Nano formulation of Paclitaxel for Targeted Drug Delivery: Advancing Precision Therapy

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Abstract

Cancer treatment dates back to the eons, as since 2500 BCE people are in search for the alleviation of such life-threatening disease, which is evident in the ancient literature and writing by scholars. Through these efforts, we were able to identify paclitaxel (Biological source – Taxus brevifolia nutt) to be a promising clinical agent, by showing efficacy in a number of solid-state tumors. Paclitaxel had an edge over other anti-neoplastic substances in the sense, it showed desirable pharmacokinetics, proving its effect to be a major cytotoxic chemical. The way it acts upon the malignant cells is quite common including, the enhancement of apoptosis/programmed cell death, potentiated by blockade of tubulin polymerization (synergism shown by these two processes is still vague to us). Though, the resistance towards single drug paclitaxel therapy is a kind of concern amidst it's notable upsides. After which, the intention to introduce nano-formulations of paclitaxel was witnessed. Basically, exploration of their additive capabilities following the minimization of its potent toxicity (Cardiac), hypersensitivity (Type 1) and human immunological intervention (mediating in the actions of B-cell, T-cell and NK cell). At this current pace, researchers hold a couple of options in terms of paclitaxel nano-formulations, starting from nanospheres several organically and inorganically amalgamated nanoparticles are readily accessible. Their activities in some popular cases namely – lung, breast and ovary carcinoma have broadened horizon towards newer therapeutic opportunities.

Keywords – Paclitaxel, Taxus, Nano-formulations, Cancer, Immune system

1. INTRODUCTION

Cancer is gradually turning out to be a catastrophic disease for the human race as a result of lack of proper treatment methodology. [1] Cancer generally is a term signifying a group of disease symptomatically uncontrolled and abnormal cell division and growth leading to a protruding mass of lump made of malignant cells, which happen to be dividing without and bounds, generally leading to mortality in most of the cases. [1,2] It has the recorded seconded highest morbidity and mortality rate in humans following cardiovascular diseases. [3]

Generally, mitosis is a spontaneous process in which new cells divide at a constant pace in order to replace the dead cells proliferating in a controlled fashion. A cell is deemed cancerous when it is somehow interrupted in its mitosis cycle causing it to uncontrollably multiply surpassing the rate of apoptosis/cell death, leading to the formation of a tumor. [3] Cancer is mostly in the form of a tumor but not all tumors are cancerous leading us to the conclusion of tumors being of 2 types- malignant/cancerous and benign/non-cancerous. Similarly, cancer can be broadly classified into 5 types- carcinoma, lymphoma, leukemia, sarcoma and melanoma. Carcinomas are the most reported cancers, developing in organs and glands, mostly skin, lungs, breasts and pancreas. [24] Lymphomas refer to the malignancies of the lymphocytes. Leukemia is a malignancy of blood, rather than typically solid-state tumours. Sarcomas develop in soft or connective tissues in the body, mostly seen in muscle, cartilage, fat, bone and blood vessels,

and are sporadic. Melanomas are malignancies, that develop in the cells that produce pigment in the skin. [1,3,24]

Traditionally in ancient India, ‘gulma’(tumor) or ‘karkat’(cancer) had been treated surgically followed by application of some medicinal herbs as mentioned in the Sushruta Samhita, leading back as up to 2500 BCE and Ayurvedic system of medicine derived from India also described the remedy to cancer being from a certain species of plants. Eber papyrus happened to state the same back in 1500 BCE. Later in the late 1940’s, certain types of cancers were recognized to be treatable by systemic chemotherapy if diagnosed. In the current scenario with highly developed treatment methodologies, therapy of cancer in advanced stages has been made possible because of new drug molecules, improved surgical systems, combinational therapies and novel drug delivery system. [2]

Paclitaxel, a broad-spectrum chemotherapeutic agent, is a microtubular inhibitor causing mitosis arrest, isolated from the bark of the yew tree Taxus brevifolia nutt, found out to be effective against a plethora of cancer cells most of which being in the form of solid-state tumors. In the late 1960’s, National Cancer Institute of India initiated new cancer drugs screening and discovery program with paclitaxel as an indicative efficacy and activity. [17]

Nanomedicine technology is currently being employed far & wide in clinical practice in improving the process of medication development for individualized treatment. At present, over 40% of new medications are either poorly soluble in water or entirely insoluble, which limits their ability to accomplish the intended therapeutic benefits. In therapeutic settings, using excessive doses or continuous infusion can lead to unwanted side effects. [8]

Nanotechnology in pharmaceutical formulations can improve medication solubility, selective targeting, and controlled release. Various micro/nano modalities, such as liposomes, polymeric/copolymeric micelles, nanospheres, nano capsules, cyclodextrin complexes, and surfactant micelles, have been utilized to make insoluble pharmaceuticals water-soluble. However, each of them has its own set of advantages and disadvantages. In-vitro tests previously demonstrated that the drug's nano formulation significantly improves its drug therapeutic efficiency, efficacy and physicochemical properties, without altering the chemical structure against cancer cells compared to paclitaxel alone. [8]

2. OBJECTIVES

- To perform therapy for malignant cells and cause apoptosis of the root malignant cells.
- To avoid dose dumping of other anti-neoplastic agents administered through topical, enteral or parenteral routes.
- To exercise target-oriented cell specific drug delivery system to enhance overall efficacy of the drug.
- To avoid the severe adverse drug reactions caused by enteral or parenteral administration of the anti-neoplastic drug.
- To provide a path for easy movement of drug from the targeted site post action for an easy elimination.

- To provide a cost-effective method of cancer treatment over the traditional ingestive chemotherapy.
- To continuously exterminate the spontaneously growing Malignant cells throughout prolonged period of time.
- To testify to the concept of Multidrug resistance and prove its inability to kill malignant cells spontaneously dividing and growing.
- Precision medicine to personalize dosage in order to tackle drugs with narrow therapeutic windows.

3. PROPERTIES OF PACLITAXEL

3.1. PHYSICAL PROPERTIES

Paclitaxel is a crystalline powder along the spectrum of white to off-white. It is biphasic, highly lipophilic, water insoluble and melts at about 216-217ºC. [1,2]

3.2. CHEMICAL PROPERTIES

Paclitaxel is a diterpenoid pseudoalkaloid with the $C_{47}H_{51}NO_{14}$ chemical formula, 853.9 g/mol molecular weight and 10-20 mg/L estimated water solubility. [2]

Systemic/ IUPAC Name of paclitaxel is (2α,5β,7β,10β,13α)-4,10-Diacetoxy-13- [(2R, 3S)-3-(benzoylamino)-2-hydroxy-3-phenylpropanoyl] oxy1,7-dihydroxy-9- oxo-5,20-epoxytax-11-En-2-yl benzoate. [2]

It is simply a taxane ring containing a 4-membered oxetane ring at sites C_4 and C_5, as well as a functional homochiral esteric side chain at C_{13}, connecting to the microtubules in a guanosine triphosphate-independent manner to produce cytotoxicity. It has an estimated water solubility of 10-20 mg/L. [1,2]

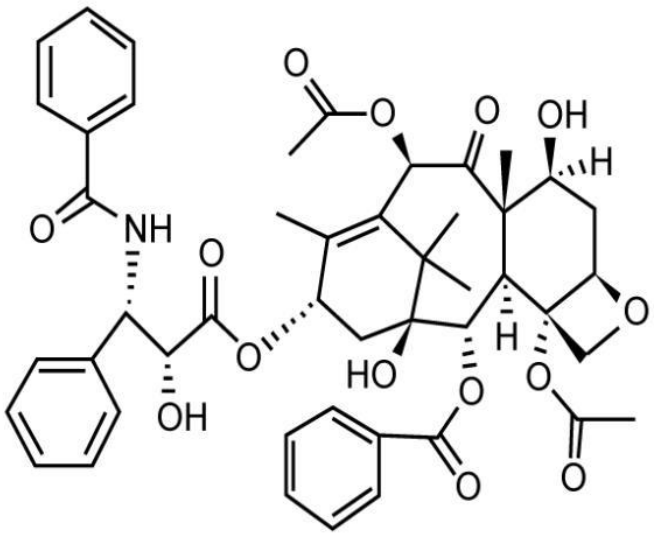

Fig. 1: Chemical structure of Paclitaxel [3]

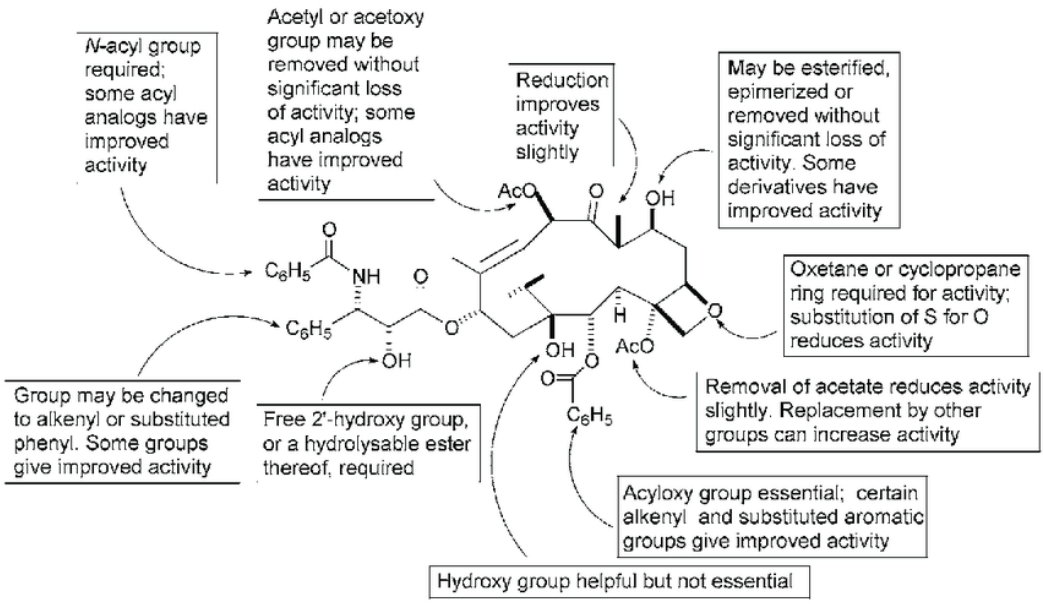

Fig. 2: Structural Activity Relationship of Paclitaxel [1]

Table 1: Significant Milestones in the Development of Paclitaxel as Chemotherapeutic Agent over the years [2]

YEAR(S)	DEVELOPMENT
1962-68	Cytotoxic agents screening obtained from natural products performed by NCI
1967	Detection of Anti-tumor activity for first time
1969	Isolation of Pure Paclitaxel
1971	Elucidation of Structure
1983	Initiation of Phase I studies
1986	Study for hypersensitivity reactions
1988	Premedication regime suggestion approved by NCI
1989	Anti Ovarian Cancer efficacy proved
1991	Anti Breast Cancer efficacy proved
1992	Anti non-small cell lung cancer efficacy proved
1992	USFDA approves Paclitaxel for Ovarian Cancer
1994	USFDA approves Paclitaxel for Breast Cancer Nicolaou and Holton independently perform Total Synthesis successfully
1994	Anti Ovarian Cancer Approval received in India
1995	Anti Breast Cancer Approval received in India

4. PHARMACOLOGY OF PACLITAXEL

4.1. MECHANISM OF ACTION

Microtubules are tubulin polymers existing in dynamic equilibrium along with heterodimers including protein subunits alpha and beta. Although the primary purpose is to aids mitotic spindle formation, during mitosis. Microtubules are significantly essential for interphase processes including cell transduction, shape, motility, intracellular transport and signal transduction. In contrary to other antimicrotubular targeting drugs causing microtubule disintegration, paclitaxel promotes tubulin polymerization. Paclitaxel-induced microtubules are highly resilient and defective, bring forth apoptosis by interrupting normal microtubules dynamics required for interphase processes aforementioned in cell division. [29]

Generally, the taxane class of drugs perform biological processes in the molecular level leading to apoptosis or programmed cell death. Even at concentration in which tubulin polymerization are not executed, cell escape the meiosis cycle but cell division spontaneously goes on. Instead, extensive DNA fragmentation of apoptosis is noted and within 2-3 days post taxane treatment, cell death is observed. [25] Taxanes have demonstrated their interaction with innumerous regulatory proteins and cancerous genes, that bind to the mitotic apparatus, while the exact mechanism conjugating the mitotic arrest to the apoptosis remains undiscovered. [25,29]

Paclitaxel alongside these also happens to induce the TNF-α gene expression, yet the structural activity relationship studies intensively indicate that the effects of paclitaxel in microtubular assembly are not akin to the slightest with these aforementioned activities. [3,29]

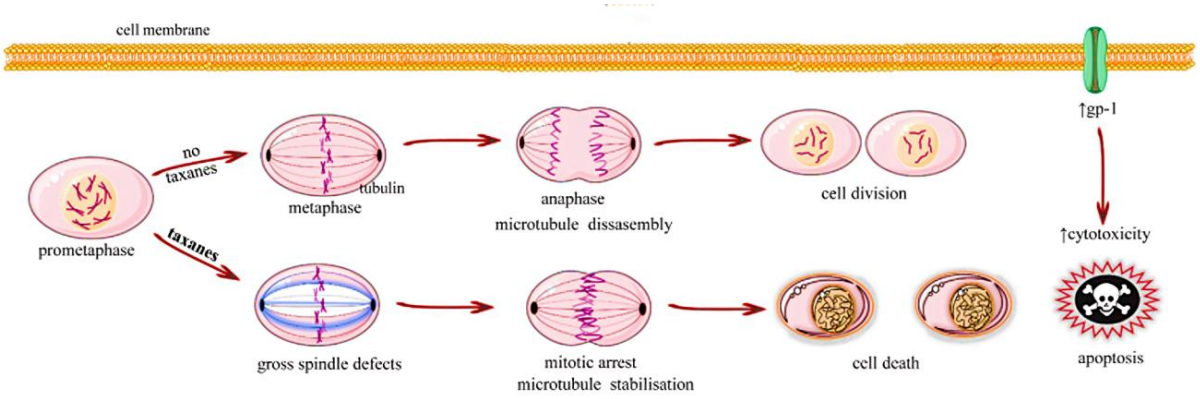

Fig. 3: Mechanism of action of Paclitaxel on the cellular level [25]

4.2. MECHANISM OF RESISTANCE DEVELOPMENT

Paclitaxel resistance can be a resultant of overexpression of ABC transporters/ ATP binding cassettes, among other factors. Mutations in β-tubulin binding areas, decreased function of apoptotic proteins (viz. p53 and Bcl2) reforms the cytokine production (viz. interleukin 6) and CYP-mediated Paclitaxel detoxification. [1,10] ABC transporters utilize ATP hydrolysis to transport substrates, penetrating the semi-permeable cell membrane. Increasing the generation

of ABC transporters caused outflow of Anticancer drugs can diminish the efficacy and establish Multidrug Resistance (MDR) cells by pumping out the drugs from the cells. [3,10]

4.3. PHARMACOKINETICS

ABSORPTION: Absorption through a 24-hour window of infusion of 135 mg/m^2 was provided to an Ovarian cancer patient was found to achieve a maximum plasma concentration of 195ng/mL and the area under curve (AUC) was 6300 ng/mL/h. [27]

DISTRIBUTION: Apparent Distribution Volume (V_d) was found to be varying between a steady range of 227-688 L/m^2 by 24-hour infusion in a steady state. Post distribution, the plasma drug complexation was achieved from around 88% up to a superficial 98% and presence of drugs like cimetidine, ranitidine, dexamethasone and diphenhydramine in systemic circulation wouldn't affect the distribution or protein binding of the Paclitaxel. [27]

METABOLISM: Paclitaxel is primarily metabolized in the liver as observed as per in-vitro investigations performed with microsomes obtained from human liver and tissue slices. Bio transformation of paclitaxel into 3 metabolites were noted-

- Paclitaxel was primarily metabolized into 6a-hydroxy-paclitaxel by the liver enzyme Cytochrome P450 Isozyme CYP2C8 through aliphatic hydroxylation. [27]
- Paclitaxel was bio transformed into 2 secondary metabolites namely- 3'-p-hydroxypaclitaxel & 6a-3'-p-dihydroxypaclitaxel, by the liver enzyme CYP3A4. [27]

ELIMINATION: The rate and route of drug elimination was observed after administration of radiolabeled paclitaxel given at a 3-hour window with 225-250 mg/m^2 infusion rate to 5 human volunteer patient and the observation was made that the elimination of 71% of the radioactivity was through fecal disposal post 120 hours administration and 14% was retrieved from the urine in several batches of traces in urine.

In a 24-hour window, 135 mg/m^2 infusion was provided to a patient with ovarian cancer, and the half-life ($t_{1/2}$) of paclitaxel was found out to be 52.7 hours.

Clearance: For infusion rate of 24 hours-

- 21.7 L/h/m2 [Dose 135 mg/m2, infusion duration 24 h]
- 23.8 L/h/m2 [Dose 175 mg/m2, infusion duration 24 h]

For infusion rate of 3 hours-

- 7 L/h/m2 [Dose 135 mg/m2, infusion duration 3 h]
- 12.2 L/h/m2 [Dose 175 mg/m2, infusion duration 3 hr]

4.4. PHARMACODYNAMICS

Paclitaxel, a taxane family anti-neoplastic agent, used for subsequent therapy of advanced carcinoma (especially breast and ovarian), alongside other forms of cancer, is a microtubular inhibitor drug stimulating the microtubular assembly from tubulin dimers and stabilizing microtubules by impeding polymerization. This stability inhibits the usual spontaneous reconfiguration of the microtubular system, required for critical interphase and mitotic processes on the cellular level. [29] Paclitaxel additionally triggers erratic microtubular arrays or "bundles" as well as countless microtubular asters during mitotic phase. The ABC transporter family comprising of ABCB1 encodes the membrane protein p-glycoprotein(p-gp), a quite renowned efflux pump, that causes multidrug resistance. [27] Cells unresponsive to Paclitaxel showcases cross resistance to other hydrophobic drugs demonstrated in higher levels of p-gp. In addition to the efflux pump, mechanisms of Paclitaxel also comprise of modification of microtubule composition or dynamics. Recent studies showcased that overexpressing β III tubulin causes resistance to Paclitaxel contributing to its loss in ability to inhibit microtubule dynamics. [27,29]

4.5. TOXICOLOGICAL STUDIES

CARDIAC TOXICITY: Paclitaxel provokes cardiac rhythmic disturbances(arrhythmias), specifically bradycardia observed in about 30% patients [27] and on further discontinuation of the drug caused isolated asymptomatic bradycardia without a shred of hemodynamic effects, which was further considered as a major contraindication for patients with cardiovascular diseases and such patients must be set up with Therapeutic Drug Monitoring for continuous supervision. [3] Furthermore, intermittent bradyarrhythmia include Mobitz Type I (Wenckebach Syndrome), Type II and 3rd degree heart block were noted as more adverse drug reactions, but on the larger database, they happened to amount up to 0.1% of complaints, deeming them a minor ADR. [28] When Paclitaxel is administered alongside Doxorubicin, patients started becoming susceptible to Congestive Heart Failure (CHF) and as observed upon cumulative administration of doxorubicin of doses ≥ 480 mg/m^2 with a rate of 25% incidence and upon further reporting, it was recorded that limiting the dose of doxorubicin to 360 mg/m^2, the probability of CHF is reduced to <5%. The mitigation of cardiac toxicity may be aided by the administration of Dexrazoxane prior to the administration of Doxorubicin, as suggested by several experimental evidences. [3,21,27,28]

HYPERSENSITIVITY REACTIONS: Rewinding to the early phase 1 studies of Paclitaxel being performed, the rate of significant hypersensitivity reactions being observed ranged along the lines of between 25 to 30%. [25] (The significant number of impacted individuals tested upon happened to develop Type 1 Hypersensitivity reactions inclusive of dyspnea, bradycardia, hypotension, urticaria and bronchospasms. [21] (HSRs frequently surface within 2-3 minutes post therapy and become noticeable by the early 10 minutes with the vast majority surfacing after the administration of the second dose. Halting Paclitaxel therapy and administrating with H_1 receptor antagonist is most effective for the treatment of major hypersensitivity reactions like bronchospasms, urticaria, dyspnea, hypertension and also minor hypersensitivity reactions viz. flushing, rashes, itching, swelling, etc. Some alternates to H1 receptor antagonist post halting paclitaxel therapy are vasopressors and fluids. Most of the hypersensitivity reactions

are overcome by using H1 antagonist, H2 antagonist and Corticosteroids as the first line drugs. [3,21,25]

HAEMATOLOGICAL STUDIES: Paclitaxel induced Neutropenia, being the prime hematological adverse drug reaction, shows its onset around 8 to 10 days post therapy and usually recovers by the beginning of the 3rd week. The most admissible dose without granulocyte-colony stimulation factor varies around 175-200mg/m^2. Paclitaxel doesn't cause any permanent damage to the immature hematopoietic cell for neutropenia being non-cumulative. [3]

MUSCULAR TOXICITY: Usually 2-5 days post therapy, paclitaxel induced myalgia occurs, which is reported as common for dosage around 170-250 mg/m2 and myopathic for doses over 250 mg/m2, especially alongside cisplatin. NSAIDs happen to offer minimal relief and further, narcotics are administered prophylactically between days 2 to 5. Myalgia can be prevented by usage of Antihistaminergic agents both (H1 and H2 antagonist). [3]

NEUROTOXICITY: Paclitaxel induced peripheral neuropathy shows sensory symptoms, such as numbness or paresthesia, in a "glove and stocking pattern". [3] It shows up within a window of 24 to 72 hours, only after high doses and observed and recorded mostly after multiple dosing containing 135- 250 mg/m^2 and for most severe cases, preferably retaliate from long term usage. Short infusion schedules around 3 hours increases the probability of neurotoxicity compared to longer infusions ranging from 1 to 4 days. [21] Drug-drug interaction observed with Cisplatin upon prolonged usage synergistically and use only under therapeutic drug monitoring and supervision of capable surgeon/doctor. In high doses, motor and autonomic effects may occur in patients with pre-existing neuropathies (e.g.: diabetes or alcoholism) and in such cases, we preferably use amifostine, glutamate and pyridoxine as they are much promising in experimental models, but currently lack strong clinical evidence regarding effectiveness backing them up. In most rare cases, it causes optic nerve dysfunction viz. scintillating scotomas (visual disturbances). [3,21,25]

5. PACLITAXEL AND HUMAN IMMUNE SYSTEM

5.1. EFFECT ON MACROPHAGES

The macrophages are available in the relative tissues and perform numerous purposes, involving the presentation of antigens, phagocytosis in the microbial body, tracking of immune mediated products and protection for malignant cells. Similar to bacteria, macrophages may eliminate cancer cells by using a variety of operations, like the lysosomal enzyme release and the production of nitric oxide (NO). In addition, if triggered by multiple variables like secretion of lipopolysaccharide from bacteria (LPS), Interferon Gamma (IFN - **γ**), two stranded RNA (ribonucleic acid), macrophages eagerly destroying tumour cells in cell culture through getting into the malignant mass and generating large quantity of reactive oxygen species intermediary substances (ROIs) and the tumour necrosis factors (TNF).

After LPS stimulation, macrophages produce various kinds of anti-inflammatory substances. PTX exhibit LPS-mimic actions notwithstanding the lack of clear structural parallels in primary

monocytes and macrophage lineages. Production of inflammatory mediators through a receptor (toll-related) that distinct initial reaction gene 88 (MyD88) dependent route. PTX and LPS both share a TLR4 and My88 dependent pathway that stimulates the transcriptional. [5]

One desirable outcome of anti-tumour immunotherapy is the stimulation of macrophages in the malignancy environment. Paclitaxel can activate immune cells such as CTL, NK to engage in battle against tumours with macrophage stimulation. PTX can also trigger the production of genes involved in transcription factor encoding.

The effects of PTX on cytokines in the management of cancer related diseases, shows magnificent outcomes. For the case of intensive paclitaxel, including Interleukin 12 shows output by tumour released macrophages could be essential to alleviate blocking or suppressing T-cells into the host tissues of tumours. With the production of Nitrous Oxide, PTX produces IL-12. Both are very useful for post-chemotherapeutic administration. [5]

5.2. EFFECT ON DENDRITIC CELLS (DCS)

DCs play an important part in the immune system of a victim belonging to the most powerful antigen - presenting cells that can be acknowledged as T lymphocytes. During the tumour rejection arises from the immunological reactions, it stimulates specifically to the tumour. Defective DCs enable tumours to escape immune system control, while DCs containing acquired cancerous cells produce CTLs from native T cells that fight tumour cells. Low dose percentage of PTX influences tumour cells to experience apoptosis, shows no negative effects on bone marrow cells or DCs. In Vitro pretreatment studies the low level PTX not only eliminates the immunosuppressive effect, but also DCs differentiation via tumour cells. [5]
DC expressed costimulator could trigger CTLs to differentiate, allowing them to recognize and eliminate tumour cells. PTX can suppress the DC activated proliferation of murine allogeneic splenic T cells in the laboratory (perform into mouse bone marrow). It performs greater production of MHC class II molecules, which are powerful typical maturation signs on DCs.

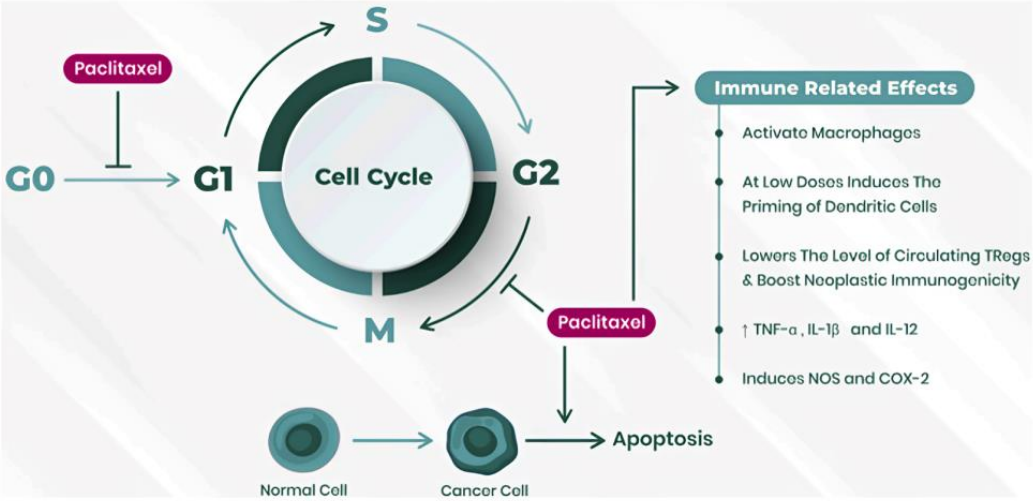

Fig. 4: Immune system affected by Paclitaxel [25]

5.3. EFFECT ON NK CELLS

A subpopulation of lymphocytes referred to as NK cells has the capacity to destroy tumour cells, virally infected cells, and cells covered with antibodies. Numerous tumour varieties are eliminated by NK cells, especially those that can escape CTL destruction due to decreased class I MHC expression. NK cells have the ability to trigger monocytes to differentiate into DCs, which have the ability to strongly elicit adaptive responses.

Despite compromising NK cell survival, it came to light that treating human NK cells successfully prevented the population in between NK cells - handled death phase of the target cells K562 under laboratory conditions. By suppressing the expression of adhesion molecules, chemotherapy under PTX can diminish the ability of human NK-like YT cells which are further bound to target cells.

On the other hand, by encouraging the mRNA and protein synthesis of perforin, a molecule implicated in NK cell-mediated cytotoxicity of human NK cells. Moreover, nuclear factor kappa B (NF kappa B) activation and indication of a significant connection between increased perforin synthesis can provide better NK cell cytotoxicity under the surveillance of PTX. [5]

5.4. EFFECT ON REGULATORY T- CELLS

Regulatory T cells have remarkable capability to inhibit both innate as well as adaptive immune responses. Lymphocytes usually regulate anti-microbial & anti-tumour feedback, whereas they also specifically preserve immunological tolerance for themselves. The Treg’s function and numerical alterations have significance for allergies, autoimmunity etc.

Higher Tregs in tumours may inhibit the immunological reaction of more CD4+ and CD8+ lymphocytes. Conversely decreasing Tregs enable effective against tumour immune responses. Consequently, the higher frequency of Tregs is the biggest reason for weakened immunity mediated by the cells in the people with cancer.

In the mouse, PTX increased the anti-tumour effects of the Toll-like-receptor 9 agonist PF-3512676 despite decreasing the quantity of Tregs and inhibitory function. Additionally, Treg population will decrease in numbers. Cell death receptor (CD 95) was upregulated that contributed to decrease in the number of Treg cells. [5]

5.5. PTX AND B CELLS

PTX reduces response under the humoral immune system in Lewis's rat recipients of an ACI cardiac transplant, despite its potential impact on T-cells. PTX based on cause cultured B lymphocytes to undergo suicide. Synthesis in the murine B lymphoma cell line triggered by PTX. When exposed to B lymphocyte cell line, enhancement of the DNA binding activity of nuclear factor activator protein 1. [5]

Paclitaxel selectively inhibits the proliferation of B-cells generated by LPS in mice, potentially via the down regulation of the intracellular pathway involving p38 MAP.

6. NANO-FORMULATIONS OF PACLITAXEL

This section is broadly classified into many state-of-the-art revelations according to the aspects

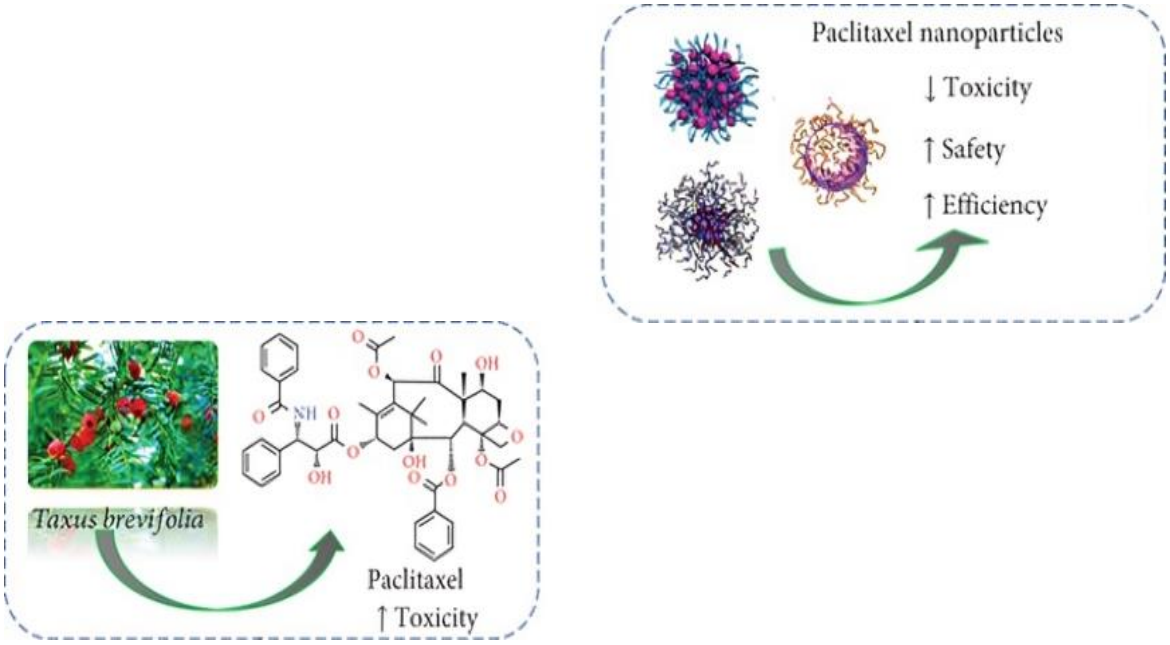

Fig. 5: Improvement of therapeutic characteristics through nano-formulation

of formulation development, which will put major relief amidst the Paclitaxel (PTX) based cytotoxic regimes. The relevant nano-formulations have been mentioned in the following:

6.1. NANOSPHERES:

Owing to its' small size, nanospheres appear to be very promising option for the Paclitaxel delivery. Nanospheres provide the ease of local, systemic as well as oral administration, due to the incorporation of biocompatible polymers. Also, the drug finds it very conducive for the entrapment and release into desired sites. Several polymers are included in the list – PLA (Poly D, L – lactic acid), PLGA (Poly D, L – lactic – co – glycolic acid), Polymer distearoyl phosphatidylethanolamine, PEG 5000 (Polyethylene Glycol 5000) [10]. These polymers add some positive features to the existing formulation like, prolonged circulation time along with enhanced activity.

6.2. ORGANIC NANO-FORMULATIONS (ALBUMIN NANOPARTICLES):

Formulation of nanoparticles truly paved the way for further study design of Paclitaxel nano-formulation. In 2005, the approval of first albumin-based nanoparticle named Abraxane was witnessed. After FDA approval, Abraxane could legally be used in case of "metastatic breast cancer" and "non-small cell lung carcinoma" (NSCLC). Such nanoparticles exhibited crucial spike in the permeability as well as retention profile of Paclitaxel which increased to more than a significant extent, compared to ordinary Paclitaxel. With as small as 130 nm size, albumin-

paclitaxel nano-formulations possess diminished ill-effect and way more pronounced useful implications (example – enhanced efficacy).

Like in the previous cases, a handful of polymers (PLGA, PLA and Chitosan) were brought on board for modifying the general albumin-based nanoparticle [10]. Incorporation of the following polymers promised improved clinical effect with possibility of fast and subsequently slow release leading to the observation of a distinct Biphasic Pattern. Along with the already stated matters of concern, enhanced cytotoxicity (Ex: PLGA – Paclitaxel), novel transplantable tumor inhibition in liver and increased cellular uptake (Ex: Chitosan coated PLGA – Paclitaxel nanoparticle) were put under serious consideration.

6.3. INORGANIC NANO-FORMULATIONS (SILVER NANOPARTICLES):

The hunt for silver nanoparticle (AgNP) was a much-anticipated thing for modern cytotoxic agent findings. Paclitaxel – is a generic drug formulated from Taxol, which is extracted from *Taxus brevifolia* (Family: Taxaceae). Taxol was discovered with the potent anti-cancer benefit of taxanes. Though, in the end of 1950's Taxol gained the popularity by NCI, researchers still need it for new age carcinoma [15]. Therefore, Paclitaxel or PTX is a wise choice for optimally treating lung, ovarian and breast cancers. Being an important anti-cancer drug Paclitaxel must mend its flaws relating to narrow therapeutic range and less solubility. Methods discussed earlier make the negative count much lowered. That is so, the utilization of polymers (Ex: PLGA), discovery of new delivery mechanisms is going to aid in clinical enhancement, reduced resistance to Paclitaxel [13]. Accompanying the photolytic and biocatalytic advantages of silver nanoparticles (AgNP), such low-cost carrier development for drugs got visible attention. In the meantime, evaluation of anti-cancer potential of AgNP-PTX (Paclitaxel based silver\ Ag nanoparticles) had been associated with study in a group of popular cell-lines, that took a big chunk of time and a number of expensive materials. Now-a-days, modern formulation considerations including "Green Synthesis" principles have lightened the way towards safer and more sustainable, approach for synthesizing Paclitaxel-AgNP.

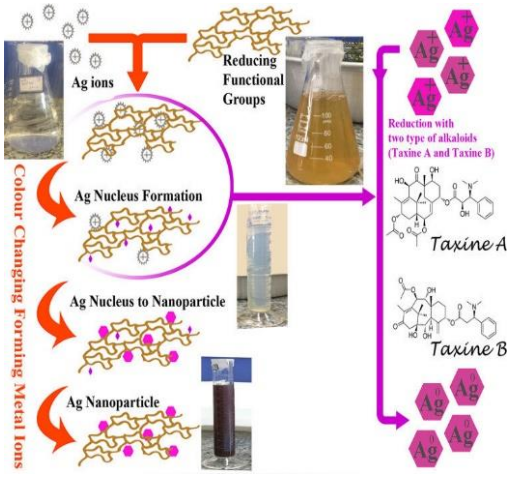

Fig. 6: Formulation of Paclitaxel silver nano-particles [15]

7. MECHANISM OF TARGETING OF PACLITAXEL NANO-FORMULATIONS

7.1. ALBUMIN – PACLITAXEL NANOPARTICLES:

Nanoformulations based on the anti-cancer property of naturally derived taxane analogues have led us to the horizon of targeted delivery of potent drug, directly to the desired site without any un-toward effect or passive clinical outcomes. A popular nanomedicine under the brand name Abraxane, holding the active ingredient of paclitaxel is a good option in ovarian, lung and breast cancers. Following the same, albumin conjugated nanoformulation got to the developmental stage. Named as nab-paclitaxel, the albumin-paclitaxel combination showed rays of hope in some severe forms of cancer. Abraxane, nab-paclitaxel was tested to show 33% more tumor uptake in comparison with conventional solvent-paclitaxel formulation (because the solvent-based paclitaxel exhibited inability to permeate cell membranes and a significant proportion of protein bound drug) [30].

Behind such attention, was the role of albumin providing increased tumor accumulation and also, in binding to secreted protein, acidic and rich in cysteine (SPARC), that enhanced the anti-cancer potential of taxane derivative, Paclitaxel.

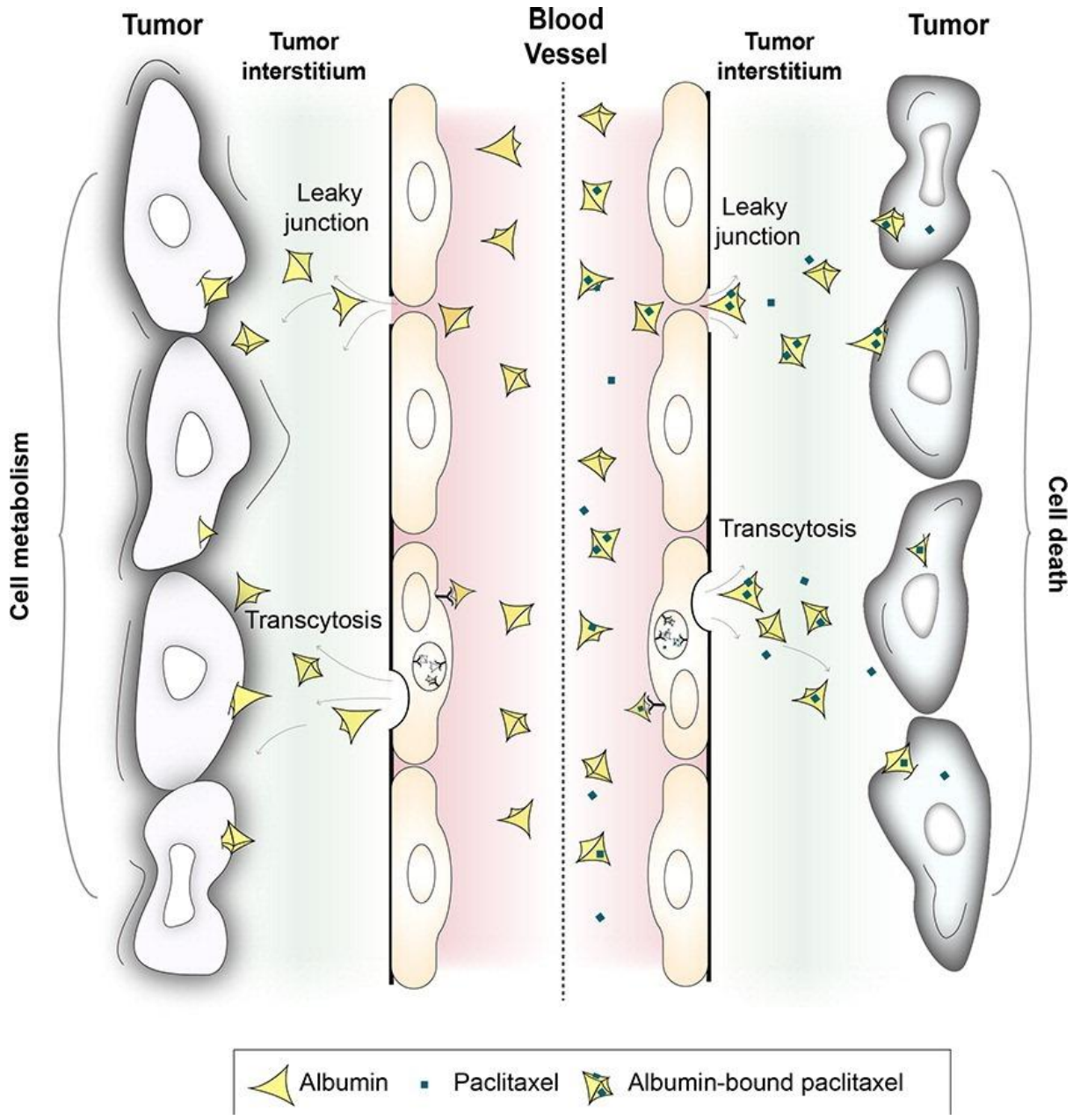

Fig. 7: Role of albumin bound Paclitaxel in induction of cytotoxicity [30]

7.2. SILVER - PACLITAXEL NANOPARTICLES:

Known by its alias, Paclitaxel-AgNP, this particular kind of paclitaxel carrying nanoformulation has been proved for the potentiating action upon the p53 controlled cell apoptosis [31]. As, the details further unfolded, the mediatory role of silver-paclitaxel on the apoptosis process became more lucid. Paclitaxel-silver nanoparticles used the reactive oxygen species (ROS) to impart superior anti-tumor and anti-metastatic effect. Production of such reactive oxygen species had association with the highly cytotoxic ROS signaling pathway,

which was liable for the main mechanism behind the exhibition of anti-cancer property [33]. Accompanying the former, researchers found out the additional effect of Paclitaxel – AgNP on cell cycle. The arrest of cell cycle had been a huge area of future interest because, it held the hidden feature of Paclitaxel-AgNP (cell cycle impairment strongly regulated by Cyclin, CdK, CAK) [32,33].

Biotoxicity of Paclitaxel-AgNP, to which we are not much familiar with, bears some sort of hindrances in the recent incorporation in anti-cancer regime. Many faces of paclitaxel-silver nanoparticle biotoxicity include, exertion of stress due to production of radical species and improper platelet functions [32]. Along with the other type of biotoxicity formulation developers encounter the potential genotoxicity too. This is the very reason, we are running at all stakes behind green synthesis of the paclitaxel loaded silver nanoparticle, also named as biogenic paclitaxel-AgNP [34].

8. DISCUSSION

Paclitaxel Nano formulations might have potential benefits, but there are several factors impeding their main stream first line usage and full-scale commercialization. Firstly, we start with the most prominent issue regarding the drug itself such that it has proven to be hepatotoxic in countless situations along with a delayed onset of action post treatment in a window of 8 to 10 days and a lengthy recovery time of estimated 15 to 21 days. Secondly, it, being noncumulative, gives no initial contradictory indications regarding permanent damage to immature hematopoietic cells. ED_{50} of Paclitaxel prior to chemotherapy ranges around 175 to 200 mg/m^2 (without G-CSF). Moreover, in the current scenario, the field of nanotechnology is still in a blooming phase with immense growth potential, currently disabling us to implement any undeciphered techniques in order to improve the stability of nano formulations or even, medical nanotechnology as a unique element and find better ways to modify, characterize and induce new ligands in targeted organs. Finally, it is currently not economical for most consumer groups and in the near future, we may see the cost of nano formulations declining faster than usual with proper supervision, innovation and expertise in this field. Furthermore, its potential might not be limited to just an anti-neoplastic agent, predetermined to be used for sarcoma therapy. [18,19]

9. CONCLUSION

Paclitaxel, being the first taxane class drug ever to ascend to clinical trials, is a pioneering antineoplastic effective against cancerous cells immune to conventional therapy. It was approved by both USFDA and several other countries (including India) for the palliative treatment of chemotherapy resistant breast and ovarian cancers. Yet, some challenges remain regarding strategical development for initial malignancy therapy to achieve permanent cure or improved chances and conditions of survival. Introducing carrier systems viz. liposomes, micelles, and particulate drug delivery could overcome current hurdles and optimize the existing methodology of treatment.

ACKNOWLEDGEMENT

We sincerely thank our supervisors, **Souvik Kundu** and **Rahul Patra**, Assistant Professor, Seacom Pharmacy College, for their valuable guidance and support throughout the preparation of this review article and helped us with resources and knowledge to bring this work to life. Their insights and expertise have been instrumental in shaping this work. We also acknowledge that all three authors viz. **Goureesh Ghosh**, **Debjit Adak** & **Aparup Das** have contributed equally to the research and writing of this article. Additionally, we appreciate the contributions of all researchers whose work has informed this study and been duly added to the list of references.

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