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Emerging Innovations in Cancer Immunotherapy

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Adjuvant Based Immunotherapeutic Approaches To Enhance Patient Survival

The immune system protects the body against illness and infection caused by foreign pathogens. The collection of reactions and responses that the body makes to damaged cells or infection is termed as an ***Immune response***. In most instances, the surveillance mechanisms are competent enough to dispose off the threat without requiring the participation of the adaptive arm of the immune system, which needs more time to become fully effective since it requires T-lymphocyte activation. In order to counter these effects, the cancer cells have evolved several mechanisms to escape Immune surveillance by initiating Immune tolerance. Tumor cells are able to ***escape*** by inducing an expansion of T-regulatory (Treg) cells, leading to delayed or failed recognition of immunogenic cancers.

The last resort to patient survival

For many years, Cyclophosphamide is widely used to treat various types of malignancies. Cyclophosphamide mediates Interferon type I secretion, cell proliferation, and regulates T-cell population in a dose dependent manner. Low doses of Cyclophosphamide have been shown to induce beneficial immunomodulatory effects (immunosuppressive) rather than high (cytotoxic) doses **(Figure 1 & 2)**.

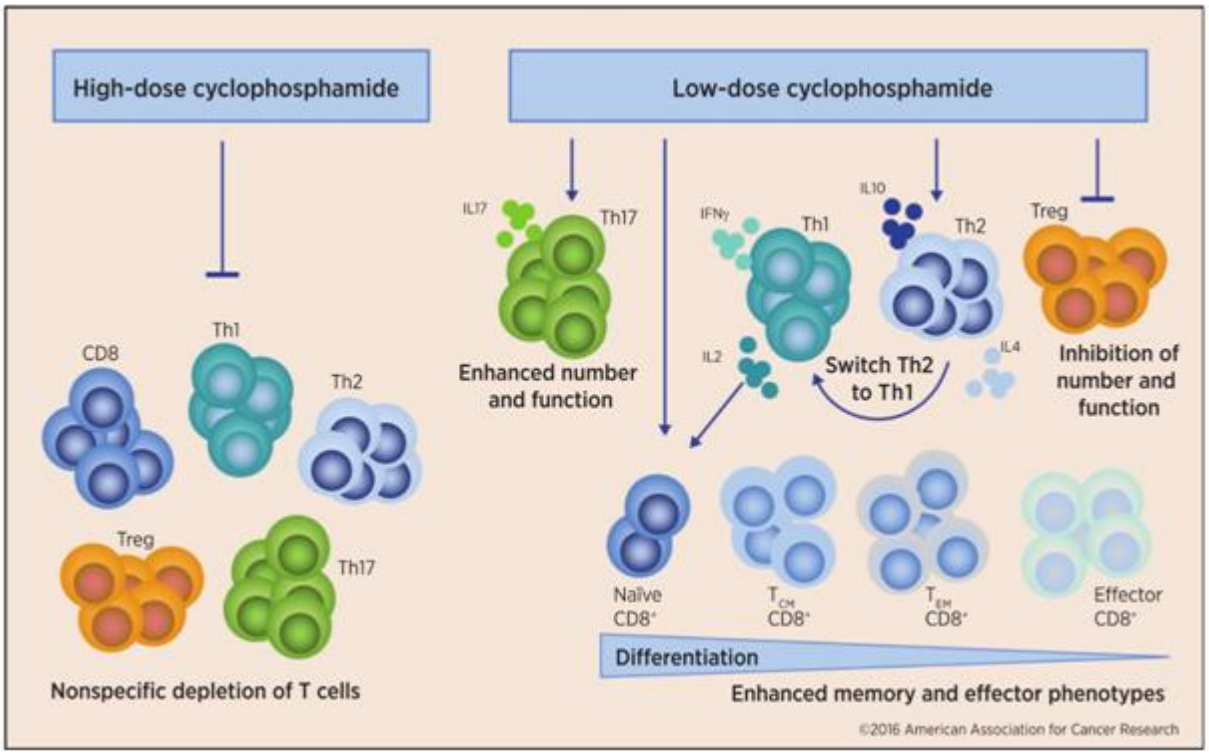

Figure 1: The immune modulatory effect of cyclophosphamide on T cells. When given in high doses, cyclophosphamide leads to nonspecific depletion of T cells. However, at low doses, it exerts a range of effects on different T-cell subsets. These include selective depletion of Tregs and inhibition of their suppressive functions; switching the secretion of cytokines from Th2 to Th1; and enhancement of Th17, memory and effector CD8 T-cell phenotypes. **Data adapted from- *Old-School Chemotherapy in Immunotherapeutic Combination in Cancer, A Low-cost Drug Repurposed (Cancer Immunol Res 2016).***

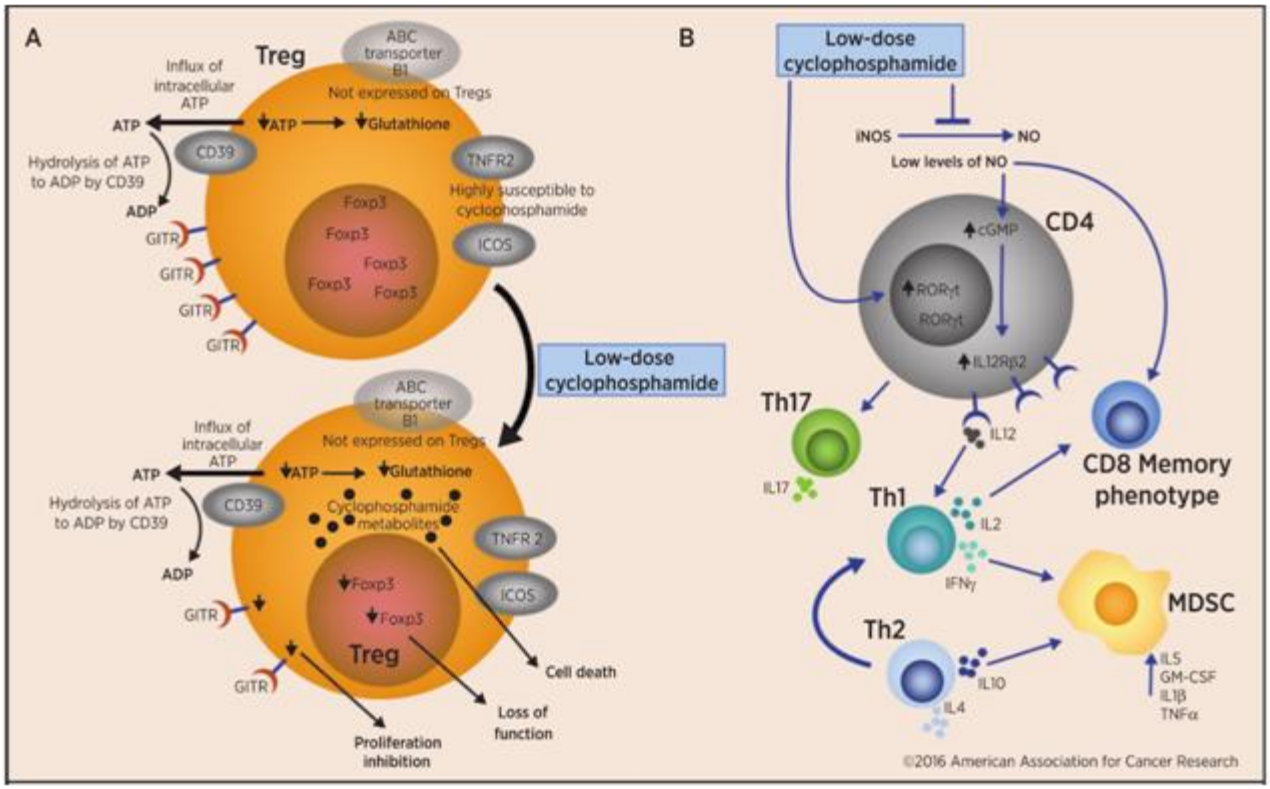

Figure 2: A. The mechanism by which low-dose cyclophosphamide inhibits the number and function of Tregs. **B**. The mechanism by which low-dose cyclophosphamide modulates different immune cells. **Data adapted from- *Old-School Chemotherapy in Immunotherapeutic Combination in Cancer, A Low-cost Drug Repurposed (Cancer Immunol Res 2016).***

Clinical data suggests that cyclophosphamide treatment is known to modulate both adaptive and innate immunity by inducing transient lymphopenia that is associated with amplified ***in - vivo*** proliferation and expansion of antigen-specific T cells coupled with reduction in the number of regulatory T cells and augmented function of tumor-infiltrating T cells in the tumor stroma. Clinical trials conducted by ***Erasmus MC Cancer Institute***, Rotterdam reported that 2 X 50 mg tablets per day of Cyclophosphamide was sufficient enough to induce significant reduction in Treg population.

Intervention- Dendritic Cells (DC) To The Rescue

DC's are specialized antigen-presenting cells that are well equipped with both initiating immunity to pathogens as well as promoting tolerance to self. They are the most potent antigen-presenting cells blessed with the

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ability to attract and interact with immune effector cells including T cells, B cells, NKT cells, and NK cells, thus inducing a primary immune response. Although having a short life span and less population, these cells are capable of dual presentation (MHC class I and MHC class II) to both T cell types thus mounting an effective long-lasting memory response.

Immunotherapy – Design & Preparation

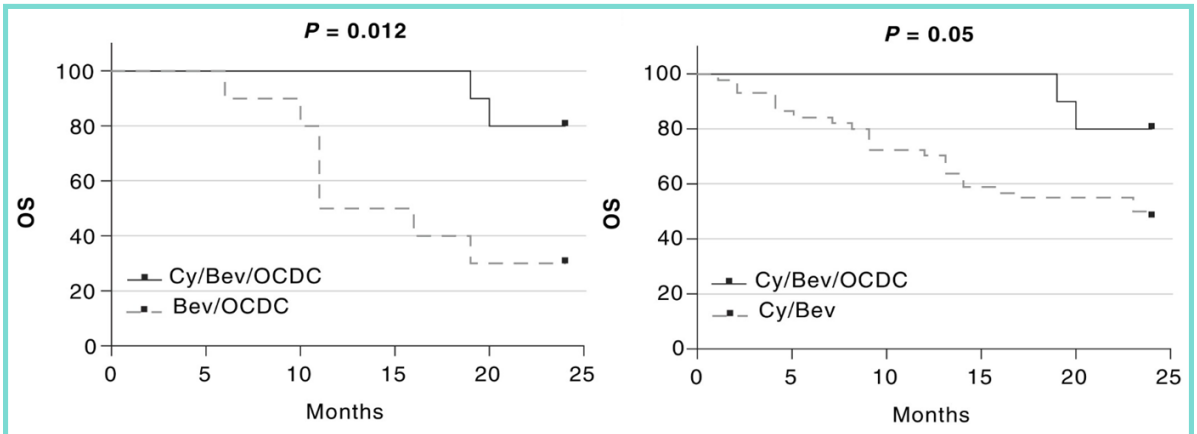
Cancer immunotherapy refers broadly to approaches that attempt to activate an immune response directed against malignant tumor. A number of different immunotherapy strategies have been developed for the same. One such approach is autologous dendritic cell-based therapy. In the last two decades, many popular and established medical companies have tried to exploit the ability of DCs to fight cancer cells. The strategy involves isolation of patient's monocytes (these act as starting materials for DC preparation) via leukapheresis followed by proliferation and maturation of DC's ***ex-vivo***. Post maturation after exposure to patient specific tumor antigens, these specialized cells take up the antigens and now express them on their surface. By the 8th day a population of mature DCs is ready in the lab that undergoes stringent quality checks before being aliquoted into multiple doses each containing a fixed number of DC's that are capable of mounting a memory bound immune response against specific tumor of the patient. Hence completely personalized and autologous (self derived). Following infusion back into the patient system, these DCs migrate to the nearest lymph nodes where they induce activation, differentiation, and expansion of naïve T cells. These activated T cells with enhanced ability to recognize the tumor cells now migrate to the site of tumor and initiate an anti-tumor immune response as well as memory response thereby boosting the patient's immune system.

A variety of strategies have been used such as different source of DCs and their maturation grade, the antigens used, the route of administration, the number of doses administered and use of adjuvants.

COMBINATION BASED THERAPY

Researchers have tried to examine the mechanisms of Cyclophosphamide augmented antitumor immunity, particularly those pertaining to T cell-DC interaction ***in-vivo***. Animal data confirmed that low doses of Cyclophosphamide can help in improving DC function ***in-vivo*** by inducing rapid activation of DC's in the liver and spleen, suggesting that therapeutic modulation of dendritic cells could also lead to altered regulatory T cell function.

In 2005, Ho¨ltl and his colleagues demonstrated that administration of low-dose Cyclophosphamide prior to DC therapy augmented DC-induced anti-tumour response in patients with metastatic Renal Cell Carcinoma (RCC) by increasing median overall survival by ~2.9 months when compared to control group. Since then many trials have been executed on similar lines.



GROUPS	CONTROL	COHORT 1	COHORT 2	COHORT 3
NUMBER	56	5	10	10
DC THERAPY	-	+	+	+
BEVACIZUMAB	+	-	+	+
CYCLOPHOSPHAMIDE	+	-	-	+
OS (12 MONTH)	60%	NA	55%	100%
OS (24 MONTH)	44%	NA	38%	78%

Figure 3: OS of patients treated with OCDC (DC Therapy) plus bevacizumab (Bev) without (Cohort 2) or with cyclophosphamide (Cohort 3). Data adapted from- *Personalized cancer vaccine effectively mobilizes antitumor T cell immunity in ovarian cancer (Sci. Transl. Med. 2018)*

Moreover, pairing a novel personalized cancer therapy with an established immunotherapy drug administered to patients has shown to favor efficient immune response in eliciting an effective adaptive immune response against tumor antigen. A group of investigators from ***University of Pennsylvania*** and the Lausanne branch of the ***Ludwig Institute for Cancer Research*** conducted a Phase II clinical trial on ovarian cancer patients to investigate the effect of combination therapy with low dose of Cyclophosphamide on patient's overall survival. Their extensive clinical findings reported that patients who were administered DC therapy in combination with Bevacizumab and low dose of Cyclophosphamide (Cohort 3) showed an increased overall survival at 12 and 24 months with no side effects when compared to Control group who received Bevacizumab and Cyclophosphamide only **(Figure 3)**.

STUDY	COUNTRY	JOURNAL
Allogeneic dendritic cell vaccination against metastatic renal cell carcinoma with or without cyclophosphamide.	Medical University of Innsbruck, Austria	Cancer Immunol Immunotherapy (2005)
Development of a dendritic cell-based vaccine for chronic lymphocytic leukemia.	Karolinska University, Sweden	Springer (2008)
Pilot Clinical Trial of Type 1 Dendritic Cells Loaded with Autologous Tumor Lysates Combined with GM-CSF, Pegylated IFN, and Cyclophosphamide for Metastatic Cancer Patients.	University of Navarra, Spain	J Immunol (2011)
Phase I/II randomized trial of dendritic cell vaccination with or without cyclophosphamide for consolidation therapy of advanced ovarian cancer in first or second remission.	University of Pennsylvania, USA	Cancer Immunol Immunotherapy (2012)
Metastatic melanoma patients treated with dendritic cell vaccination, Interleukin-2 and metronomic cyclophosphamide: results from a phase II trial.	Copenhagen University Hospital, Denmark	Cancer Immunol Immunotherapy (2012)
Phase I/II clinical trial of dendritic-cell based immunotherapy (DCVAC/PCa) combined with chemotherapy in patients with metastatic, castration-resistant prostate cancer.	Charles University, Czech Republic	Oncotarget (2015)
Extended Tumor Control after Dendritic Cell Vaccination with Low-Dose Cyclophosphamide as Adjuvant Treatment in Patients with Malignant Pleural Mesothelioma.	Erasmus MC Cancer Institute, Netherlands	Am J Respir Crit Care Med (2016)
Personalized cancer vaccine effectively mobilizes antitumor T cell immunity in ovarian cancer.	University of Pennsylvania, USA	Sci. Transl. Med. (2018)
Low-dose cyclophosphamide depletes circulating naïve and activated regulatory T cells in malignant pleural mesothelioma patients synergistically treated with dendritic cell-based immunotherapy.	Erasmus MC Cancer Institute, Netherlands	Oncoimmunology (2018)

Figure 4: Table summarizing key researches on combining Immunotherapy with low dose Cyclophosphamide over the past 15 years.

Closing Remarks

We anticipate that Cyclophosphamide is an applicable agent that allows the host immune system to reduce the proportion of Treg in tumor-bearing patients, thereby allowing for a more complete, sustained and robust antitumor immune response initiated by immunotherapy that is paramount to eliminate the tumor ***in-vivo***. Preclinical and clinical studies have already shown great promise for the combination of this olds chool chemotherapeutic agent with cancer therapies and immune checkpoint inhibitors. Conjugation of immune modulators such as Cyclophosphamide with DC-based immunotherapy harbours excellent potential to significantly enhance the mean and overall survival in cancer patients. These findings now form the basis of new treatment regimens aiming the depletion of Treg population by Cyclophosphamide in combination with DC-based immunotherapy as a multimodality treatment in cancer patients after chemotherapy **(Figure 4)**..