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Original Article

Beyond Alkylation – Cyclophosphamide's Expanding Role in Cancer Therapy and Immunomodulation

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ABSTRACT

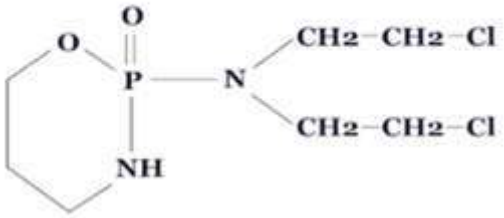
Cyclophosphamide (CTX) is an alkylating cytotoxic agent that primarily targets proliferating lymphocytes. It has been widely used as a chemotherapeutic and disease-modifying agent against several malignancies, lymphomas, and some autoimmune diseases. Depending on the dose and timing of administration, CTX can also improve immune responses. Although the hypothesized mechanism of tumor specificity (activation by cancer cell phosphamidases) turned out to be irrelevant to its activity, it was initially created to selectively target cancer cells. Nonetheless, cyclophosphamide's distinct cytotoxic properties are attributable to its unique metabolism and inactivation by aldehyde dehydrogenase. This review was conducted to identify cyclophosphamide-related toxicities as reported in prior research on that topic. Ninety-one articles were obtained, but only six studies met the inclusion criteria. The studies included a total of 3,531 participants. Cyclophosphamide was linked to a number of toxicities, including liver toxicity, urotoxicity, cardiac toxicity, hematological, and non-hematological toxicities. The toxicity of cyclophosphamide varied depending on the regimen and combination drugs, as well as some gene variants.

Keywords: Cyclophosphamide, toxicity, predictors, outcomes, cancer therapy; drug stability; prodrugs; vesicular systems; nanoparticles; trastuzumab

INTRODUCTION

Cyclophosphamide (CTX) belongs to a class of cytotoxic alkylating nitrogen mustard chemicals, and is also marketed under the trade names ENDOXAN®, CYTOXAN®, ROCYTOX®, and NEOSAR®. The amount of CTX given in animal models and in humans varies according on the treatment objective and plan. Due to its selective cytotoxicity on lymphocytes without being myeloablative, high-dose CTX therapy (≥200 mg/kg) was originally developed as a conditioning regimen for allogeneic bone marrow transplantation to prevent graft-versus-host disease (GvHD).[1] One of the greatest anti-cancer medications ever created is cyclophosphamide. Even fifty years after its invention, it is still widely used in chemotherapy and in the conditioning and mobilization procedures for blood and marrow transplantation (BMT). Cyclophosphamide was found to be the most effective molecule after testing 1,000 chosen compounds and antibiotics against 33 tumors. In 1958, the first clinical trials of cyclophosphamide for the treatment of cancer were carried out, and in 1959, the FDA authorized it as the eighth cytotoxic anticancer drug. [6]

Cyclophosphamide Structure



The purpose of this work is to analyze the English literature on CTX cardiomyopathy and present two instances of this illness that have occurred at Barnes Hospital since the introduction of bone marrow

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transplantation. Despite the high incidence and prevalence of ALL, the therapies are still restricted in their effectiveness and do not have enough support. [4] The effectiveness of cyclophosphamide has been demonstrated in individuals with ALL, and it has also demonstrated encouraging efficacy. However, a systematic review has not yet been conducted to assess the effectiveness of higher-level evidence with more compelling findings. Thus, a thorough review and meta-analysis are necessary to determine the effectiveness and safety of cyclophosphamide in treating ALL. [5] Cancer is a collection of disorders distinguished by aberrant cells that proliferate uncontrollably, invade surrounding tissues, and metastasize to other parts of the body. Cells in the body typically grow, divide, and die in a controlled manner, but this process fails in cancer, resulting in the development of tumors. Tumors can be either benign (non-cancerous) or malignant (cancerous), and malignant tumors have the ability to metastasize, or spread, to other parts of the body and establish new tumors there. The term "anti-cancer" can apply to things that are believed to aid in the prevention or reduction of cancer risk, such as certain foods or lifestyle changes, or to drugs that inhibit the growth of cancer cells during treatment. Chemotherapy, radiotherapy, and immunotherapy are all examples of anti-cancer treatments. In addition to lifestyle changes like quitting smoking, cutting back on alcohol use, and maintaining a healthy level of physical activity, anti-cancer dietary regimens frequently highlight fruits, vegetables, and whole grains as key components in preventing cancer. The "rational use of cancer" is the evidence-based, scientific approach to creating and using cancer therapies that are based on a thorough understanding of cancer biology and the interactions between tumors and their environment. It concentrates on cutting-edge medication delivery methods, targeted approaches, and combination therapies to increase treatment efficacy, get over resistance, and reduce adverse effects. By combining knowledge of cellular mechanisms, gene regulation, and the complicated tumor environment to create more effective and individualized treatment plans, the history of cancer has moved beyond empirical methods.

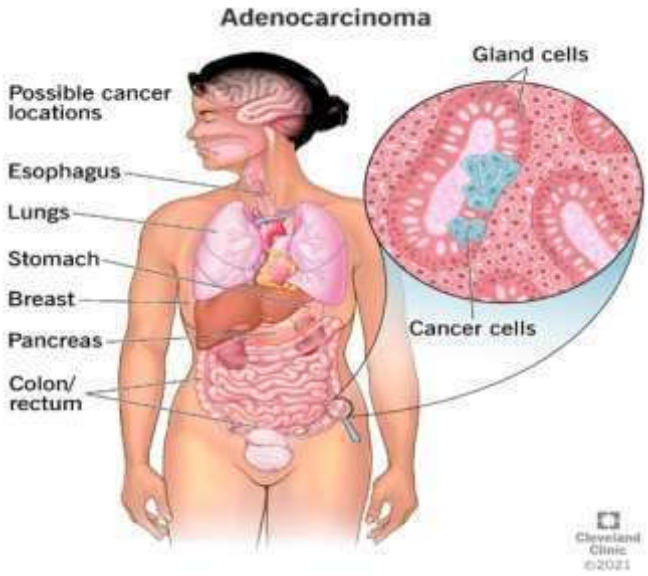

Fig.no 1. Location of the cancer in human body.

2.The History of Cancer

Demonstrates how, from antiquity to the present, our understanding of and methods for treating this complex condition have evolved. Early reports describe the physical effects of malignancies, whereas modern research concentrates on immunotherapy and genetic causes. prehistoric up until the year 500 AD.

• Fossil Evidence: The oldest evidence of cancer comes from human mummies from ancient Egypt and fossilised bone tumours found in dinosaurs over 70 million years ago.

• Egyptian Papyrus: Dating to about 3000 BC, the Edwin Smith Papyrus recounts breast tumours and ulcerations and declares that "There is no treatment."

• Hippocrates and Humoral Theory: About 400 BCE, the Greek physician Hippocrates used the term "carcinos" (Greek for "crab") to describe cancer. He connected the illness to an imbalance in the body's four humours, particularly an overabundance of black bile.

The Middle Ages to the 18th Century

• Galen's Contributions: Building on Hippocrates, the Greek physician Galen referred to tumours as "onkos" (which means swelling) and continued to rely on the humoral theory.Cancer was often seen as a deadly sickness with few treatment options throughout the Middle Ages, when medical knowledge of the disease stalled. Early 20th and 19th Century

• Cell Theory: In the 1840s, pathologist Rudolf Virchow made significant findings, characterising cancer cells as separate cells descended from earlier cells and hypothesising that they resembled the normal cells from whence they arose.

• Surgical Advances: The development of anaesthesia in the 1800s made it possible to perform more complex and frequent cancer surgeries.

• X-rays and Radium: Wilhelm Roentgen's discovery of X-rays and the Curies' discovery of radioactive materials like radium in the late 19th century made radiation treatment conceivable.

• First Radiation Therapy: In 1899, Swedish physicians Tage Sjogren and Tor Stenbeck successfully treated skin cancers with X-rays.

20th and 21st Centuries

• DNA and Genetics: It became evident that cancer is a disease brought on by DNA mutations that cause a lack of control over cell development after Watson and Crick discovered DNA in the middle of the 20th century.

• Chemotherapy and Targeted Therapies: Considerable progress in chemotherapy, followed by targeted medications like tyrosine kinase inhibitors and monoclonal antibodies, allowed for more successful treatment alternatives.

• Increased Cancer Prevalence: The 20th century's higher life expectancy, together with increased environmental and lifestyle factors, led to a notable increase in the incidence and burden of cancer.

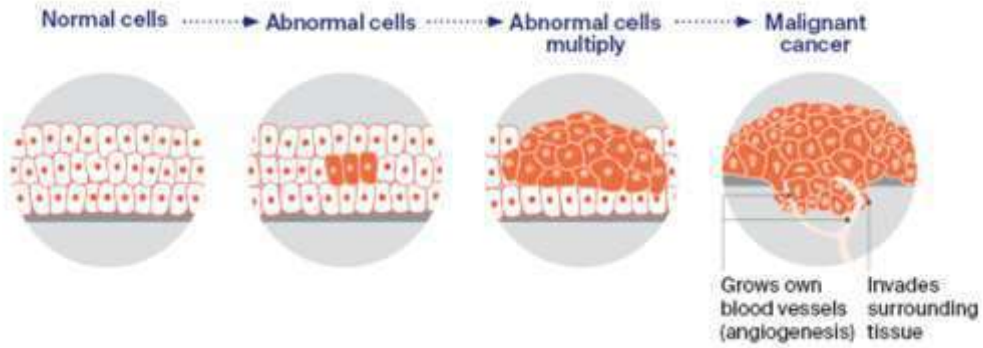

Fig.no 2. Development of cancer cell.

3.Stability of Anticancer Drugs

Most anticancer compounds have had their stability assessed in a variety of experimental conditions. Given the concerning concentration of these chemicals, a number of studies have focused on assessing the presence of certain antineoplastic drugs or their breakdown products in hospital wastewater or sewage systems. In this case, the medications were subjected to benign circumstances, such as room temperature and the natural pH of the water employed as a solvent, for all stability tests. For example, it has been discovered that several cytostatic medications, such as daunorubicin, doxorubicin, vinblastine, chlorambucil, vincristine, irinotecan, and melphalan, are extremely unstable in milli-Q water (pH of 6.3) because their chemical structures contain reactive groups that promote hydrolytic reactions. Specifically, daunorubicin, doxorubicin, irinotecan, and vincristine have rapidly deteriorated; just 10% of the initial concentration remains after only five minutes of exposure. On the other hand, melphalan, vinblastine, and chlorambucil have all been broken down in the first 240 minutes. By altering variables like pH and/or temperature, the stability has been evaluated in an aqueous environment. It has been

demonstrated that mitoxantrone, an alkylating drug widely used to treat chronic myelogenous leukaemia, is very unstable in aqueous formulations. It has been demonstrated that the alkylating agent busulfan (1,4-butanediol dimethanesulfonate), which is frequently used to treat chronic myelogenous leukaemia, is very unstable in aqueous formulations. Because diluted solutions become less stable as storage temperatures rise, the deterioration seems to be temperature-dependent. Although busulfan is taken by infusion, once it is put in a formulation using a concentrate, its shelf life is fairly short. The stability of the solution only marginally increases when held at 2–8 °C, regardless of the kind of container used. The stability profile and presence of cytostatic compounds of platinum (CPC), antineoplastic drugs often employed in therapeutic applications, in hospital wastewater were assessed using inductively coupled plasma mass spectrometry (ICP-MS). Even at low quantities, these chemicals have a negative impact on biota when they enter aqueducts and sewers through the excretion of treated patients. Even though the molecular structures of all the chemicals of the CPCs class, including oxaliplatin, carboplatin, and cisplatin, are identical, they behave quite differently in the environment. These substances really go through diverse processes in the environment, such as hydrolysis, photolysis, dilution, adsorption, sedimentation of suspended particles, and biodegradation, which produce different unchanged compounds or degradation products. Products containing cisplatin are more readily absorbed. [7] Action Mechanism A review of cyclophosphamide's chemistry and pharmacology is required to completely understand its wide range of therapeutic applications. In an attempt to improve nitrogen mustard's selectivity for cancer cells, cyclophosphamide was developed in 1958. 3, 4 The objective was to develop prodrug that was chemically inert but could be metabolised into an active form, primarily the activemolecule in cancer cells. Because some cancer cells express high levels of phosphamidase, which can cleave the phosphorus-nitrogen (P-N) bond and release nitrogen mustard, the chemical design of cyclophosphamide—which replaces the methyl group of nitrogen mustard with an oxazaphosphorine ring—was justified. [3] As a result, cyclophosphamide was among the first drugs that were logically developed to specifically target cancer cells.

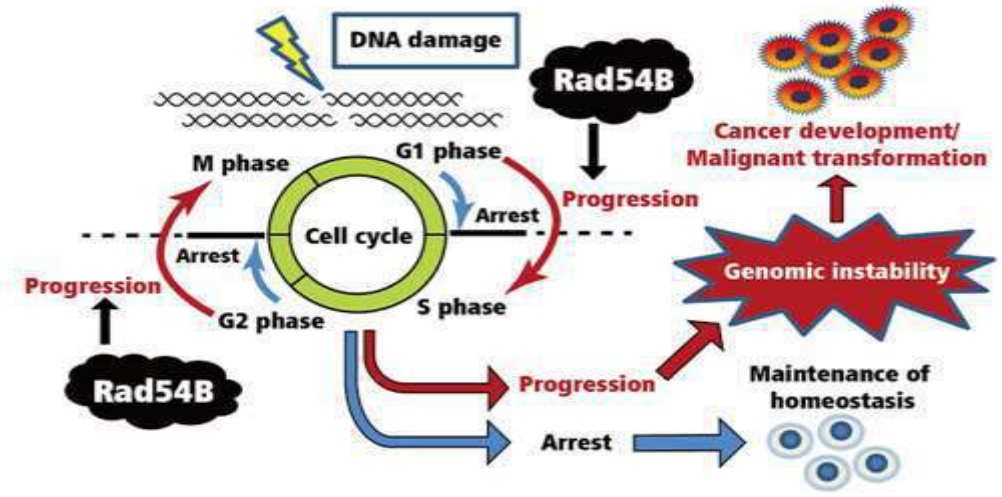

Fig.no 3. Mechanism of action of cyclophosphamide.

The initial theory that cyclophosphamide would work as targeted anticancer therapy via phosphamidase activation turned out to be false, even though it is a prodrug that requires metabolic activation. The reactivity of the 2-chloroethyl groups affixed to the central nitrogen atom is intimately linked to the cytotoxic effect of nitrogen mustard. Under physiological circumstances, nitrogen mustards undergo intramolecular cyclizations by removing chloride to create a cyclic aziridinium (ethyleneiminium) cation. Numerous nucleophiles, including DNA guanine residues, may easily attack this very unstable cation on one of the carbon atoms of the three-membered aziridine ring. [5] By releasing the alkylating agent's nitrogen, this reaction allows it to react with the second 2-chloroethyl side chain and establish a second covalent connection with another nucleophile. This prevents DNA replication by

creating intrastrand and interstrand DNA crosslinks. Cyclophosphamide is an inert prodrug that has to be chemically and enzymatically activated in order to produce active phosphoramide mustard, in contrast to aliphatic (or open chain) nitrogen mustards. The hepatic cytochrome P-450 system hydroxylates the oxazaphosphorine ring to produce 4-hydroxycyclophosphamide, which coexists with its tautomer, aldophosphamide. [2] These unstable transport precursors readily permeate into cells, where they break down aldophosphamide into acrolein and phosphoramide mustard. Six While acrolein causes hemorrhagic cystitis, one of the main toxic effects of cyclophosphamide, phosphoramide mustard creates the intrastrand and interstrand DNA crosslinks that give cyclophosphamide its cytotoxic qualities. [2]

4. Immunomodulatory roles of drug

Immunomodulatory effects

Numerous cellular subgroups, some immunosuppressive and others immunostimulatory, are seen in the tumour microenvironment (TME). The role of these subtypes in MM and how cyclophosphamide affects their behaviour will be discussed in the next section. Tregs, or regulatory T cells By suppressing both innate and adaptive immunological processes, Tregs—an immunosuppressive subset of T-lymphocytes that express CD4 and Foxp3—help promote tolerance to self-antigens and stop the emergence of autoimmune reactions. In particular, memory cells and cytotoxic T lymphocytes with a strong affinity for antigens are disrupted. Tregs are known to be higher in people with MM and monoclonal gammopathy of unknown significance (MGUS), which permits immune evasion and advances the illness, despite some conflicting associations with disease progression. For example, individuals with Treg levels of 5% or greater had a statistically significant shorter time to advancement than those with lower Treg levels, according to a research including about 200 patients with MGUS or MM. However, a smaller experiment with ten patients with MGUS or MM and five healthy subjects could not replicate the results. It has been demonstrated that MM cells stimulate Treg proliferation and activation by releasing interferon-1. The effect of cyclophosphamide on Tregs was initially observed in a 1974 research, prior to the identification of Tregs. 2,4-dinitrofluorobenzene (DNFB) is used topically to induce touch sensitivity in animal models. In this experiment, the sodium salt of dinitrobenzene-sulfonic acid (DNBSO3) was injected intravenously to avoid the development of a cutaneous reaction and to promote tolerance to subsequent DNFB administration. This effect might be reversed by administering cyclophosphamide three days prior to sensitiser exposure. This was shown to be associated with enhanced T cell proliferation, which is thought to be brought on by cyclophosphamide's reduction of a suppressive cellular component. By transmitting the as-yet-unidentified immune-suppressive Tregs, lymph node cells from sensitised mice also decreased future responses in non-sensitized animals. In a mouse model, a cyclophosphamide-sensitive T-cell population was found to be capable of suppressing antigen-specific cytotoxic T-cell lymphocytes (CTLs). The combination of 150 mg/kg cyclophosphamide with the transfer of tumor-specific immune cells resulted in tumour regression in a T cell-deficient L5178Y cyclophosphamide-resistant lymphoma mouse model; cyclophosphamide or immune cells alone had no impact. This showed that suppressor T cells may be eliminated by cyclophosphamide, enabling tumor-sensitized CTLs to operate. It is thought that since Tregs contain less ATP than effector T cells, they are more vulnerable to cyclophosphamide. This is caused by high levels of CD39, which converts external ATP into ADP and creates an ATP sink and intracellular ATP efflux, and low amounts of microRNA (miRNA)-142-3p, which inhibits the conversion of intracellular ATP to cyclic AMP. The production of GSH, which is required to offset the dangerous effects of cyclophosphamide, is hampered by the decreased quantity of ATP. Tregs have fewer efficient DNA repair mechanisms than effector T cells, making them more susceptible to the cyclophosphamide's DNA cross-linking effects. Low dosages of cyclophosphamide can also inhibit Tregs' ability to suppress. The downregulation of the glucocorticoid-induced TNFR family-related gene (GITR), a costimulatory protein that stimulates Treg proliferation, was associated with decreased proliferative ability in a mouse model administered intraperitoneally. The time and amount of cyclophosphamide have an impact on Treg function. Continuous daily treatment may lead to drug resistance and impaired immunomodulation. In one

study, for example, individuals with breast cancer were administered 50 mg of cyclophosphamide twice a day for alternate weeks. Treg function declined and there were fewer Tregs as a result. However, in a trial of individuals with advanced cancer who received 50 mg of the medication every day for almost three months, the percentage of Tregs dropped although their functional ability did not [4]. In an animal model, a 6-day drug-free period was observed to provide consistent CTL levels when compared to intervals of 9 or 12 days. In conclusion, Tregs, an immunosuppressive kind of T cell that is prevalent in MM patients, have been connected to the development of disease and immune escape. Compared to other T cells, they are especially vulnerable to cyclophosphamide-mediated death because to low intracellular ATP levels and compromised DNA repair pathways.

- **Effector T cells**

CD8-expressing T lymphocytes recognise antigen presented by MHC Class I (major histocompatibility complex), which is present on most nucleated cells. Following activation, they release their cytotoxic granules' contents, which kill cells. Effector T cells are severely compromised in MM. People who have a modest tumour burden, monoclonal gammopathy of undetermined significance (MGUS), the pre-malignant type of MM, and longer lifespans are more likely to have tumor-specific T cell expansions. Additionally, as the disease progresses, T cells from MGUS patients produce a lot of cytokines when exposed to their own cancer cells, but T cells from MM patients do not, suggesting that their function is compromised. It has been demonstrated that low-dose cyclophosphamide enhances T-cell responses to T cell receptor (TCR) activation and increases the quantity of tumour antigen-specific T cells in cancer patients. This is only partially due to a decrease in Treg-mediated immune suppression. It has been demonstrated that low-dose cyclophosphamide biases T helper cells from a Th2 profile to a Th1 profile, which is identified by the secretion of IL-2, which encourages memory CTL proliferation. The inhibition of inducible nitric oxide synthase (iNOS), which is necessary for the production of nitric oxide (NO), may help to explain this. Soluble guanylyl cyclase (sGC), which changes guanosine-5-triphosphate (GTP) into 3',5'-cyclic guanosine monophosphate (cGMP), is activated by low NO levels. Following cyclophosphamide treatment, elevated numbers of CD4+ helper T cells that produce IL-17 have also been seen. Interestingly, this discovery may be related to gut flora, specifically gram-positive Lactobacilli johnsonii and Enterococcus hirae. Higher numbers of these Th17 cells and lower levels of Tregs have been associated with improved survival in MM patients. In a mouse investigation, intestinal permeability increased after a modest dosage of cyclophosphamide, and bacteria were shown to move to lymph nodes, where they elicited Th1 and Th17 cell immune responses. Vancomycin, a glycopeptide antibiotic, stopped this effect, although it's still unclear what causes cyclophosphamide-induced intestinal permeability. The incapacity to produce a sufficient adaptive immune response is a common mechanism of immune evasion across cancer subtypes. Immunogenic cell death (ICD) is a kind of apoptosis that can initiate an adaptive immune response against antigens derived from cancer or infections. The ability of cytotoxic chemicals to cause an immunogenic kind of controlled cell death varies. ICD's underlying procedures are well known and have already been discussed. In summary, one of the main triggers is the endoplasmic reticulum (ER) stress response. ER stress occurs when a cell has an excessive number of unfolded or misfolded proteins. Significant ER stress is brought on by MM cells' high intracellular immunoglobulin levels. Calreticulin travels to the cell surface due to compensatory mechanisms, where it acts as a "eat me" signal, encouraging phagocytosis and dendritic cell activity, which in turn increases the activation of tumor-specific cytotoxic T lymphocytes. Danger-associated molecular patterns (DAMPs) are essential supplements to this process in the context of cancer. Cyclophosphamide has been shown to induce ER stress and signs of ICD in mice models of thymoma and high-grade lymphoma, but there is no solid evidence that this mechanism plays a role in the in vivo response to cyclophosphamide observed in multiple myeloma. More study is required in this area, because ICD and subsequent antigen presentation to the adaptive immune system are thought to be an important first step in a cascade of immunomodulating effects due to cyclophosphamide, given the dependence of myeloma cells on ER stress pathways. In summary, effector T cell responses are

compromised in MM patients. Low-dose cyclophosphamide increases tumor-specific T cell activity by reducing the quantity and activity of Tregs, changing the phenotype of T helper cells from Th2 to Th1, increasing the amount of Th17 cells, and inducing ICD.

- **Dendritic cells (DCs)**

DCs are skilled antigen-presenting cells (APCs) that create an essential link between the innate and adaptive arms of the immune system. High levels of IL-6 in the blood have been shown to impair DC generation and function in myeloma patients. As a result, CD34+ cells develop into monocytic cells that can phagocytose but cannot deliver myeloma epitopes to T cells, activating them. Furthermore, DCs come in two primary subtypes: myeloid and plasmacytoid. More plasmacytoid DCs, which may potentially aid in the development, survival, and dissemination of MM cells, are seen in the bone marrow of MM patients. Large amounts of PD-L1 (programmed death ligand 1), which inhibits T cells, are produced by these plasmacytoid DCs. DCs isolated from treated animals generated stronger allogeneic and antigen-specific T cell proliferation and released more IL-12 than mice not exposed to cyclophosphamide. Cyclophosphamide has been used to increase responses to DC vaccine-based immunotherapies in murine models and early-stage human studies for renal cell carcinoma with encouraging results. In conclusion, cyclophosphamide can restore the compromised DC activity that results in decreased T cell activation in individuals with multiple myeloma.

Macrophages

Macrophages influence the development and course of MM and other malignancies. Cytokines and chemokines, produced by tumor cells and bone marrow stromal cells (BMSCs), recruit and activate circulating monocytes to form tumor-associated macrophages (TAM). When macrophages are activated, they become polarized with either an M1 or an M2 phenotype. M1 macrophages are pro-inflammatory and frequently produce high amounts of TNF-α and IL-12 in response to illnesses. Increased levels of M2 macrophages, which have immunosuppressive effects and encourage angiogenesis that promotes tumor growth, have been observed in MM patients with progressive disease as compared to those in remission. In general, TAMs resemble M2 macrophages more closely and provide pro-tumorigenic signals. In vitro experiments have revealed that IL-12, which is often produced by M1 macrophages as opposed to M2 macrophages, may suppress angiogenesis in myeloma cells and, in a mouse model, can slow tumor development after injection with numerous myeloma cell lines. The previously mentioned function of IL-12 in fostering the growth of Th1 helper T cells, which promote the proliferation of memory CTLs, has been discussed. Additionally, Th1 cells release IL-2, interferon-gamma, and TNF-β, all of which activate macrophages. Furthermore, lenalidomide, an immunomodulatory agent, has been associated with the predominance of M2 macrophages, which are known to be resistant to combination therapies like the anti-CD38 monoclonal antibody daratumumab. In a murine model, one group assessed macrophage phenotype and function after giving cyclophosphamide at a dose of 50 mg/kg. These macrophages exhibited higher levels of pro-inflammatory IL-6 and IL-12, which are linked to the M1 phenotype, as well as lower amounts of anti-inflammatory IL-19 and TGF-β, which have been found to cause immune-suppressive Tregs. The study by Pallasch et al., which utilized a malignant B-cell line that was resistant to the CD52 monoclonal antibody alemtuzumab, revealed that the secretion of prostaglandin E2 (PGE2) by the malignant B-cells prevented macrophage-mediated phagocytosis. Cyclophosphamide and alemtuzumab worked together to nearly eradicate the malignant cells, something that other alkylating agents were unable to achieve. The cell line's exposure to cyclophosphamide resulted in an 'acute secretory activation phenotype' (ASAP), which was characterized by the B-cells' production of tumor necrosis factor-α (TNF-α) and vascular endothelial growth factor A (VEGF A), as well as a decrease in the expression of the macrophage-suppressive PGE2. In a phase 1b clinical experiment of upfront daratumumab with cyclophosphamide, bortezomib, and dexamethasone in transplant eligible patients (NCT02951819), a low dose of cyclophosphamide has similarly been shown to induce a secretory response in MM cells, leading to enhanced macrophage-mediated antibody dependent cellular phagocytosis (ADCP) in daratumumab-

treated MM cells both in vitro and in vivo. Cyclophosphamide-conditioned macrophages were found to have higher levels of CD64 Fc gamma receptor expression, which is necessary for ADCP, while MM cells had lower levels of the 'don't eat me' antigen CD47, which may have further improved phagocytosis. Furthermore, the increased expression of SLAM-F7 on the surface of MM cells points to a potential synergy with elotuzumab (anti-SLAM-F7 monoclonal antibody). In conclusion, MM patients with progressive disease exhibit elevated amounts of anti-inflammatory M2-phenotype TAMs. At low dosages, cyclophosphamide causes a rapid secretory response in MM cells, which increases anti-tumor phagocytic activity.

- **Cells that suppress myeloid origin (MDSCs)**

MDSCs are a diverse collection of myeloid-derived cells that are CD33 positive and are often either CD14 positive monocytic MDSCs or CD14 negative granulocytic MDSCs, with the latter being more common in individuals with MM. These cells are immunosuppressive in a variety of ways, helping tumors survive. Through arginine depletion, nitric oxide production, and reactive oxygen species, effector T cells are weakened. MDSCs create PGE2, which inhibits macrophage phagocytosis. They also induce NK cell energy through PGE2 and TGF-β signaling, as well as promote the proliferation of immunosuppressive Tregs via TGF-β-dependent and independent pathways. Although cyclophosphamide has a variety of ways to improve immune function in myeloma, it has also been shown to cause MDSCs, which compromise T-cell antitumor responses. But in a mouse model, this was seen using 100–300 mg/kg, but not at lower dosages (10–40 mg/kg). The authors hypothesized that cytokine release in response to leucodepletion may be contributory, and perhaps this does not occur with lower doses. In conclusion, MDSCs possess a number of immunosuppressive properties. High quantities of cyclophosphamide may cause them, but this has not been observed at lower doses.

- **Cells that kill naturally (NK)**

The innate immune system's essential component is NK cells. Their activity is governed by a delicate balance between signals generated by activating and inhibitory NK receptors, which recognize ligands expressed by tumor cells or virally-infected cells. In MM, a number of processes impair NK cellular function. TGF-β, which is produced by MM cells and Tregs, downregulates NK-activating receptors and impairs cytotoxicity. IL-6, which inhibits NK cell function, and PGE2 from MDSCs both inhibit NK activation via the natural cytotoxicity regulators (NCR), NKG2D and CD16/FcγRIIIA receptors (reviewed in) (18). In a study of nine chemotherapy-resistant cancer patients who received metronomic cyclophosphamide at 50 mg twice daily on alternate weeks, the absolute number of circulating Tregs decreased after 30 days, along with a corresponding increase in NK cytotoxicity, which improved to levels that were not significantly lower than those seen in healthy donor controls. Selective depletion of this population did not further improve NK function, indicating that the remaining Tregs had lost their NK inhibitory capacity. In their study, Pallasch et al also demonstrated that pre-incubation of NK cells with conditioned media from cyclophosphamide-treated leukemia cells significantly improved alemtuzumab-induced NK-mediated ADCC. While the presence of PGE2 markedly reduced ADCC, the addition of VEGF or TNF-α, which were produced by the leukemia cells in response to cyclophosphamide, greatly enhanced it. To sum up, in MM patients, the production of TGF-β by Tregs, IL-6 by MM cells, and PGE2 by MDSCs impairs NK cell activity. Cyclophosphamide enhances NK cell activity by decreasing the frequency of Tregs, lowering the production of PGE2, and increasing the release of pro-inflammatory cytokines. [8]

5.Cyclophosphamide Uses

- Cancer
- Malignant Melanoma
- Nephrotic Syndrome
- Hodgkin’s Disease
- Multiple Myeloma
- Brain Tumor
- Blood Cancer
- Breast Cancer
- Non-Small Cell Lung Cancer
- Ovarian Cancer
- Lung Cancer

6. The mechanism of action of cyclophosphamide

Cyclophosphamide is an anti-cancer medication. The alkylating chemical stops cancer cells from reproducing and developing by damaging their genetic material (DNA and RNA). It combats cancer in this way.

7.Advantages

- Cyclophosphamide is a crucial component of several combination chemotherapy regimens used to treat a wide range of malignancies. It works against.
- Leukemias and lymphomas include multiple myeloma, mycosis fungoides, Hodgkin's and non-Hodgkin's lymphoma, and acute and chronic leukemias.
- Breast, ovarian, and lung cancers are examples of solid tumors.
- Children's tumors: retinoblastoma and neuroblastoma.
- Strong Immunosuppression: The drug's capacity to inhibit the immune system makes it extremely helpful in treating severe autoimmune and inflammatory disorders in which the immune system is hyperactive. It works especially well for:
- Vasculitis: Frequently seen as the medication of choice for illnesses such Wegener's granulomatosis (granulomatosis with polyangiitis) and polyarteritis nodosa.
- Systemic Lupus Erythematosus (SLE): Particularly in cases of severe organ involvement, such as lupus nephritis.
- Other Autoimmune Disorders: Severe ocular inflammatory diseases, systemic sclerosis-associated interstitial lung illness, and certain kinds of rheumatoid arthritis.

❖ Corticosteroid-Sparing Effect: Cyclophosphamide therapy can frequently reduce the amount of systemic corticosteroids used in the treatment of autoimmune diseases, which helps to lessen the adverse effects of prolonged steroid use.

❖ Usage in Transplantation: To prepare the patient's immune system and avoid graft rejection prior to bone marrow or hematopoietic stem cell transplantation, it is an essential component of conditioning protocols.

❖ Adaptable Delivery: It can be given orally (tablets or capsules) or intravenously, giving treatment regimens the flexibility to meet the particular illness and patient needs (e.g., monthly intravenous pulses or daily oral dosages).

❖ Proven Effectiveness: Cyclophosphamide was first used in clinical practice in the 1950s and has a long history of documented effectiveness, which has allowed for a thorough understanding of its risk-benefit ratio. [9]

8. Disadvantages

• Gastrointestinal Issues: Common problems include mouth ulcers, nausea, vomiting, diarrhoea, and appetite loss.
• Hair Loss: Alopecia, or hair loss, including eyelashes and eyebrows, is a common side effect even though hair usually comes back after therapy.
• Myelosuppression (bone marrow suppression): The drug lowers the bone marrow's ability to produce enough blood cells, which leads to:
• Low white blood cell counts (neutropenia/leukopenia) increase the risk of infection.
• Anaemia: Fatigue and dyspnoea are brought on by low red blood cell levels.
• Thrombocytopenia, or low platelet count, is the cause of bruising and bleeding.
• Weariness and Debility: A general lack of vigour and strength.
• Modifications to the Nails and Skin: Possible symptoms include a skin rash, darkening of the skin and nails, and trouble healing wounds. [10]

9. Patient Precautions

• Hydration: Urinate frequently, especially after taking the drug, and drink a lot of water (2–3 quarts per 24 hours, or as directed by your doctor). You might be able to prevent serious bladder problems like hemorrhagic cystitis by doing this.
• Dose Timing: Take oral cyclophosphamide first thing in the morning to guarantee adequate fluid intake and bladder emptying throughout the day.
• Contraception and pregnancy: Cyclophosphamide may result in serious birth defects. Women should use effective contraception during treatment and for up to a year after the last dose. Men should utilise effective

contraception during therapy and for four months following the last dosage.

• Breastfeeding: Avoid breastfeeding while taking this drug and for at least a week following the final dosage since it may pass into breast milk and harm the infant.

• Risk of infection: Cyclophosphamide increases the risk of infection by weakening the immune system. Wash your hands often, avoid persons who have active illnesses like the flu, chickenpox, or common cold, and notify your doctor immediately if you get any signs of an infection (fever, chills, cough, etc.).

• Risk of Bleeding: The drug may lower platelet levels, which increases the risk of bleeding. Use a soft toothbrush, exercise caution near sharp objects (razors, nail cutters), and notify your doctor immediately if you see any unexplained bleeding or bruises.

• Alcohol and Other Drugs: Talk to your doctor about your alcohol consumption because certain liquid preparations contain alcohol and excessive alcohol consumption may interfere with therapy. Tell your doctor about any prescription, over-the-counter, herbal, or vitamin supplements you use to avoid potentially dangerous medication interactions.

• Immunisations: Do not receive any vaccinations or immunisations while taking cyclophosphamide without your doctor's approval.

• Dental Work: Inform your dentist that you are taking this medication before undergoing any dental procedures.

• Handling: Take oral pills or capsules whole. Do not break, eat, or open them. To avoid direct exposure, carers should wear gloves when giving the medication.

• Sun Exposure: Use sunscreen and spend as little time in the sun as possible because the drug may make your skin more vulnerable to the sun.

FUTURE DIRECTIONS

Investigating its use in metronomic regimens, combining it with more recent immunotherapies like PD-1 antagonists, and developing more tailored versions to reduce toxicity are a few examples of cyclophosphamide development. Additionally, research is being done on customised medicine techniques that use patient-specific data to determine who might benefit most from cyclophosphamide therapy. It is being researched for a number of applications, including high-dose post-transplantation treatment to support immunological recovery after stem cell transplants and the development of new medications. Targeted drug design: Scientists are developing new, safer, and more potent cyclophosphamide-based drugs. To improve efficacy, some more recent versions, for example, aim to increase the concentration of phosphoramide mustard (PAM), the active metabolite, within tumour cells. Reducing harmful byproducts: Attempts are undertaken to stop the creation of dangerous byproducts such chloroacetaldehyde, which can have negative consequences including hemorrhagic cystitis. [12]

CONCLUSION

Because CTX may directly target immune cells without inducing myelosuppression, it is a potent immunosuppressive drug. 28, 31 As a result, it is now utilised therapeutically as a conditioning regimen for allogeneic bone marrow transplantation and to treat some autoimmune illnesses. Cyclophosphamide was linked to a number of negative side effects, including haematologic and non-hematologic toxicity, liver damage, urotoxicity, and cardiotoxicity. The toxicity of cyclophosphamide varies according to the regimen, combination medications, and specific genetic variants. Further investigation is required to investigate the gene variations associated with cyclophosphamide-induced toxicity, as certain gene polymorphisms do not affect toxicity. Additionally, it was shown that while mensa therapy does not reduce the associated adverse effects, cyclophosphamide dosage reduction does. This is the first comprehensive investigation and study of the toxicity of cyclophosphamide. Further study is required since little is known about the toxicity of cyclophosphamide.

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