Chapter – 2

AIMS & OBJECTIVES
2.1 GOALS TO BE ACHIEVED FROM THE CURRENT RESEARCH WORK

- To develop nanosized carrier systems for anticancer drugs
- To target it to the desired tumor site either by active or by passive means
- To compare its efficacy with already available formulations
- *in vivo* and *ex vivo* toxicity studies

2.2 ORIGIN OF PROPOSAL

The choice of suitable pharmaceutical formulation with high toxicity for tumor cells and less toxicity to the normal cells is an essential requirement of chemotherapy. To enhance the effectiveness of the available chemotherapeutics, and to overcome the various shortcomings as described in section 1.7.1, we can carry out targeting to cancer cell specifically and not in general to the whole body. Broadly, we can classify the targeting into two types: Biological targeting and physical targeting. Biological targeting includes Tyrosine kinase receptors as in case of human epidermal receptors (HER) in breast cancer are responsible for the production of number of proteins inside the cell and thus their blockage will not allow the cell to grow eg: Herceptin®; angiogenesis i.e. formation of the new blood vessels for the fast growing cells with a higher metabolic rates. If the blood supply to such cells is blocked, then the growth of the cells will surely stop eg: Avastin® and lastly the proteosomes i.e. the cells which are responsible for the removal of cell garbage and processing the peptides and recycling the amino acids back for the formation of new proteins eg: Velcade® but many a times such targeting are achieved with the help of biological molecules which are not very safe. The controversies
regarding the safety issues of such molecules have been going for long despite of their continuous usage by oncologists.

**Physical targeting:** It is the one which is achieved by allowing the drug to act on the cells which have a particular microenvironment i.e. group of cells have a particular temperature and pH. This involves either the drug moiety directly which is active in either acidic or basic pH or formulating the drug in such a way that it is released to act on the cells only at a particular temperature or pH. To achieve the purpose many such polymers have been designed.

Despite the concerted efforts put in by various researchers in designing the different therapeutic modalities, the success rate of certain cancer treatment regimen is only partial with limited effect on long term prognosis. Therefore, the scientists have been trying to work out not only new methods but to put the already available ones in such combinations that would cure as well as prevent the recurrence of the disease.

Conventionally, the whole knapsack of cancer treatments can be redistributed into three packets namely: surgery, chemotherapeutics and mechanophysical killing of cancer cells. Different permutations and combinations of the material of these three packets have been tried to achieve the desired response in a patient. The combination is designed with the view that synergistic response of the participants kills maximum number of cells as well as prevents the recurrence of the problem.

Surgery is primarily effective only at the early to middle stages of the tumors of particular sites like breast, neck, colon etc. It cannot be used for deep seated sites inaccessible to the surgeons or as in the case of leukemia where such type of procedures could not be performed. The major drawback of surgery is the risk of metastasis due to
accidental migrating of cells which may lead to spread of tumor to other organs and its recurrence. Oncologists for long have exclusively relied on chemotherapy for treating the later or advanced stages of cancer. Till date, chemotherapeutic regimens that are used alone, suffer from number of shortcomings. Several obstacles frequently encountered with singular use of anticancer agents include normal tissue toxicity, poor specificity and stability and a high incidence of drug-resistant tumor cells. The delivery itself is really painful and discomforting and often compromises the quality of life (QOL) of the patients. To circumvent these problems the chemotherapeutic drugs are often formulated into prodrugs controlled drug delivery systems, targeted drug delivery system, stimuli responsive delivery systems and cytosolic acting drugs etc. Moreover various mechanophysical methods which rely on physical means like change in temperature, pH or by giving the energy from external source like from ultrasounds etc to kill cancerous cells are also being employed.

One of the classical examples out of the various permutations and combinations that have been used for decades includes radiotherapy with surgery. Radiation therapy shrinks a tumor and is used in conjunction with surgery to ensure that remnants of cells that may remain after surgery are destroyed. It has been used for the treatment of hundreds of different conditions. Similar attribute could be extrapolated wherein a particular combination of targeted drug delivery and mechanophysical killing, referred to as “mechanophysical targeting” could be customized and appropriately used for effective treatment of various cancer conditions. Mechanophysical targeting is a hypothetical term implied to explain that how “a single arrow can hit two targets.” i.e. a mechanophysical arrow will kill tumor as well as target the drug to the desired site.
2.3 SELECTION OF THE DRUG

Taxanes (Paclitaxel and Docetaxel) are potent anticancer drugs with proven activity against a broad range of human malignancies, including ovarian and breast cancer and non-small cell lung carcinoma.\(^1,2\) Paclitaxel is an effective anti-cancer drug against a wide range of solid tumors, which promotes polymerization of tubulin dimers to form microtubules and stabilizes microtubules by preventing depolymerization. In other terms, Paclitaxel is one of several cytoskeletal drugs that target tubulin. Paclitaxel-treated cells have defects in mitotic spindle assembly, chromosome segregation, and cell division. Unlike other tubulin-targeting drugs such as colchicine that inhibit microtubule assembly, paclitaxel stabilizes the microtubule polymer and protects it from disassembly. Chromosomes are thus unable to achieve a metaphase spindle configuration. This blocks progression of mitosis, and prolonged activation of the mitotic checkpoint triggers apoptosis or reversion to the G-phase of the cell cycle without cell division.\(^3,4\)

The ability of paclitaxel to inhibit spindle function is generally attributed to its suppression of microtubule dynamics,\(^3\) but recent studies have demonstrated that suppression of dynamics occurs at concentrations lower than those needed to block mitosis. At the higher therapeutic concentrations, paclitaxel appears to suppress microtubule detachment from centrosomes, a process normally activated during mitosis.\(^5\) The binding site for paclitaxel has been identified on the beta-tubulin subunit.\(^5\)
2.4 RATIONALE OF DRUG SELECTION:

Currently marketed formulation of Paclitaxel (Taxol®) and Docetaxel (Taxotere®) are administered intravenously at different dosages and infusion schedules. Currently global sales of taxanes (Taxol® and Taxotere®) are approximately $3.0 billion. The basic problems associated with the use of taxanes for the treatment of cancer are:

- Poor bioavailability of Taxanes derivatives (e.g. Paclitaxel and Docetaxel).
- Hypersensitivity reactions associated the currently marketed formulations of Taxol® and Taxotere®.
- Patient non-compliance.
- Highly toxic surfactants of already available formulations (Cremophore EL of taxol is hepatotoxic, nephrotoxic etc.)

The ability to administer taxanes in a better targeted formulation would offer considerable advantages, particularly with paclitaxel where the chemophore EL vehicle can be responsible for hypersensitivity reactions. Co-administration of local hyperthermia by the oncologists with the thermosensitive formulation will cause the drug to be released in the larger amounts only at the tumor site. Taxanes such as ‘Paclitaxel’ were selected as feasible drug candidates for pH/temperature dependent targeting:

- Taxanes are potent and most widely used anticancer drugs in various malignancies.
- Taxanes has low aqueous solubility and low permeability, belongs to BCS class IV.
- Currently marketed formulations are administered intravenously and suffer with number of chemotherapy related side effects.
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- Hypersensitivity reactions, nephrotoxicity etc. like problems are associated with the currently marketed formulations
- Patient non-compliance due to heavy dose schedules

2.5 SELECTION OF THE SYSTEM

Nano sized drug delivery system were selected to be formulated as they offer a number of advantages. Nanotechnology has tremendous potential to make an important contribution in cancer prevention, detection, diagnosis, imaging and treatment. It can target a tumor, carry imaging potential to document the presence of tumor, sense pathophysiological defects in tumor cells, carry therapeutic genes or drugs based on tumor characteristics, react to external triggers to liberate the agent and document the tumor response and recognize residual tumor cells. Nanoparticles are important because of their nanoscaled structure but nanoparticles in cancer are still bigger than many other drugs in different ailments. Their "large" size can make it difficult for them to evade organs such as the liver, spleen, and lungs, which are persistently clearing foreign materials from the body. Toting up, they must be able to take advantage of understated differences in cells to distinguish between normal and cancerous tissues. Indeed, it is only recently that researchers have begun to successfully engineer nanoparticles that can effectively evade the immune system and actively target tumors. Active tumor targeting of nanoparticles engages attaching molecules, known communally as ligands to the outsides of nanoparticles. These ligands are special in that they can identify and bind to complementary molecules, or receptors, found on the surface of tumor cells. When such targeting molecules are added to a drug delivery nanoparticle, more of the anticancer
drug finds and enters the tumor cell, increasing the efficacy of the treatment and reducing toxic effects on surrounding normal tissues. Although the past 30 years of innovation in nanotechnology has made the carriers capable of carrying a whole host of new anticancer drugs directly to tumors, we are still searching for the ideal delivery nanosystem. Nanotechnology studies are not new. In essence, all drug molecules can be considered as Nanoengineered structures. What is new is the inclusion of a number of other nano-based approaches to medical studies.

Different drug delivery systems available with specific emphasis on nano sized drug delivery systems are discussed in chapter 3.

2.6 RATIONALE FOR SELECTION OF SYSTEM 1

Micellar drug delivery systems enjoy several advantages over other particulate configurations. Some of them are as follows: They can significantly enhance the water solubility of hydrophobic drugs for improved bioavailability.

- They usually exhibit low critical micelle concentration (CMC), rendering the drug-loaded micelles stable in the bloodstream to achieve long circulation time. This in turn makes it possible to accumulate the encapsulated drugs in the required regions via an active and/or a passive mechanism.

- There is also conjecture that their small size (usually less than 100 nm) makes the drug-loaded micelles less susceptible to uptake by the reticuloendothelial system (RES)

- Temperature / pH sensitivity of the formulation will prevent the drug release at normal sites
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- Expected decrease in the side effects.
- Expected decrease in the dose of the drug formulation.
- Temperature dependency can be very successful with adjuvant hyperthermia therapy currently used in the treatment of breast cancer.
- Nano-size dimensions results in permeability enhancement.

2.6.1 PERSPECTIVES OF TEMPERATURE / PH DEPENDENT CONTROLLED RELEASE ANTICANCER FORMULATIONS

Temperature rise and acidic pH at the site of the tumor as compared to other tissues in the body, are the two most common observations by the number of cancer physicians. These characteristics however can be utilized for targeting as well as controlled or timed release of the anticancer drug at that particular site. Adverse effects of all anticancer formulations have been well known for centuries. Many, a times the chemotherapy adds to the miseries of the patient. Though, it has a cytotoxic effect at the tumor site but invariably it also kills other cells of the body and thus makes the therapy very harsh and painful. If any such method can be designed that will cause the formulation when administered intravenously to release the drug only at the tumor site, then definitely it will though not vanish but decrease number of side effects and will also increase the efficacy of the drug. For reasons of patient convenience, controlled release formulations seems a valuable addition to standard formulations. Besides, the decreased frequency of dose administration of anticancer agents may potentially reduce total healthcare system costs. In addition, controlled release formulations are of benefit in therapies that require prolonged exposure by means of a protracted treatment course.
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The positive perspectives of the patient, the oncologist and the pharmaceutical industry on the use of temperature / pH dependent controlled and timed release formulations are as follows:

a. The patient
As these types of the formulations will cause the drug to be released at the site of a particular temperature and pH, we can expect of sparing the patients to an extent with the systemic side effects involved normally with the chemotherapy. Sustained release for the longer period of time will also reduce the number of in-patient and out-patient hospital visits with their associated medical and nursing administrative costs. In cancer most of the times, it is seen that patient do not dies of the cancer itself but with the side effects of the chemotherapy. So, it is the need of an hour that the therapies to be made more gentle to the patient but at the same time harsh on the tumor.

b. The oncologist
The concern of oncologist regarding bioavailability in the tissue cells is understandable due to invariable changes in the temperature of the tumor site. The well known hyperthermia therapies in such cases can be used adjuvantly and is expected to be highly effective and highly target specific as compared to the ones which are used alone with the chemotherapeutic agents. Moreover, the size of the drug designed will enable the drug to cross the so, called leaky vasculature of the tumor and will allow the moiety to be retained inside for a longer period of time due to well high on scientists mind fact these days of "enhanced permeation and retention effect."
c. The pharmaceutical industry

From the formulation perspective, initially such formulations may be more expensive to develop as compared to their normal counterparts. As these require investment of as much as few hundred millions of dollars, for over decades of research and technology from pharmaceutical companies. Therefore, they would be initially expensive when finally reach the market. However, in long term perspective, such targeted chemotherapy appears to be more economical than the normal one due to expected decrease in the frequency of administration and systemic side effects. So, greater preference would be given to such formulations by oncologists and cancer patients. There are at least 10 such targeted drugs as shown in table 1 which have already achieved the so-called 'blockbuster' status, worth billions in annual sales.

A significant cost differential will emerge in favor of targeted therapy in terms of administrating treatment, monitoring the patient and managing adverse effects. The net result is that controlled release targeted-cancer therapy will increase the revenues for the manufacturer. Furthermore, these novel targeted formulations could be patent protected and are a source of revenues for manufacturers.
### Table 1: Different targeted drugs in market and their uses

<table>
<thead>
<tr>
<th>No.</th>
<th>Product</th>
<th>FDA-approved use for cancer</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Imatinib mesylate (Gleevec®)</td>
<td>Novartis gastrointestinal stromal tumor (tyrosine kinase inhibitor)</td>
</tr>
<tr>
<td>2</td>
<td>Dasatinib (Sprycel®)</td>
<td>Bristol-Myers Squibb chronic myeloid leukemia (Bcr-Abl kinase inhibitor)</td>
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<tr>
<td>3</td>
<td>Nilotinib (Tasigna®)</td>
<td>Novartis chronic myeloid leukemia and acute lymphoblastic leukemia (tyrosine kinase inhibitor)</td>
</tr>
<tr>
<td>4</td>
<td>Trastuzumab (Herceptin®)</td>
<td>Roche Pharmaceuticals certain types of breast cancer (HER-2), non-small cell lung cancer (tyrosine kinase inhibitor)</td>
</tr>
<tr>
<td>5</td>
<td>Gefitinib (Iressa®)</td>
<td>AstraZeneca to treat some patients with CML</td>
</tr>
<tr>
<td>6</td>
<td>Erlotinib (Tarceva®)</td>
<td>Roche Pharmaceuticals to treat patients with advanced non-small cell lung cancer (tyrosine kinase inhibitor)</td>
</tr>
<tr>
<td>7</td>
<td>Cetuximab (Erbitux®)</td>
<td>Bristol-Myers Squibb Co. Squamous cell carcinoma of the head and neck or colorectal cancer (blocks the binding of ErbB2 receptors)</td>
</tr>
<tr>
<td>8</td>
<td>Panitumumab (Vectibix®)</td>
<td>GlaxoSmithKline advanced or metastatic colorectal cancer (Mechanism of action: prevents its growth signals, which may inhibit signal transduction and lead to uncontrolled protonation)</td>
</tr>
<tr>
<td>9</td>
<td>Lapatinib (Tykerb®)</td>
<td>AstraZeneca metastatic breast cancer (This small-molecule drug inhibits several tyrosine kinases, including the tyrosine kinase activity of HER-2, which leads to the uncontrolled growth of cancer cells)</td>
</tr>
<tr>
<td>10</td>
<td>Tensirolimus (Torisel®)</td>
<td>Wyeth Pharmaceuticals to treat patients with advanced renal cell carcinoma. (This small molecule drug is a specific inhibitor of a kinase called mTOR that is activated in tumor cells and stimulates their growth and proliferation)</td>
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</table>
2.7 RATIONALE FOR SELECTION OF SYSTEM II

Solid lipid nanoparticles (SLN) made from biodegradable solid lipids exist in the submicron size range have attracted increasing attention in recent years. The advantages of SLN are as follows: possibility of controlled drug release and drug targeting, protection of incorporated compound against chemical degradation, no biotoxicity of the carrier, and no problems with respect to large scale production.\textsuperscript{11-13}

References:


