UNIVERSITI PUTRA MALAYSIA

EFFICACY OF DENDRITIC CELL-BASED VACCINE ON ACUTE MYELOID LEUKAEMIA IN A MURINE MODEL

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By

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

EFFICACY OF DENDRITIC CELL-BASED VACCINE ON ACUTE MYELOID LEUKAEMIA IN A MURINE MODEL

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July 2013

Chair: Maha Bt Abdullah, PhD

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Acute myeloid leukaemia (AML) has a high rate of relapse despite current chemotherapy and haematopoietic stem cell transplantation. This emphasizes the need of alternative therapeutic strategies in improving the long-term survival of AML patient. Dendritic cells (DCs) are professional antigen presenting cells that able to elicit specific T cell immunity. Their use in immunotherapy is to direct immune response against residual tumour cells and eventually lead to complete tumour eradication. This unique capability of DCs renders them as an attractive adjunct for the treatment of AML. Therapeutic efficacy of syngeneic DC-based vaccine alone or in combination with chemotherapy to eradicate AML was evaluated in vivo. DC-based vaccine were generated *in vitro* by culturing murine bone marrow cells in the presence of granulocytes-macrophages colony-stimulating factor (GM-CSF), interleukin-4 (IL-4) and tumour necrosis factor-α (TNF-α) and subsequently DC were pulsed with AML cell (C1498) lysate at a ratio of one DC to three AML cells.

In vitro data showed that generated DC-based vaccine was functionally intact with the capability to induce T cell proliferation and elicit cytotoxicity effect against murine C1498 cell line. In vivo experiments carried out on groups of 6 mice showed that monotherapy with Ara C alone failed to control tumour growth. Although DC-based vaccine monotherapy was also able to control aggressive tumour development ($P < 0.05$; Man-Whitney Test), it provided only minimal survival benefits with median survival time (MST) of 12 days compared to 10 days with PBS treatment. In contrast, the antitumour effect was enhanced by combination therapy when Ara C treatment was given prior to DC-based vaccine treatment (AraC-DC) ($P < 0.10$; Man-Whitney Test) with MST greatly improved to 15 days. Strikingly, repeated combined therapy by intervening DC-based vaccine treatment preceding Ara C treatment and a repeat of DC-based vaccine (DC-AraC-DC) showed greater antitumour effect and dramatically improved long term survival (MST of 20 days) of AML mice than in response to either monotherapy alone ($P < 0.05$; Man-Whitney Test) or combined therapy ($P < 0.05$; Man-Whitney Test). These novel findings reveal the value of incorporating DC-based vaccine with cytosine arabinoside at different treatment schedule in treating AML patient clinically.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

KEBEKESANAN VAKSIN BERASASKAN SEL DENDRIT UNTUK MIELOID LEUKEMIA AKUT DI DALAM MODEL MENCIT

Oleh

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Walaupun remisi lengkap dapat dicapai dengan rawatan kemoterapi dan pemindahan sel tunjang hematopoitik, mieloid leukemia akut (AML) masih mempunyai kadar rerap yang tinggi. Kadar penyembuhan rawatan yang rendah ini menekankan keperluan strategi baru yang lebih berkesan untuk mencegah relap sementara memanjangkan hayat pesakit AML. Sel dendrit (DC) adalah sel penampil antigen (APC) yang profesional untuk menstimulasi respon sel T. Penggunaan DC dalam terapi immuno adalah untuk mengarahkan respon imun membasmikan baki sel-sel tumor untuk tumor remitan lengkap. Keupayaan DC yang unik ini membenarkannya menjadi calon yang berpotensi untuk rawatan AML. Kajian ini bertujuan untuk menentukan kebekesanan vaksin DC sahaja atau dalam gabungan kemoterapi untuk membasmikan AML di dalam model mencit. DC vaksin dihasilkan *in vitro* dengan mengkultur sel sumsum tulang mencit di dalam kultur medium mengandungi sitokin '*granulocytes-macrophages colony-stimulating factor*' (GM-CSF), '*interleukin-4*' (IL-4) dan '*tumour necrosis factor-α*' (TNF-α) diikuti oleh '*pulsing*' DC dengan lisat

sel mencit AML (C1498) pada nisbah satu DC dengan tiga sel AML (tumor lisat:DC). Keputusan kajian *in vitro* menunjukkan bahawa vaksin DC yang dijana mempunyai keupayaan untuk mengaktifkan proliferasi sel T dan menghasilkan kesan sitotoksik terhadap sel mencit C1498. Kajian *in vivo* dengan menggunakan 6 ekor mencit dalam satu kumpulan rawatan turut meunjukkan bahawa monoterapi dengan Ara C sahaja gagal unituk mengawal pertumbuhan sel sel tumor. Walaupun penggunaan vaksin DC sahaja berjaya untuk mengawal pertumbuhan tumor secara progresif ($P < 0.05$; Uji Man-Whitney) tetapi median tempoh hayat (MST) mencit AML hanya dipanjangkan ke 12 hari berbanding dengan 10 hari kumpulan kawalan PBS. Manakala, kombinasi terapi dengan menggabungkan Ara C dengan vaksin DC (AraC-DC) ($P < 0.10$; Uji Man-Whitney) dapat menunjukkan antitumor yang lebih berkesa dan memnjangkan MST mencit kepada 15 hari. Tambahan pula, rawatan ulanagan dengan vaksin DC berikutan dengan Ara C dan ulangi vaksin DC (DC-AraC-DC) menghasilkan penindasan tumor yang lebih berkesan dan memanjangkan hayat mencit AML secara signifikan (dengan MST 20 hari) jika dibandingkan dengan monoterapi ($P < 0.05$; Uji Man-Whitney) atau kombinasi terapi (AraC-DC) ($P < 0.05$; Uji Man-Whitney). Penelitikan baru in dengan kombinasi AraC dan vaksin DC pada jadual rawatan yang berbeza menyokong penggunaannya dalam merawat pesakit AML secara klinikal.

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APPROVAL

I certify that a Thesis Examination Committee has met on 9 July 2013 to conduct the final examination of Ng Wei Yi on her Master of Science thesis entitled "Efficacy of Dendritic Cell-based Vaccine on Acute Myeloid Leukaemia in a Murine Model" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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DECLARATION

I declare that the thesis is my original work except for the quotations and citations which have been duly acknowledged. I also declare that is has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other Institution.

NG WEI YI

Date: 9 July 2013

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Ag	antigen
AML	acute myeloid leukaemia
APC	Antigen Presenting Cell
AraC	Cytosine Arabinoside
ATCC	America Type Culture Collection
BM	bone marrow
BMDC	bone marrow dendritic cell
BSA	bovine serum albumin
CD	clusters of differentiation
CD40L	CD40 ligand
cDC	conventional dendritic cell
CLIP	class II-associated invariant chain peptide
ConA	Concanavalin A
CTL	Cytotoxic T lymphocyte
DAMPs	damaged-associated molecular pattern
DC	dendritic cell
FBS	fetal bovine serum
FDA	Food and Drug Administration
FITC	fluorescein isothiocyanate
G	gauge
GM-CSF	granulocyte macrophage-colony stimulating factor
GMP	Good Manufacturing Practice
GO	gemtuzumab Ozogamicin
H-2	mouse Major Histocompatibility Complex I
HLA	human leukocyte antigen
hr	hour
HSCT	Haematopoietic Stem Cell Transplant
hTERT	human telomerase reverse transcriptase
IA	mouse Major Histocompatibility Complex II

IFN-ɣ	interferon-alpha
IL	interleukin-1
IMDM	Iscove's Modified Dulbecco's Medium
i.p.	intraperitoneal
i.t.	intratumoral
LAA	leukaemic-associated antigen
ME	mercaptoethanol
MHC	major histocompatibility complex
MIIC	MHC class II compartment
min	minute
mL	mililitre
mo	monocyte
MST	median survival time
NKT	Natural killer T cell
NK	natural killer cell
NLRs	nucleotide oligomerization domain -like receptor
NOD	nucleotide oligomerization domain
PAMPs	pathogen associated molecular patterns
PBS	phosphate-buffered saline
pDC	plasmacytoid dendritic cell
PE	phycoerythrin
PerCP-Cy 5.5	peridinin chlorophyll protein-Cy 5.5
PI	propidium iodide
pMHC	peptide- major histocompatibility complex
PRRs	pattern recognition receptors
RLU	relative luminescence
rpm	rotation per minute
RPMI-1640	Roswell Park Medical Institute
s	second
s.c.	subcutaneous
S:R ratio	Stimulator-to-Responder ratio
SD	standard deviation

T:E ratio	Target-to-Effector ratio
TAA	tumour associated antigen
TCR	T cell receptor
T_{FH}	T follicular helper cell
T_H	T Helper cell
TLRs	toll-like receptors
TNF- ɣ	tumour necrosis factor-alpha
TNF-α	tumour necrosis factor α
Treg	regulatory T cell
WT1	Wilms' tumour protein 1

CHAPTER 1

INTRODUCTION

Acute Myeloid Leukaemia (AML), a myeloproliferative disorder, is characterized by a drastic increase in malignant non-lymphoid haematopoietic progenitor cells that will eventually lead to the failure of normal haematopoiesis (Houtenbous *et al.*, 2005). The American Cancer Society estimated that about 47,150 new cases of leukaemia will be diagnosed in the United States in 2012 with about 1 out of 3 diagnosed with acute myeloid leukaemia (AML) and more than 70% of them will eventually die of AML (http://www.cancer.org). Indeed, according to SEER Cancer Statistics Review, the prognosis of adults with AML remain precarious, with a 5-year overall survival rate of about 25%. And the overall survival is much worse in the subgroup of elderly AML patients (>65 years) with only a 5.1% survival after 5 years of treatments (Howlader *et al.*, 2011). In addition, the third report from the National Cancer Registry 2008 showed that leukaemia is one of the top ten cancers in Malaysia in terms of incidence, in which, six in every 100,000 individuals were diagnosed with myeloid leukaemia (Lim *et al.*, 2/7008). These dismal outcomes despite current therapeutic approaches underlie the need for new yet less toxic treatment alternatives.

Current therapeutic treatments for AML consisting high-dose chemotherapy with or without stem cell transplantation manage to achieve complete remission in about

80% of AML patients (Houtenbos et al., 2005). However, the majority of patients, especially in the elderly, will eventually suffer a relapse due to minimal residual disease (MRD), despite having initially achieved complete remission (Estey & Döhner, 2006). This phenomenon is believed to happen due to survival of a small reservoir of resistant leukaemic cells after chemotherapy (Westers et al., 2006). Although allogeneic haematopoietic stem cell transplant (HSCT) can be used to clear MRD and lengthen the survival, it is still limited to younger patients with less co-morbidity and patients with suitable stem cell donors. Furthermore, currently there is still lacking of standard post-remission therapy to prevent relapse due to MRD (Estey & Döhner, 2006). These observations underscore the suboptimal state of the current AML therapeutic strategy and the need for novel strategies to prevent relapse and lengthen the survival of AML patients. Therefore, newer treatments such as immunotherapy, alone or in combination with chemotherapy, presents an attractive alternative in treating MRD in AML patients.

Traditionally, cancer immunotherapy focused on interleukin-2 or other T cell-activating cytokines that are capable of increasing the number of T cells specific for tumour associated antigens. Such activated T cells will then be able to infiltrate the tumour and cause specific destruction to the tumour cells. However, such passive immunotherapy methods have all failed to yield long lasting memory T cells to control the advanced-stage disease in a significant way (Rosenberg, 2001). In contrast, active immunotherapy with dendritic cell (DC) vaccines targeting residual leukaemic cells has better potential as therapy because it aims to elicit and boost the immune response to specifically attack residual tumour cells (Westers *et al.*, 2006).

Dendritic cells (DCs), derived from bone marrow stem cells, are superior antigen presenting cells (APCs) of the immune system. Professor Ralph Steinman was awarded with the Nobel Prize in 2011 for his discovery of the dendritic cell and its role in adaptive immunity. This important discovery has rekindled hope for development of vaccines and therapeutics against cancer by using DCs. DCs are key regulators for immunity induction and tolerance maintenance while possessing the unique ability to capture and transfer antigens to the regional lymph nodes, to stimulate B and T cells through antigen presentation via the appropriate co-stimulatory surface molecules (Banchereau and Steinman, 1998). Additionally, only DCs are able to prime naïve T cells and induce differentiation to form antigen-specific effector cells. These unique capabilities of DCs potentially make them an ideal agent for initiation of an immune response to tumour-associated antigens (TAAs) leading to tumour rejection (Chen et *al.*, 2004). Thus, DC-based immunotherapy is a viable alternative in cancer treatment.

Cancer vaccine is developed based on the concept that the induction of tumour antigen-specific T cell response would ultimately lead to tumour elimination or rejection (Rosenberg, 2001). Leukaemia cells generally express leukaemia-associated peptides, but most of them fail to initiate an efficient immune response (Westers *et al.*, 2006). Consequently, cancer vaccines help in overcoming this problem by channelling tumour antigens into DCs and encourage optimum maturation of DCs. A number of clinical trials utilizing tumour-antigen loaded DCs as vaccines in human showed no significant toxicity (Jack *et al.*, 2007). These findings indicate that DCs

are attractive target for therapeutic manipulation of the immune system to enhance immunity in cancer.

Many studies have shown that DC vaccines are able to elicit positive immune responses against AML, however, recent reviews of clinical studies suggested that immune responses induced by such approach is still inconsistent and many of them failed to meet clinical end points. The poor therapeutic efficacies of current DC vaccination might be due to the dominance of immunosuppressive mechanism that hampers the induction of effective anti-leukaemic immunity (Anguille *et al*., 2011). Roddie *et al*. (2006) postulated that the anti-leukaemic responses elicited by DC-based approaches might be abrogated by the presence of regulatory T cells (Treg) in AML patients. These findings highlight the need to improve current DC-based vaccine strategy. Isolated DCs loaded with tumour antigen *ex vivo* and administered to murine model as a cellular vaccine in treating AML as well as their antitumour effects in conjunction with chemotherapy remains largely unexplored. The major goal of this study is to evaluate the efficacy of the AML DC-based vaccine plus chemotherapy in a murine model. It is hypothesized that AML DC-based vaccine plus chemotherapy can induce a better antitumour effect in an AML mouse model. The general objective of the study is to determine the efficacy of AML DC-based vaccine alone and in conjunction with chemotherapy to eliminate AML in a murine model.

The Specific Objectives are:

1. To generate and biologically characterize bone marrow-derived dendritic cells (BMDCs) from C57BL/6 mice.
2. To establish and evaluate the functionality of murine AML DC-based vaccine *in vitro*.
3. To investigate the efficacy of the DC-based vaccine with and without chemotherapy intervention in AML murine model.

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