Introduction
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The immune system is a network of cells that arise from hematopoietic stem cells (HSCs), tissues and mediators that defend the host from potentially harmful pathogens and provides protection against diseases. The effector functions of the immune system works precisely for the host as it is capable of distinguishing self from non self. Failing to mount an effective immune response puts the organism at risk of developing disease, while a breakdown in development of tolerance puts the organism at risk for developing immunity to self (autoimmunity). Antigen presenting cells (APCs) play a decisive role in the initiation and modulation of adaptive immune responses. Dendritic cells (DCs) are the sentinels and key regulators of T and B cell immunity, pertaining to their superior ability in uptake, processing and presentation of antigens compared with other APCs. They were first described by Ralph Steinman and Zanvil Cohn in 1973 (1). DCs are not only critical for the induction of both primary and secondary T and B cell mediated immune responses, but are also crucial for the elicitation of immunological tolerance. DCs arise from both myeloid as well as lymphoid precursors. They represent the connecting link between the innate and the adaptive arms of the immune system, playing a central role in B and T lymphocyte activation, differentiation into plasma cells, memory cells, effector T helper and cytotoxic T cells, respectively. Progenitors of DCs in the bone marrow give rise to circulating precursors that home to different tissues, where they reside as immature cells and upon antigen encounter they mature. Maturation stimuli lead to up regulation of co-stimulatory molecules and surface expression of chemokine receptors that promote migration to nearest lymph node. This unique function of DCs has offered the possibility of developing clinical protocols involving their use in tumor immunotherapy. They were introduced as adjuvants in vaccination strategies that aimed to induce antigen specific effector and memory cells. Dendritic cell therapy represents a new and promising immunotherapeutic approach for treatment of advanced cancer as well as for prevention of cancer.

In the last two decades large numbers of clinical trials have been conducted using DC vaccines on different kinds of tumors and it was found that they were able to initiate promising clinical response against a number of diseases like renal cell carcinoma, melanoma, HIV, multiple myeloma, acute myeloid leukemia, breast cancer, etc. (2-11).
Allogeneic DCs pulsed with tumor antigens based immunotherapies to generate specific T cell responses have also been tested in clinical trials for patients with solid tumors as well as for different haematological malignancies (12-13). Improvements in the \textit{in vitro} DC generation methodology and better cognition of DC biology have resulted in their widespread use as DC vaccines in the clinical trials. Clinical use of DCs requires recurrent vaccination regime to yield relatively high number of tumor antigen specific Cytotoxic T lymphocytes (CTLs) and a complete or minimal residual disease response (11-21). For the subsequent vaccinations, clinical grade DCs are required in large number, which has to be generated \textit{ex vivo} and made available. Vaccination strategies involving DCs have been developed on account of the special attributes of these cells in orchestrating innate and adaptive immune responses. The propose of DC vaccination is to generate tumour specific armed effector T cells that can curb the tumour mass specifically without affecting the healthy cells and that can actuate immunological memory to control tumour relapse. The first step in this process involves priming of DCs with tumour associated or specific antigens. Priming can be accomplished either by culturing DCs \textit{ex vivo}, that have been derived from patients or from an allogenic source with the desired tumour specific antigen and later on these cells then can be introduced back into the patient, or in an \textit{in vivo} scenario by stimulating DCs to take up the tumour specific antigen (3).

The major sources of DC are autologous CD14$^+$ or CD34$^+$ cells. Repeated vaccinations are required for the initiation of clinical response and therefore use of CD14$^+$ cells from the patient, limit the number thus reducing their potential for clinical application. Further drawback of this method is that it involves repeated blood drawing to collect monocytes. The other major sources of DCs are the CD34$^+$ progenitor cells from bone marrow or G-CSF mobilized peripheral blood (PBL). DCs generated from CD34$^+$ cells have been used for clinical trials and they are able to generate clinical response. However G-CSF mobilized CD34$^+$ cells from patients (22-24) have the drawback of skewing towards the Th2 response, which is detrimental for immunotherapy purposes. In order to overcome this disadvantage, use of allogenic DCs is being increasingly used. Allogenic DCs generated from healthy donors which are HLA matched or partially matching have been used for clinical trials. Allogeneic DCs can also be generated from CD34$^+$ cells derived from umbilical cord blood (UCB) (25-36). Thus UCB can be exploited as an additional source
for the generation of allogeneic DCs. UCB has many advantages over other allogeneic donors for DC generation like no risk to the donor and ready availability. Cord blood banks can be exploited for this purpose to look for HLA matching or partially matching grafts from across the globe. Being in the frozen state, their transportability becomes easier. UCB-derived DCs have been used in the pilot phase of clinical trials as well, in hematological disorder like AML, as a therapeutic agent to increase the survival of patients (37-38).

In our laboratory we have already standardized methods for large scale generation of DCs from UCB-derived CD34⁺ cells as well as MNCs (mononuclear cells) (35-36). These DCs were characterized by immunophenotyping and functional assays like MLR (mixed lymphocyte reaction), antigen uptake and chemotactic migration. However, for preparation of DC vaccines the basic requirement is that the DCs should generate effector and memory Cytotoxic T lymphocytes to elicit a comprehensive immune response. As far as cord blood cells are concerned, looking at their naïve nature, there was a concern as to whether the DCs generated from them will have enough potential to elicit a proper T cell response. If yes one needs to compare whether the response is as potent as that of DCs generated from, the standard source i.e. autologous/allogenic CD14⁺ cells from PBL. In one of the present studies (Part A) we undertook a systematic approach to compare UCB-DCs/CTLs with PBL-DCs/CTLs using various parameters. PBL and UCB were collected throughout the study, and subsequently screened for HLA-A*0201 polymorphism. Since the basis of CTL assay was HLA-A*0201 restricted, which is an MHC class I polymorphism. DCs and CTLs were generated from HLA-A*0201 positive samples. CTLs were characterized and in-depth study on their functionality was undertaken. Our data demonstrate that UCB-DCs/CTLs are as potent as standard PBL-DC/CTLs and could therefore be used as an allogenic source for therapeutic purposes.

In an attempt to step-up the efficacy of cellular vaccine, in Part B of our study we carried out a new set of experiments to enhance functionality and efficacy of DCs as a therapeutic agent. Although the current literature describes dozens of methodology and scope to improve DC based vaccines, yet some obstacles remain that need to be circumvented. The complexity of the DC system requires noetic manipulation of DCs to accomplish
protective or therapeutic immunity by modulating the immune responses to get the desirable outcome. There have been multiple reports stating that the DCS generated in vitro are functionally compromised as compared to their equivalent counterparts in vivo. Despite the in-depth understanding of the intricacy of DC mediated orchestration of host immune responses, in vitro generated DCs may not truly depict the equivalent state of migratory DCs in vivo, thereby limiting their use as adjuvant to ameliorate the precision and effectiveness of cancer immunotherapy. Recent experimental evidence demonstrates that human monocyte derived DCs (MoDCs) may be handicapped in their migratory property. IL-4 is a substantive cytokine used in the terminal differentiation of DCs. It inhibits many of the downstream pathways of arachidonic acid (AA) metabolism resulting in the afflicted production of eicosanoids and platelet activating factor (PAF), by affecting the release of AA from the membrane. The first step of PGE2 biosynthesis is the release of AA from membrane phospholipids by phospholipases such as phospholipase A2 (PLA2). The inadequacy of PGE2, PAF and eicosanoids may be responsible for the observed impairments of MoDCs (39-40). We speculated that exogenous addition of AA to our cultures at the differentiation step may skew towards a favorable change i.e. help in further improving the functionality of DCs. The rationale for adding exogenous AA was that being a fatty acid it could be readily taken up by the cells and it may get metabolized into prostaglandins in a COX-1 (Cyclooxygenases) or COX-2 dependent manner. To validate this hypothesis, we tested the effect of AA addition on in vitro DCs generation. Our data demonstrated that indeed AA⁺DCs are superior in functions and has a more favorable Th1 cytokine profile than AA⁻DCs.

Data generated from these studies clearly indicate that the UCB-DCs/CTLs are as potent as their standard vaccine counterparts i.e. PBL monocyte DCs/CTLs, and AA⁺ DCs/CTLs are superior to AA⁻ DCs/CTLs. Findings from the two studies highlights the importance of UCB, hence UCB could therefore be used as an allogenic source for therapeutic applications. Thus our data may help to increase the clinical utility of our methodology, by translating the technology from laboratory to the clinics, thus taking us one step closer towards the personalized therapy by better contriving of DC based cancer vaccine.
Aims of this thesis

- *In vitro* generation of CTLs using lysate pulsed DCs
- Comparison of *in vitro* CTL activity of UCB-derived DCs with standard PBL monocyte-derived DCs
- Characterization of CTLs
- *In vitro* analysis of the antitumor activity of the generated CTLs
- Cryopreservation of *in vitro* generated antigen pulsed DCs
- *In vivo* CTL assay
- To increase the efficiency of *in vitro* generation DCs by addition of AA in culture medium during differentiation.