up and transport cancer-related antigens to T lymphocytes, activating anti-tumor effector responses. After being activated by DC, cytotoxic T lymphocyte cells (CTL) will secrete the cytolytic granzyme B that can effectively induce rapid apoptosis of target cells. On the other hand, DC also secrete several cytokines and a large number of exosomes, which together operate as a whole antigen-presenting entity. The efficacy of the vaccine's treatment may be affected by the sources used for DC vaccines. Umbilical cord blood (UCB) from healthy donors can be employed when autologous cancer patient's peripheral blood (PB) cannot be used as a source for isolating DC due to genetic abnormalities. Comparing UCB to other sources, there is a painless method of collecting sources as opposed to PB, which necessitates a venipuncture or leukapheresis procedure to isolate the blood. Many studies related to the use of PB-DC have been carried out, but research on potential comparisons between PB-DC and UCB-DC is still very limited. In this review, the potential of PB- and UCB-derived DC and their secretomes for cancer will be discussed.

**Keywords:** dendritic cells, vaccines, umbilical cord blood, peripheral blood**Introduction**

Cancer is the rapid creation of aberrant cells that outgrow their normal bounds, infect nearby bodily components, and ultimately spread to other organs. Immunotherapy has emerged as an effective treatment for many types of cancer.<sup>1</sup> Dendritic cells (DC) vaccine was used as an immunotherapy agent that has the capacity to pick up and deliver antigens

associated with cancer to T lymphocytes, inducing potent effector responses against cancer. The important role of DC is to mediate innate immune responses, induce adaptive immune responses, and activate memory cells. In addition, DC can modulate cytokine and chemokine gradients to control inflammation and lymphocyte homing. All of these signals play an important role in maintaining systemic anti-cancer effects.<sup>2-4</sup>

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Peripheral blood (PB) is typically used for autologous DC vaccine generation. If autologous cells cannot be used to generate DCs due to a genetic problem, umbilical cord blood (UCB) from a healthy donor can be used as an alternative.<sup>5</sup> UCB has several advantages compared to other sources. PB collection requires more invasive procedures than UCB, such as leukapheresis or venipuncture. Moreover, the number of monocytes in adult PB is less than the one in UCB.<sup>6</sup>

Like other cell types, DC also secretes several cytokines and EV called secretome. The secretome of DC contains a large number of exosomes which are referred to as dexosomes. Dexosomes derived from human cells contain cargos that together function as an entire antigen-presenting entity. Tetraspanin is one of them, like all identified proteins for presenting antigenic material, such as the cluster of differentiation (CD)1a, CD1b, CD1c, and CD86 costimulatory molecules, as well as the major histocompatibility complex class I/II (MHC I/II).<sup>7</sup>

The use of allogeneic DC secretome as cancer vaccine is now referred to as cell-free therapy with the same mechanism as DC, namely through T-cell activation or indirect activation of DCs. Secretomes from allogeneic DC are more stable because they can be stored at cold temperatures for more than 6 months compared to cells that can lose their viability if not directly injected into patients.<sup>8</sup> Although many studies related to the use of PB-DC have been carried out, research on potential comparisons between PB-DC and UCB-DC is still very limited. In this review, we will discuss the potential of PB- and UCB-derived DC and their secretomes in cancer.

## DC vaccine for cancer

Compared to traditional anti-bacterial or viral vaccines, cancer vaccines are different in a few ways. As a type of immunotherapy, cancer vaccines can assist in educating the immune system about the "look" of cancer cells so that it can identify and eradicate them.<sup>9-10</sup> In fact, by targeting cancer cells, therapeutic vaccines may offer hope to patients whose malignancies have progressed or relapsed and are no longer responsive to traditional immunotherapies.<sup>9,11,12</sup>

DC vaccines are a promising adjuvant cell therapy for cancer. DC can gather up and transport cancer-related antigens to T lymphocytes, thereby activating anti-tumor effector responses. The activation of cytotoxic T lymphocyte (CTL) or CD8<sup>+</sup> by DC is mediated by 4 signals. These 4

signals are: 1) the interaction between T cell receptor (TCR) on the T cells and MHC; 2) co-stimulation through CD28 and CD80/86, which are expressed on T cells and DC respectively; 3) secreting pro-inflammatory cytokines such as interferon (IFN) and interleukin (IL)-12, which can stimulate the expansion function and memory formation of CTL; or 4) DC control the activation of particular integrins and chemokine receptors on T cells to guide migration in the direction of targeted organs.<sup>13-18</sup> After being activated by DC, CTL cells will secrete the cytolytic granzyme B that can effectively induce rapid apoptosis of target cells. On the other hand, DC also secrete several cytokines and a large number of exosomes, which together operate as a whole antigen-presenting entity.

Recently, DC-based cancer therapy has been a promising post-chemoradiation adjuvant cell therapy due to the reports that it can increase median overall survival.<sup>19,20</sup> In order to take advantage of DCs' action against tumor cells, various strategies have been tried to use them as therapeutic vaccines. The most common approaches are using entire tumor cells or pure/recombinant antigen peptides to pulse DC before injecting them back into the patient.<sup>9</sup>

As of January 2024, data of ClinicalTrials.gov listed roughly 610 clinical trials on DC treating cancer, demonstrating the growing use of DC as immunotherapy. There are two categories of whole tumor cell vaccines: allogeneic and autologous. The FDA has approved Sipuleucel-T DC vaccination (Provenge®) as the first cell-based cancer therapy. These autologous active cellular immunotherapies target prostatic acid phosphatase (PAP), which is only expressed in prostate cancer cells, by stimulating T-cell responses via antigen-presenting cells (APCs). Patients' median survival in 2010 was four months. Another DC-based vaccination, called APCEDEN®, has been licensed by the Indian government agency (Central Drugs Standard Control Organization), for the treatment of prostate, ovarian, colorectal, and non-small cell lung cancer.<sup>9,19,21,22</sup>

## Cellular and molecular interactions between DC, T cells, B cells, and cancer cells

Naïve Th cells interact with activated DC in the lymph node zone or follicular region. After being activated by DC, CTL contains preformed lysosomal granules in their cytoplasm, which are ready to be degraded after activation. Proteins, including CD107a, serve as coatings on the granules, acting

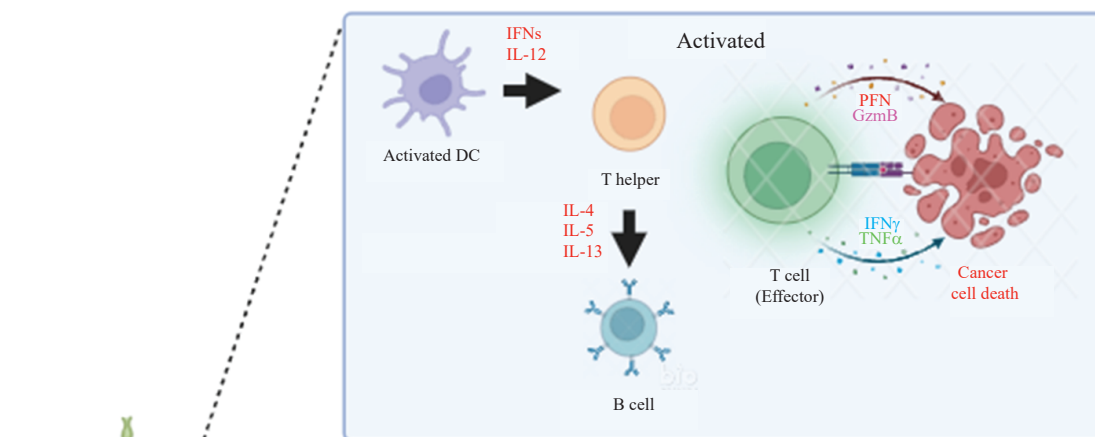
as a degranulation marker transiently expressed on the cell surface during granule release. Within these granules, the cytolytic granzyme B is tightly packed, effectively inducing rapid apoptosis of target cells. CTL also release effector cytokines such as IFN- $\gamma$  and tumor necrosis factor (TNF)- $\alpha$ , which are involved in the preparation and differentiation of CTL and directly kill tumor cells (Figure 1).<sup>23,24</sup>

Signals from infected cells or cancer cells cause the release of cytotoxins such as perforin, granzymes and granulysin by CTL. CTL activation is mediated by the interaction of various co-stimulatory molecules as well as immune cells. Tumor-specific proteins are expressed in cancer cells. Tumor peptides are processed and presented on the cell surface by human leukocyte antigen (HLA) molecules. Then the peptide-presented HLA is recognized and bound by CTL. This binding causes a cascade reaction to express Fas ligand (FASL), involving Fas receptors present on tumor cells, thus initiating the activation of procaspase 8 and 10, resulting in apoptosis. In addition, perforin produced by CTL causes polymerization and forms pores in cancer cells so that granzymes can enter cancer cells through these pores and cut functional proteins and DNA, resulting in apoptosis.<sup>25</sup>

## DC vaccine derived from PB and UCB

The main sources of DC are monocytes and CD34<sup>+</sup> cells. Both can be obtained from PB, bone marrow blood, and UCB of newborns. PB is widely used as a source of autologous DC. Due to the limited potency of the patient's immune cells, another alternative shall be proceeded to increase the efficacy of immunotherapy. UCB from healthy donors can be used when autologous cells cannot be used as a source for making DC.<sup>5,26</sup> UCB-DC has several advantages compared to PB-DC (Table 1).

In comparison to PB collection, which needs a leukapheresis or venipuncture process to isolate the blood, UCB collection is a non-invasive method of obtaining sources. On the other hand, the proliferative capacity and repopulation potential of UCB hematopoietic cells is higher than that of bone marrow. UCB-derived DC has lower immunogenicity, a greater proportion of CD3<sup>+</sup>, and CD56<sup>+</sup> cells which are the primary functional fractions. UCB-derived DCs also have stronger antitumor activity against different cancers than that of PB so they could provide better T cell activation by providing high HLA compatibility with patient.<sup>26,28,30</sup> In addition, the risk of graft



**Figure 1. Mechanism of DCs vaccine as cancer therapy.** Activated DCs migrate to the lymph nodes to interact with naïve T cells and activate effector T cells by secreting proinflammatory cytokines, such as IFNs and IL-12. After activated by DC, Th1 and Th2 cells then release IL-12, IFN $\gamma$ , IL-4, IL-5, and IL-13 to induce CTL and B cell responses. Then cytotoxic T cells release effector cytokines such as IFN $\gamma$  and TNF $\alpha$ , which are involved in the preparation and differentiation of CTL to kill tumor cells. The tumor cells killing process involves activated CTL that release cytotoxins such as perforins and granzyme B, so that they can cut functional proteins and DNA resulting in apoptosis (figure created in Biorender.com).

DC vaccine injection in lymph node

**Table 1. Comparison of PB-DC and UCB-DC.**<sup>5,26-29</sup>

Comparison	PB-DC	UCB-DC
Source isolation	Invasive, requires a process of leukapheresis or venipuncture	Non-invasive
Exposure to tumor microenvironment (TME)	Yes	No
Number of monocytes in source	Low	High
Culture period	Rapid	Slow
Proliferative capacity	Low	High
Immunogenicity	High	Low
Antitumor activity	Low	High
Secretion rates of IL-12, IFN $\gamma$ , and TNF $\alpha$	Low	High

versus host reaction (GvH) in UCB with the same (HLA) matching is weaker than in bone marrow and PB.<sup>5,27</sup> The number of DC in human PB is only 1%, and the monocyte cells in adult PB is less than monocytes from UCB.<sup>29</sup> The maturation of monocyte cells in adult PB may be inhibited in the interim by the tumor microenvironment (TME).<sup>26</sup> On the other hand, UCB monocytes are more naïve and can be differentiated to DC easily.<sup>6,26</sup> Under optimal conditions, UCB monocytes express similar amounts of CD11c, CD163, CD80, and HLA-DR with PB monocytes. It was conveyed that monocytes derived from UCB showed a disturbance of homeostatic extravasation compared to mature monocytes.<sup>29</sup>

DC from UCB has a different frequency and function than DC from PB. In UCB, the DC phenotype tends towards immaturity, characterized by reduced antigen uptake as indicated by expression of CD32 and CD64 immunoglobulin (IgG) receptors, and lower expression of co-stimulatory molecules, including MHC class II, intercellular adhesion molecule (ICAM)-1, CD80, and CD86.<sup>6,29</sup> UCB-derived DC have a better chance of being used in immunotherapy due to their high availability.<sup>27</sup> Efficacy and tolerability of UCB-DC combined with chemotherapy for the treatment of patients with gastric cancer have been reported.<sup>28,31</sup> UCB-DC showed that there are no serious adverse effects and UCB-DC has a similar phenotype, lower immunogenicity, stronger proliferation, and a similar antitumor activity compared with PB-DC. Other studies suggest that an immunotherapeutic approach based on UCB-derived DC may be useful in treating human papillomavirus (HPV)-associated esophageal cancer (EC). It was possible to induce T-cell proliferation and HPV18E7-specific cytotoxic T-cell

responses *in vitro* using UCB-derived DC transfected with HPV18E7-DNA.

### The Potential of DCs-derived Secretomes in Cancer

DC, like other cell types, secretes a variety of cytokines and extracellular vesicles (EV) known as secretomes. It contains a large number of exosomes, also known as dexosomes. Human dexosomes harbor various cargos that collectively function as an entire antigen-presenting entity so it is also highly beneficial for offering good efficacy as a cancer vaccine. Dexosomes showed similar co-stimulatory molecules and cytokines as owned by their parent cells. This dexosomes could provide off-the-shelf treatment with similar potency through direct or indirect interaction with immune cells.<sup>26,30</sup> Due to their increased resilience to tumor-mediated suppression and improved anti-tumor activity in pre-clinical animal studies, dexosomes have been hailed as a possible replacement for DC-based vaccinations.<sup>32</sup> Compared to DC vaccinations, dexosomes provide additional benefits, including exhibit 10-100 times more peptide major histocompatibility complex (pMHC) complexes on their surface and have been demonstrated to be enriched in ligands that activate natural killer (NK) cells.<sup>33-34</sup> Due to particular sorting and loading mechanisms, dexosomes also have a more constrained and controllable molecular composition, a significantly longer shelf life, and can more effectively reach the desired spot on secondary lymphoid organs. Dexosomes might also be simply adapted to carry their cargo to particular, predetermined locations.<sup>30,32</sup>

Dexosomes can either directly or indirectly stimulate the T cell response. It is possible to directly excite CD4<sup>+</sup> T lymphocytes or T helper cells (Th cells) and CTL via MHC class I/II molecules on their secretome surface.<sup>30,32</sup> However, the direct dexosome-T cell pathway is less likely to occur extensively *in vivo* and has been reported to be ineffective in generating naïve T cells. Presumably, dexosomes cannot interact with T cells until they are taken up by other DC, which then process and extract antigenic material from tumor-associated antigens-major histocompatibility complex (TAA-MHC) and use it to prime specific T cells.<sup>7,30</sup> There are 3 ways that dexosomes can indirectly excite T lymphocytes. The antigen is initially taken up by DC through the endocytic route, processed, and then presented to T lymphocytes on the DC surface. Another option is using dexosomes that use DC cross-linking. Moreover, tumor cells can also deliver antigen by presenting dexosomes to DC. Tumor cells engulf antigens and present them to T lymphocytes via cross-presentation.<sup>7,30</sup> Secretomes also contain cytokines, such as TNF- $\alpha$ , IL-6, and members of the IL-12 family, which can be used as a cell-free therapy to activate the patient's immune system against cancer cells.<sup>30,35</sup>

## Conclusion

The use of UCB-DC has advantages in terms of source isolation, exposure to TME, monocyte count, proliferative capacity, immunogenicity, and antitumor activity. However, in terms of culture period, PB-DC is superior. In addition, the secretome from DC also has the same potential role as the antigen presenting cells so that it can be used as a cell-free cancer vaccine.

## Authors Contribution

RH, CRS, and MIB were involved in the conception and planning of the research. RH drafted the original manuscript. MIB performed the final editing of the manuscript. CRS, AF, YEH, A, and AW gave critical and contextual suggestions for the manuscript. All authors read and approved the final manuscript.

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