CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE
1.1 Introduction

The World Health Organization estimated 14.1 million cancer cases around the world in 2012; of these 7.4 million cases were in men and 6.7 million in women [1]. This number is expected to increase to 24 million by 2035 [2]. This growing cancer burden within the overall context of non-communicable diseases is a key focus of research throughout the globe. Breast cancer is the most common cancer worldwide in women contributing more than 25% of the total number of new cases of cancer diagnosed in 2012 [3]. Breast cancer, unlike cancer of lungs, liver, bone, brain, and skin, invades locally and spreads initially through the regional lymph nodes, bloodstream and may metastasize to affect almost any organ in the body [4-6].

Breast cancer is treated using a multimodal approach combining surgery with adjuvant systemic treatment depending upon the stage of the cancer [7-14]. Adjuvant chemotherapy is one of the most commonly prescribed systemic treatments for breast cancer wherein chemotherapeutic drugs such as anthracyclines, alkylating agents, 5-Flurouracil, gemcitabine, capecitabine, vinorelbine, eribulin and taxol are administered [14-30]. Paclitaxel (Ptx), a commonly used taxane, is an anti-mitotic agent showing great promise as an antineoplastic drug for breast cancer [31]. However, the hydrophobicity of Ptx demands its administration in an organic solvent Cremophor EL®, which causes solvent induced toxicity [32]. Additionally, Ptx is also associated with therapy antagonism through the activation of inflammatory signals in cancer, culminating in cancer progression [33]. In this context, a combinatorial approach which essentially aids the clinical management of Ptx-induced severities by inhibiting inflammatory signals causing cancer progression is desirable. This strategy would allow the continued use of Ptx as a potent neo-plastic drug for breast cancer, however, with lesser side-effects. In the present clinical scenario, Ptx-induced inflammatory responses are managed by the administration of non-steroidal anti-inflammatory drugs which by themselves are associated with adverse side-effects
This alerts the need for an agent that is safer than the conventional drugs and could augment the therapeutic effects of Ptx.

Epidemiological studies have observed multispectral attributes in plant polyphenols such as Curcumin, Epigallocatechin gallate (EGCG), Resveratrol etc which can be exploited for multifaceted effects such as anti-cancer, anti-oxidant and anti-inflammatory therapy [35-39]. Polyphenols are qualified chemopreventives [40] which can be ideal candidates for combination chemotherapy with conventional drugs. Therefore, a strategy to combine Ptx with natural chemopreventives would aid harness the benefits of the individual drugs and thereby essentially overcome Ptx adversities. However, polyphenolic compounds are in general afflicted by their extremely low plasma availability and instability in the in vivo system [41]. These disadvantages can be alleviated significantly through an appropriate nanoencapsulation approach, wherein drugs can be delivered individually or in combination, loaded within biocompatible nanocarriers [42]. This strategy would enable the delivery of drugs to the diseased site without hampering their chemical integrity, and thereby increase the drug availability. Ideally, nanodelivery systems will allow for more specific targeting of the drug, resulting in improved efficacy and minimized side effects [43]. Various biomaterials are reported for the development of nanocarriers for drug delivery, of which biodegradable polymers have an upper hand given their biocompatibility and ease of controlled dissolution [44]. Additionally, designing multiple compartments in nanocarriers would enable accommodation of combination drugs within them favorable to retain chemical integrity of distinct drugs [45]. Such a co-delivery mode has several inherent advantages beneficial for the biological system such as enhanced synergistic therapeutic efficacy, better drug-resistance management, and the capacity to temporally regulate drug release.

1.2 Scope and outline of the thesis

Combination strategies play a crucial role in cancer therapy, wherein multi-target therapy combines a cocktail of standard chemotherapeutic drugs, or
other novel agents having different mechanisms of action. However, patient non-compliance with multiple drug administration schedules is perceived as a realistic difficulty. In addition, the difference in the schedule of administering individual drugs in the combination makes it challenging to control the pharmacokinetics and pharmacodynamics of the combination [46]. Cross-resistance induced by multiple drugs is yet another concern [47], which together with toxicity issues due to high doses of these drugs can impair quality of life for little clinical benefit [48]. Reducing dosages to alleviate these side-effects would, however, lead to unsuccessful therapy. Hence, to address these concerns, a rationally engineered therapeutic vehicle that could transport desired dosages of drugs in combination, and release them in a differential, yet controlled manner, would be an ideal strategy. The present thesis describes the strategy adopted for engineering such a combination nanomedicine based on a core/shell scheme which would facilitate desirable outcomes.

Chapter 1 describes the design, synthesis and characterization of the core/shell nanomedicine. A core/shell nanocarrier based on Poly-l-lactide-co-glycolic acid (PLGA) and Casein optimally loading Ptx and EGCG in the core and shell respectively was synthesized and characterized for its size, size distribution, colloidal stability, viscosity, casein quantification, casein degradation, drug entrapment (qualitative and quantitative) and in vitro drug release profile.

Chapter 2 portrays an extensive analysis of the in vitro and in vivo biocompatibility of PLGA-Casein core/shell nanocarriers. Herein, a detailed panel of hemo- and immuno-compatibility assays were performed to investigate the response of the nanocarriers towards blood and immune response cells in vitro. Further, any possible toxicity caused to vital organs by the nanocarriers and the response of the nanocarriers to cytokines was assessed in vivo. This chapter also discusses in details the biodistribution of PLGA-Casein core/shell nanocarriers and the in vivo pharmacokinetics of the core/shell nanomedicine done to investigate the differential release of the drugs from the core and the shell.
Chapter 3 deals with a complete evaluation of the anticancer effect of the combination nanomedicine on breast cancer cells (MDA-MB-231 and MCF7). A detailed study of how the combination nanomedicine tackled the inflammation pathway which regulates cancer progression has been performed. This was done by investigating the effect on selected molecules and genes using techniques such as immunoblotting, real-time-PCR and flowcytometry.

Lastly, Chapter 4 is a translation study done on breast cancer patient derived samples. The breast cancer tissues obtained were first immunohistologically characterized for their receptor status viz, estrogen receptor (ER), progesterone receptor (PR), Her2 receptor and epidermal growth factor receptor (EGFR), which are generally considered for designing therapy in the clinic. Following this, the cells isolated from the respective tissues were subjected to combination nanomedicine treatment both in the non-targeted and targeted forms. For targeting, Her2 and EGFRs were considered and the targeted nanocombinations were bioconjugated with similar anti-Her2 and anti-EGFR antibodies. These receptors were considered because Her2 positive and triple negative (EGFR positive) breast cancers are generally the aggressive forms of the disease. An initial evaluation of the combination drugs in nanoform was done on 14 patient samples, to establish the proof of concept.

1.3 Review of Literature

1.3.1 Breast cancer epidemiology

Nearly 1 in 3 cancers diagnosed in women in the United States is of the breast, with over 100,000 new breast cancer patients estimated to be diagnosed annually in India [49-52]. By 2020, 70% of the world’s cancer cases will be in the underdeveloped and developing countries, with India facing a breast cancer epidemic [53]. The National Cancer Registry programme of the Indian Council of Medical Research estimates that about 31% of all cancers registered in India are that of breast cancer [54]. In India the average age of the high risk is 43-46 years, unlike in the west where women aged 53-57 years are more prone to breast cancer.
The incidence of breast cancer in India is reported to be alarmingly high in the metropolitan cities, than the rural areas (Figure 1.1).

![Incidence of breast cancer in India](Image)

Figure 1.1: Breast cancer incidence in India by the International Agency for Research on Cancer (WHO) (Image courtesy: The Hindu, 2007)

### 1.3.2 Molecular subtypes of breast cancer

Breast cancer is a clinically heterogeneous disease [56-60]. Histologically, similar tumors may have different prognoses and may respond to therapy differently. It is believed that these differences in clinical behavior are due to the molecular differences between histologically similar tumors. Studies aimed at classifying the molecular subtypes of breast cancer have confirmed that breast cancer represents a group of molecularly distinct neoplastic disorders. A partnership from the Department of Genetics, Stanford University, USA and The Norwegian Radium Hospital proposed five molecularly distinct classes of breast cancer [61]. While the molecular classification of breast cancer closely corresponds to divisions already made using traditional pathological methods, the classification has important implications for our understanding of the heterogeneity of breast cancer.

The five main molecular classes of breast cancer are:

- Basal type: ER-negative, PR-negative and Her2-negative tumors
- Luminal A: mostly ER-positive and histologically low grade
- Luminal B: mostly ER-positive and may express low levels of hormone receptors and are high grade tumors
- Her2 positive: exhibits high amplification and expression of Her2 gene and several other genes of the Her2 amplicon
- Normal breast like: high expression of many genes known to be expressed by adipose tissue and other non-epithelial cell types. These tumors also show strong expression of basal epithelial genes.

More recently, a new subtype classified as “claudin-low” has also been identified [62]. Figure 1.2 represents the various molecular subtypes of breast cancer.

![Molecular classification of breast cancers](Image courtesy: Perou et.al., Nature, 2000)

The proposed molecular classification does appear to reflect both prognosis and response to therapy [63, 64]. The low grade luminal tumors are indolent and sensitive to antiestrogens, while Her2 positive tumors are more aggressive and are sensitive to trastuzumab, an anti-Her2 therapy [65]. Basal tumors are very aggressive types though they can be sensitive to chemotherapy [66].

### 1.3.3 Breast cancer treatment

In the recent years, life-saving treatment strategies for breast cancer advanced dramatically bringing new hope and excitement. Various treatment options are available for breast cancer and the choice of treatment is generally based on the factors like age, physiological condition of patients and stage of
cancer. The treatment options for breast cancer could be characterized into two: *local treatment* and *systemic treatment* [67-69].

1.3.3.1  **Local treatment**

This type of treatment specifically targets the tumor and the rest of the body parts remains unaffected.

- **Surgery**

  Surgical removal is suggested to patients having a localized tumor below 4 cm. Lumpectomy is a type of surgery referring to surgical removal of the tumor in breast along with negligible amount of surrounding tissue. Another version, partial mastectomy is extensive and removes more amount of normal tissue surrounding the tumor. This is also referred to as quadrantectomy [70]. These two surgical procedures constitute the ‘breast conserving surgery’ as the removal of complete breast is avoided. Furthermore, patients with a more advanced stage of breast cancer may undergo a total mastectomy or complete removal of breast with a sentinel lymph node biopsy. For a more advanced tumor radical mastectomy is advised that includes removal of breasts along with removal of lymph nodes in the armpits and chest. Surgeries are the preferred mode of treating breast cancer and are accompanied by radiotherapy or chemotherapy.

- **Radiation therapy**

  Surgical removal of tumor is often followed by radiation therapy to remove residual microscopic cells. Superiority of this combination has been proved by data demonstrating a recurrence rate of 14.3% for those patients undergoing breast conservation therapy followed by radiation as compared to 39.2% for those undergoing surgery alone [71].

1.3.3.2  **Systemic treatment**

The systemic treatment option makes use of drugs, hormones, antibodies and enzymes to treat breast cancer wherein, these therapeutic agents are administered through blood vessels or are given orally.
• **Chemotherapy**

Chemotherapy is usually recommended for all women with an invasive breast cancer that is hormone receptor-negative. It is usually administered intravenously or given orally. On the other hand, regional chemotherapy is the administration of drugs directly into the cerebrospinal fluid, an organ, or a body cavity such as the abdomen, mainly affecting cancer cells in those areas. Several therapeutic drug options are available for the treatment through chemotherapy and are discussed in the ensuing sections.

• **Hormone therapy**

Hormone therapy removes hormones or blocks their action and stops cancer cells from growing. For example, hormone therapy with tamoxifen is often given to patients with early stages of breast cancer and those with metastatic breast cancer [72]. Moreover, hormone therapy with an aromatase inhibitor is given to some postmenopausal women who have hormone-dependent breast cancer. Presently, more than 90% of patients with breast cancer require either chemotherapy or hormonal therapy [73].

• **Targeted therapies**

Therapies targeting a specific downstream protein or a metabolite comes under this category and is being studied extensively. Some of the frequently used therapeutic strategies are targeting Her2 and VEGF protein that play important role in tumor progression and chemoresistance [74]. Some of the commonly used targeted therapies for breast cancer are trastuzumab (Her-2), lapatinib (Her-2), everolimus (mTOR) and bevacizumab (VEGF) [74].

1.3.4 **Chemotherapy: a ‘mainstay’ in breast cancer treatment**

Breast cancer treatment is often a combination of different types of therapies to ensure eradication of the entire tumor. However, interestingly,
Chemotherapy has been for years, a major modality of treatment in the breast cancer clinic. Chemotherapy can be of two major types: neo-adjuvant and adjuvant therapy [75]. Neo-adjuvant chemotherapy is commonly used to reduce the size of a tumor prior to surgery and also to prevent development of micrometastasis [76, 77]. Neo-adjuvant chemotherapy is being increasingly utilized in patients with locally advanced breast cancer and many centers are favoring treatment with cytotoxic agents and radiotherapy before surgery to “downstage” tumors, opening the possibility of limited surgery such as lumpectomy and thus to avoid mastectomy. Adjuvant chemotherapy is given after surgery to reduce the risk of recurrence and to eliminate micrometastasis thereby preventing distant metastasis [78]. Of the many cytotoxic drugs used, Taxanes are one such class of drugs which have demonstrated exceptional activity towards breast cancers [79].

1.3.4.1 Taxanes

Taxanes are diterpenes produced by the plants of the genus Taxus (yews), and are widely used as chemotherapy agents [80]. Taxane agents include paclitaxel (Taxol) and docetaxel (Taxotere) [81]. Taxol, a brand name of paclitaxel with chemical structure as depicted in Figure 1.3, is one of the most effective anticancer drugs used in the clinic to treat a variety of solid tumors. Since its discovery in 1960’s, paclitaxel has been used extensively and is well established as a potent anticancer agent.

Paclitaxel interferes with normal function of microtubule growth, and stabilizes their structure. This destroys the ability of the cell to use its cytoskeleton in a flexible manner. Specifically, paclitaxel binds to the β-subunit of tubulin [82]. Tubulin is the building block of microtubules, and the binding of paclitaxel locks these building blocks in place [83]. The resulting microtubule-paclitaxel complex does not have the ability to disassemble [83], which is a requisite for microtubule dynamic function. This adversely affects cell function because the shortening and lengthening of microtubules is necessary for their function as a mechanism to transport other cellular components.
1.3.5  Paclitaxel related adversities

Although the use of paclitaxel in chemotherapy has reported commendable success rate as single agent therapy (20-40%), its use is generally associated with major and minor side effects in patients receiving this therapy. The common side-effects include [84]:

- an increased risk of getting an infection due to a decline in white blood cell count
- tiredness and breathlessness due to a drop in red blood cells (anaemia)
- bruising more easily due to platelet decrease
- numbness or tingling in fingers and toes
- aching joints (arthralgia) and muscles (myalgia)
- inflammation
- tiredness and weakness (fatigue)
- hair loss (alopecia)
- low blood pressure during the treatment
- mouth sores, ulcers and diarrhea
- liver changes
- loss of fertility

Some of the above stated side-effects although diminish after the therapy, they may mark the beginning of other severe situations as is the case with the inflammation caused by paclitaxel which can trigger cancer progression and/or recurrence [85, 86]. This has been established by several researchers who have studied the links between chemotherapy-mediated cancer progression especially in the case of paclitaxel [85].
1.3.6 Paclitaxel and NF-kB signaling pathway

Recent studies have showed that paclitaxel is able to induce early reactive oxygen species (ROS) production in cancer cells, and hydrogen peroxide (H₂O₂) was found to be involved in paclitaxel-induced cancer cell death in vitro and in vivo [87,88]. Though this has been claimed as to be a ‘bystander effect’ of ROS generation by paclitaxel [89] there are evidences which strongly support the view that presence of ROS in cancer cells could trigger the activation of the inflammatory pathway lead by the transcription factor nuclear factor-κappa B (NF-κB) [90-92].

Rel or NF-κB proteins comprise a family of structurally-related eukaryotic transcription factors that are involved in the control of a large number of normal cellular and organismal processes, such as immune and inflammatory responses, developmental processes, cellular growth, and apoptosis [93-98]. In addition, these transcription factors are persistently active in a number of disease states, including cancer, arthritis, chronic inflammation, asthma, neurodegenerative diseases, and heart disease [96].

Known inducers of NF-κB activity are highly variable and include ROS, tumor necrosis factor alpha (TNFα), interleukin 1-beta (IL-1β), bacterial lipopolysaccharides (LPS), isoproterenol, cocaine, ionizing radiation etc [99]. Paclitaxel, like many other cytotoxic drugs, is a known activator of NF-κB [100]. The induction of ROS during paclitaxel therapy is a major link to the activation of
NF-κB and its downstream (Figure 1.4). The activity of NF-κB is primarily regulated by the interaction with inhibitory IkB proteins [101, 102]. Once activated, the transcription factor NF-κB translocates to the nucleus and binds at the κB site in the DNA to eventually regulate gene expression [101, 102].

![Image of the activation process of NF-κB](Image courtesy: Cassidy et. al., 2003)

**Figure 1.4:** ROS generated in cells lead to the activation of NF-κB and its downstream

### 1.3.6.1 NF-κB signaling and cancer

NF-κB is widely used by eukaryotic cells as a regulator of genes that control cell proliferation and cell survival [101]. In cancer, NF-κB is increasingly recognized as a crucial player in many steps in cancer initiation and progression thereby potentially preventing the success of anti-cancer therapy (Figure 1.5) [103]. NF-κB is engaged in tumorigenesis via the promotion of cell proliferation and suppression of cell death [104, 105]. NF-κB controls certain key cell cycle regulatory genes, including cyclin D1, cyclin D2, cyclin D3, cyclin E1, c-myc, CDK2, CDK4 and CDK6 [106-108]. Recent biological evidences reveal that the pro-survival function of NF-κB is related to its functional interaction with the PI3K-AKT-mTOR signalling pathway, one of the key elements in promoting cell proliferation and cell growth [109-111]. When cells are treated with cytokines and growth factors, AKT engages mainly IKKα in promoting NF-κB activation. The anti-apoptotic function of NF-κB is mainly achieved through the transcriptional regulation of an array of anti-apoptotic proteins, which can be divided into two groups. The first group mainly includes inhibitor of apoptosis proteins (IAPs),
Ciap1, Ciap2, XIAP and CFLIP [112]. The second group mainly refers to Bcl-2 family members, including Bcl-2 and Bcl-xL [113].

NF-κB also regulates the process of metastasis via its transcriptional activation of target genes, including VCAM-1, ICAM-1, MMPs and CXCR4 [116-120]. More importantly, it has been demonstrated in a mouse model that IKKβ/IκBα/ NF-κB pathway is required for the induction and maintenance of epithelial mesenchymal transition (EMT) [121-125]. IKK-dependent but NF-κB transcription-independent function is also involved in the control of metastasis. It has been shown that genetic inhibition of IKKα kinase activity promotes Maspin expression and reduces metastatic potential of the cancer cells [126]. Moreover, NF-κB is involved in angiogenesis by controlling key angiogenesis factors such as VEGF, IL-6, MCP-1 and MMPs [120, 127-130]. Similar to IKK-dependent but NF-κB transcription independent control of metastasis, there is also a specific link between IKK and angiogenesis. It has been showed that IKKβ upregulates mTOR activity through direct phosphorylation of TSK1 at ser487 and ser511 [131].

Figure 1.5: Nuclear factor kB (NF-κB) activation affects all six hallmarks of cancer through the transcription of genes involved in cell proliferation, angiogenesis, metastasis, inflammation and suppression of apoptosis as identified in both cell lines and tissue samples (Image courtesy: Baud et.al, 2009)

Another significant area of cancer research is multidrug resistance and NF-κB regulates several genes which mediate resistance to chemodrugs [132-134]. A major gene set transcribed by NF-κB in this regard is the ATP binding cassette
(ABC)-containing drug efflux transporters which play important roles in regulating intracellular drug concentrations that determine cell sensitivity to chemotherapeutic agents [135, 136]. Of particular relevance to cancer chemotherapy are the transporters P-glycoprotein (Pgp) encoded by multidrug resistance 1 (MDR1) gene [137].

In recent years, cancer stem cells (CSCs) have gained immense interest as key tumor-initiating cells that may also play an integral role in recurrence following chemotherapy [138-145]. As such, a number of mechanisms of chemoresistance have been identified in CSCs. Breast cancer stem cells are identified by virtue of their expression of the cell surface markers epithelial-specific antigen (ESA) and CD44 and the absence of expression of CD24 [146]. The expression of aldehyde dehydrogenase is also considered a relevant marker [147, 148]. Studies have reported that activated NF-κB pathway causes self-renewal in breast cancer cells thereby permitting them the property of ‘stemness’, as depicted schematically in Figure 1.6. The interleukin IL-8 which is transcribed by NF-κB is regarded as a chief player in this process [149].

Figure 1.6: NF-κB may regulate self-renewing breast cancer stem cells and induce production of extracellular proteins for generating cancer stem cell niche (Image courtesy: Hinohara et.al, 2012)
1.3.7 Clinical manifestations of NF-κB activation and its management

In the breast cancer clinic, patients receiving paclitaxel chemotherapy are often presented with chronic inflammation manifested in the form of arthralgia, myalgia and neuropathy [151, 152]. Literature indicates that all these physical conditions caused by paclitaxel therapy are linked to the activation of the inflammatory pathway mediated by NF-κB [153, 154].

Presently, such patient conditions are managed by the administration of non-steroidal anti-inflammatory drugs (NSAIDs), opioids, corticosteroids, immunosuppressive agents and anti-histamines for treating the inflammation associated with Ptx [155-157]. However, continued administration of such agents leave patients at risk of adverse effects [156, 157]. In the last few years, researchers have embarked on proteosome inhibitors, antisense oligodeoxynucleotides, cell penetrating peptides and small molecule inhibitors to address NF-κB (Figure 1.7) [158-161].

![Figure 1.7: A variety of drugs that inhibit NF-κB have been classified according to their molecular targets. (Image courtesy: D’Aquisto et.al, 2002)](image)

1.3.8 Natural compounds as NF-κB inhibitors

Amidst all the research on synthetic anti- NF-κB agents, the quest for naturally derived compounds especially polyphenol, have also gained high
reputations, owing to their anti-NF-κB effects at comparable concentrations as that of the classical anti-inflammatory drugs. Data from pre-clinical and clinical studies have strongly supported some of the polyphenols such as Curcumin, Epigallocatechin gallate (EGCG), Capsaicin and Resveratrol as potent inhibitors of NF-κB [162-179]. Of these compounds, EGCG needs special mention because of its multifaceted inhibitory effects [161, 163-165, 180-185]. It is a compound originally derived from green tea and is reputed for its multiple signaling inhibitory properties demonstrated in a wide range of cancer types including breast cancer (Figure 1.8).

![Figure 1.8: Effect of EGCG on EGFR, MAPK cascades, and activation of the transcription factors AP-1 and NF-κB. (Image courtesy: Khan et. al, 2006)](image)

### 1.3.8.1 Green tea and Epigallocatechin gallate (EGCG)

Green tea is a popular drink consumed by millions around the world. It is chemically characterized by the presence of large amounts of polyphenolic compounds known as catechins (Figure 1.9). A typical cup of brewed green tea contains, by dry weight, 30–40% catechins including epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epigallocatechin-3-gallate (EGCG) [186]. EGCG is the most prominent catechin found in green tea
accounting for about 60% of the total catechin in green tea [187]. Research claims that EGCG is responsible for the majority of the potential health benefits attributed to green tea consumption [188]. Chemical structure of EGCG consists of three heterocyclic rings- A, B, and C, with multiple hydroxyl groups.

![Chemical structures of the major tea polyphenols. (Image courtesy: Bigelow et.al, Oncogene, 2006)](image)

**Figure 1.9: Chemical structures of the major tea polyphenols. (Image courtesy: Bigelow et.al, Oncogene, 2006)**

### 1.3.8.1.1 Benefits of EGCG

EGCG possesses the strongest antioxidant activity and is a stronger antioxidant than other well known dietary antioxidants such as ascorbic acid (vitamin C), α-tocopherol (vitamin E) and β-carotene (a vitamin A precursor) [189]. The antioxidant property of EGCG arises from the phenolic groups that can oxidize to generate quinone compound [190, 191]. This is further enhanced by the presence of the trihydroxyl group in the B ring and the gallate moiety at the 3’ position in the C ring [192].

EGCG is widely renowned as a potent chemopreventive agent and this is well supported by results from epidemiological, cell culture, animal and clinical studies [193-195]. The antioxidant property of EGCG is strongly regarded as a major attribute that drives its multifaceted properties. Various mechanisms have been proposed to explain the chemoprevention process of EGCG, of which the generally accepted proposals include:-

(i) EGCG may enhance gap junctional communication between cells and thus protect cells from tumor development [196]. Experimental studies
suggest an effect of this polyphenol in blocking the promotion of tumor growth by sealing receptors in the affected cells [197];

(ii) Another possible mechanism indicates that this compound may facilitate direct binding to certain carcinogens [198].

Its inhibitory influences may ultimately suppress the final steps of carcinogenesis as well, viz., angiogenesis and metastasis. Various animal studies have revealed that treatment with EGCG inhibits tumor incidence and multiplicity in different organ sites such as skin (UV radiation and chemically induced), lung, liver, breast, prostate, stomach and colon based on preclinical, observational, and clinical trial data [199, 200]. In vitro cell culture studies showed that EGCG potently induces apoptosis and promotes cell growth arrest, by altering the expression of cell cycle regulatory proteins, activating killer caspases, and suppressing NF-κB activation [180]. EGCG was also shown to affect several biological pathways, including growth factor-mediated pathway, the mitogen activated protein (MAP) kinase-dependent pathway, as well as the ubiquitin/proteasome degradation pathway [180].

With regard to the effect of EGCG on NF-κB, EGCG has been shown to inhibit the activation of NF-κB in head and neck and MDA-MB-231 breast cancer [201-203]. Recently, EGCG has been reported to inhibit invasion via suppression of NF-κB-mediated matrix metalloproteinase-9 expression in cancer [204]. EGCG also directly targets tumor vasculature mediated by the inhibition of HIF-1α and NF-κB activation, thereby inhibiting tumor growth, proliferation, migration, and angiogenesis [205]. The mechanistic advance of EGCG on inhibiting tumor angiogenesis is proposed to be very unique, wherein EGCG does not target angiogenesis in normal tissue [205]. This claim is supported by the view that the presence of oxidative stress is much higher in cancer tissue than at normal sites of the body [206], letting the antioxidant EGCG to be more active at the diseased site. Figure 1.10 depicts the various molecules on which EGCG has been reported to exhibit antagonistic effects.
1.3.8.1.2 Limitations of EGCG: Stability and bioavailability

EGCG is highly stable in the acidic pH range of 2.0–5.5. In neutral and alkaline pH, however, EGCG is easily autooxidized [207]. In HBSS (pH 7.4) at 37°C, oxidative products are formed in a time-dependent manner and these products are evaluated to be EGCG dimers which form in mild alkaline fluids or after reaction with the 1,1-diphenyl-2-picryl-hydrazyl radical (found in small intestine) [208-210].

The polyphenolic structure of EGCG although is a major contributor to its multispectral biological effects, is also a reason for its low bioavailability. It is understood that the polyphenolic structure of EGCG allows it to form hydrogen bonds with water molecules thereby forming a large hydration shell around it [211]. This drastically reduces intestinal absorption which eventually affects EGCG bioavailability [212]. According to Lipinski’s Rule of 5, compounds with a molecular weight > 500, >5 hydrogen bond donors or 10 hydrogen bond acceptors would have poor bioavailability due to their large actual size (high molecular
weight) or large apparent size (due to the formation of a large hydration shell) [213].

EGCG is also subject to extensive biotransformation including methylation, glucuronidation, sulfation and ring-fission metabolism [214, 215]. Studies on the enzymology of EGCG methylation have shown that it is methylated to form 4″-O-methyl-(−)-EGCG and 4′,4″-O-dimethyl-(−)-EGCG [214].

Several other factors, including temperature, oxygen concentration, antioxidant concentration, metal ions, among others also affect stability of EGCG [216, 217]. Hard water with high concentrations of Ca$^{2+}$ and Mg$^{2+}$, or even drinking milk together with EGCG leads to its inactivation [218]. Furthermore, new findings on bioavailability have shown that EGCG absorption takes places mostly in the small intestine, and that in subjects with a functioning colon it passes to the large intestine where it is broken down to phenolic acids by the action of colonic microflora [219]. Figure 1.9 depicts a list of factors that drastically influence the bioavailability of EGCG. Until now, the restricted use of polyphenols in clinical practice is mainly due to their poor systemic bioavailability and relative instability in a given microenvironment. Nevertheless, diverse methods have been investigated to describe how to overcome these restrictions e.g. addition of the absorption enhancer such as piperine, vitamin A, fish oils, fluorine chemistry and so on (Figure 1.11). Development of nano-scaled carrier systems for EGCG has also been widely attempted and found to be successful in overcoming the limitations of EGCG (discussed in details subsequently).

Figure 1.11: Factors influencing EGCG bioavailability: Factors enhancing plasma levels of EGCG are listed to the left, those that diminish bioavailability can be found on the right (Image courtesy: Mereles et.al, Int. J Mol. Sci., 2011)
1.3.9 **Nanotechnology in Drug Delivery**

Nanotechnology, the science, usually refers to research at the scale of 1-1000 nm. Nanotechnology is unique in that it represents not just one specific area, but a vast variety of disciplines ranging from basic material science to personal care applications. One of the important areas of nanotechnology is ‘nanomedicine’ which is a promising improvement to the conventional concept of drug delivery. The National Cancer Institute (NCI) has recognized nanotechnology as an emerging field with the potential to revolutionize modern medicine for detection, treatment, and prevention of cancer [220].

1.3.9.1 **Nanomedicine**

Nanomedicine has an incredible potential for revolutionizing the therapeutics and diagnostics under the premise of developing ingenious nanodevices. Drug delivery nanosystems constitute a significant portion of nanomedicine. The importance of nanotechnology in drug delivery is in the concept and ability to manipulate molecules and supramolecular structures for producing devices with programmed functions. Nanoscale delivery vehicles can:
(1) enhance the therapeutic efficacy and minimize adversities associated with available drugs;
(2) enable new classes of therapeutics; and
(3) encourage the re-investigation of pharmaceutically suboptimal but biologically active new molecular entities (eg. Polyphenols) that were previously considered unimportant [221].

Compared to conventional drug delivery, nanosystems provide a number of advantages. In particular, they can enhance the therapeutic activity by prolonging drug half-life, improving solubility of hydrophobic drugs, enhancing bioavailability of hydrophilic drugs, reducing potential immunogenicity, and/or releasing drugs in a sustained or stimuli-triggered fashion [222-225]. These advantages can help in reducing the toxic side effects of drugs, as well as the administration frequency. In addition, nanoscale particles can passively accumulate in specific tissues (e.g. tumors) through the enhanced permeability and retention (EPR) effect which is also known as passive targeting [226, 227].
Beyond these clinically efficacious nanosystems, nanotechnology has been utilized to enable new therapies and to develop next generation nanosystems for “smart” drug delivery [228]. In addition to all these attributes, nanoparticles can be modified with cell/tissue-specific ligands which render them potential enough to enhance the therapeutic efficacy and reduce the side effects relative to conventional therapeutics [230]. In cancer therapy, the presence of targeting ligands can greatly enhance the retention and cellular uptake of nanoparticles via receptor-mediated endocytosis even although tumor accumulation is largely determined by the physicochemical properties of nanoparticles [231, 232]. This can then lead to higher intracellular drug concentrations and increased therapeutic activity, which is particularly important for bioactive macromolecules that require intracellular delivery for bioactivity [221]. Figure 1.12 depicts passive and active targeting that is achievable by nanocarriers.

Figure 1.12: Passive and active targeting approaches of nanoprobes in cancer diagnosis. Passive tumor targeting is achieved by extravasation of nanoprobes through increased permeability of the tumor vasculature and ineffective lymphatic drainage (EPR effect). Active targeting, also called ligand-mediated targeting, involves utilizing affinity ligands on the surface of NPs for specific retention and uptake by the targeted disease cells. (Image courtesy: Park, Quant Imaging Med Surg., 2012)
There are several types of biocompatible nanosystems commonly suggested for use in drug delivery. These are generally developed using a variety of materials including polymers (polymeric nanoparticles, micelles, or dendrimers), lipids (liposomes), viruses (viral nanoparticles), and even organometallic compound (nanotubes) (Figure 1.13) [233-239].

Figure 1.13: Types of nanocarriers for drug delivery. A, polymeric nanoparticles: polymeric nanoparticles in which drugs are conjugated to or encapsulated in polymers. B, polymeric micelles: amphiphilic block copolymers that form to nanosized core/shell structure in aqueous solution. The hydrophobic core region serves as a reservoir for hydrophobic drugs, whereas hydrophilic shell region stabilizes the hydrophobic core and renders the polymer to be water-soluble. C, dendrimers: synthetic polymeric macromolecule of nanometer dimensions, which is composed of multiple highly branched monomers that emerge radially from the central core. D, liposomes: self-assembling structures composed of lipid bilayers in which an aqueous volume is entirely enclosed by a membranous lipid bilayer. E, viral-based nanoparticles: in general structure are the protein cages, which are multivalent, self-assembles structures. F, carbon nanotubes: carbon cylinders composed of benzene rings (Image courtesy: Cho et al., Clin. Can. Res., 2008)

Various anticancer drugs such as doxorubicin, taxol, docetaxel, 5-flourouracil and daunorubicin have been loaded into appropriate nanocarriers to improve their solubility, efficacy etc [240] (Table 1.1).
Table 1.1: List of nanoparticles developed using various biocompatible materials


<table>
<thead>
<tr>
<th>System</th>
<th>Structure</th>
<th>Characteristics</th>
<th>Examples of compounds</th>
</tr>
</thead>
</table>
| Polymeric nanoparticles       | Drugs are conjugated to the side chain of a linear polymer with a linker | (a) Water-soluble, nontoxic, biodegradable
(b) Surface modification
(pegylation)
(c) Selective accumulation and retention in tumor tissue (EPR effect)
(d) Specific targeting of cancer cells while sparing normal cells—receptor-mediated targeting with a ligand | Albumin-Taxol (Abraxane)
PGA-Taxol (Yotax)
PGA-Camptothecin (CT-2106)
HPMA-DOX (PK1)
HPMA-DOX-galactosamine (PK2) |
| Polymeric micelles            | Amphilic block copolymers assemble and form a micelle with a hydrophobic core and hydrophilic shell | (a) Suitable carrier for water-insoluble drug
(b) Biocompatible, self-assembling, biodegradable
(c) Ease of functional modification
(d) Targeting potential | PEG-pluronic-DOX
PEG-PAA-DOX (NK911)
PEG-PLA-Taxol (Genexol-PM) |
| Dendrimers                    | Radially emerging hyperbranched synthetic polymer with regular pattern and repeated units | (a) Biodistribution and PK can be tuned
(b) High structural and chemical homogeneity
(c) Ease of functionalization, high ligand density
(d) Controlled degradation
(e) Multifunctionality | PAMAM-MTX
PAMAM-platinate |
| Liposomes                     | Self-assembling closed colloidal structures composed of lipid bilayers     | (a) Amphiphilic, biocompatible
(b) Ease of modification
(c) Targeting potential | Pegylated liposomal DOX (Doxil)
Non-pegylated liposomal DOX (Myocet)
Liposomal daunorubicin (Dauno/Xome) |
| Viral nanoparticles           | Protein cages, which are multivalent, self-assembled structures           | (a) Surface modification by mutagenesis or bi conjugation—multivalency
(b) Specific tumor targeting, multifunctionality
(c) Defined geometry and remarkable uniformity
(d) Biological compatibility and inert nature | HSP-DOX
CPMV-DOX |
| Carbon nanotubes              | Carbon cylinders composed of benzene ring                                 | (a) Water-soluble and biocompatible through chemical modification (organic functionalization)
(b) Multifunctionality | CNT-MTX
CNT-amphotericin B |
1.3.10 Nanotechnology in combination-delivery of drugs

Combination therapy has shown several potential advantages (e.g. synergistic effects and reversal of drug resistance) and may prove more effective than single drug therapy [241]. However, due to the distinct pharmacokinetic profiles of individual drugs, the synergistic drug ratio optimized in vitro will undoubtedly change after the conventional administration of drug ‘cocktails’—an outcome that could in turn lead to insufficient therapeutic results in vivo. To this end, lipid- and/or polymer-based nanoscale systems, previously developed for single drug delivery, have been applied to facilitate co-delivery. For some drug combinations, successful tuning of the relative dosage of various drugs in single particle level is possible, enabling simultaneous delivery to target sites with a maintained drug ratio [242]. For certain other combinations, novel delivery vehicles with desired functionalities that enable co-encapsulation of hydrophobic and hydrophilic drugs, active targeting, and/or temporally controlled release have been developed [243-245]. In recent years, several types of nanocarriers loaded with multiple chemotherapeutic agents have been developed which exhibited improved anticancer activity. Certain representative case studies are discussed here owing to their therapeutic value.

1.3.10.1 Liposomes, Dendrimers, Micelles, Polymeric and Solid-lipid nanoparticles

Liposomes encapsulating curcumin and resveratrol have been formulated and, upon systemic administration, their chemopreventive effect was exemplified in prostate-specific PTEN knockout mice in vivo, as a result of PTEN loss and/or activated p-Akt signalling pathways [246]. In another study, Wu et al. formulated a transferrin-conjugated liposome by co-entrapping doxorubicin and varapamil (a P-gp inhibitor). They evaluated the effectiveness in doxorubicin-resistant K562 cells revealing enhanced cytotoxicity caused by overcoming P-gp-mediated multidrug resistance [247]. The ratiometric approach involving the co-delivery of two different drugs with differing molar ratios following systemic administration is also an important factor for improving the therapeutic efficacy of the dual drug
in a combination approach for cancer treatment [248]. In this regard, a Phase I study was performed by using a liposomal formulation of two different drugs [i.e. irinotecan and floxuridine in the ratio of (1:1)], and the maximum tolerated dose as well as pharmacokinetic parameters of the liposomal formulation were determined in patients with advanced solid tumors. The results demonstrated that the above dual-drug-loaded liposomal formulation was well tolerated, showing enhanced anticancer activity in patients [249].

Owing to the presence of a highly branched structure, dendrimers can be used as a suitable targeted drug delivery vehicle by conjugating with different ligands. The presence of multivalent branches with cage-like structures has been used as a suitable platform for simultaneous delivery of hydrophobic and hydrophilic drugs. Taking this into consideration, Tekade et al. have formulated dual-drug-loaded dendrimers by co-encapsulating methotrexate (MTX; a hydrophobic drug) and all-trans retinoic acid (ATRA; a hydrophilic drug) inside the polyamidoamine (PAMAM) dendrimer. They demonstrated reduced hemolytic toxicity of the dendrimer and enhanced the cytotoxicity profile in HeLa cells, compared with free drug [250].

Polymeric micelles have also been successful in anticancer drug delivery. Recently, Wang et al. investigated the efficiency of the simultaneous and targeted delivery of paclitaxel, along with verapamil, by using a micellar system to overcome MDR and demonstrated enhanced cytotoxicity in drug-resistant tumor cells [251]. Further, Katragadda et al. formulated paclitaxel and 17-allylamino-17-desmethoxygeldanamycin (17-AAG)-loaded PEG-distearoylphosphatidylethanolamine/tocopheryl polyethylene glycol 1000 (PEG-DSPE/TPGS) mixed micelles and demonstrated that the dual-drug-loaded mixed micelles effectively blocked the proliferation of human ovarian cancer SKOV-3 cells [252]. In another study, Shin et al. developed block co-polymeric micelles (PEG-b-PLA) by entrapping three different drugs (paclitaxel, 17-AAG and rapamycin), and they have shown the above drug-loaded micelle acting as a three-in-one nanocontainer for solubilizing multiple drugs [253]. They have demonstrated that this micelle consisting of three drugs had a high synergistic
effect in MCF-7 and 4T1 breast cancer cells so that the above formulation provided a simple and efficacious three-in-one nanomedicine for cancer therapy.

Biocompatible polymers have been very attractive carriers for drug delivery especially in the form of nanoparticles (NPs) encapsulating a range of drugs. Acharya et al. demonstrated the synergistic effect of dual drugs entrapped in polymeric NPs resulting in enhanced cytotoxicity of the combination formulation as compared with the free drugs at a low dose against leukemic K562 cells [254]. The synergistic effect of co-formulation of doxorubicin and curcumin in poly (d,l-lactide-co-glycolide) (PLGA) NPs promoted cytotoxicity of both drugs in leukemic K562 cells in vitro by overcoming the MDR phenotype [255]. Polymeric NPs have been used to deliver a combination of small interfering (si)RNA and chemotherapy. In this regard, poly(ethylene oxide)-modified poly(beta-amino ester) (PEO-PbAE) and PEO-modified poly(epsilon-caprolactone) (PEO-PCL) NPs encapsulated with MDR-1 silencing siRNA and paclitaxel significantly enhanced the cytotoxic activity of paclitaxel in resistant SKOV3 cells similar to that observed in drug-sensitive SKOV3 cells [256]. Song et al. have reported reversion of multidrug resistance by co-encapsulation of vincristine and verapamil in PLGA nanoparticles [257].

Solid-lipid nanoparticles (SLNPs) formulated by encapsulating cholesteryl-butyrate (a prodrug of butyrate), doxorubicin or paclitaxel were evaluated for their antiproliferative effect on the human colorectal cancer cell line HT-28. They have shown higher cytotoxicity than the equivalent amount of free-drug treatment as a result of the synergetic effect [258]. Currently, polymer–lipid hybrid NPs (PLN) are an emerging new form of SLNPs. A novel PLN was formulated by encapsulating doxorubicin and a chemosensitizer, GG918 (elacridar), for the treatment of MDR breast cancer cells and the results demonstrated that doxorubicin and GG918 co-encapsulated PLN formulation showed a greater efficiency when compared with the single drug formulation on a MDR breast cancer cell line [259].
1.3.10.2 Combinatorial nanoparticles

Combinatorial nanoparticles containing multiple cytotoxic drugs with different mechanisms of action and chemical natures are also relevant in this regard. Recently, core/shell nanoparticles made of PLGA and bovine serum albumin were reported for entrapping the small molecule inhibitors, everolimus and sorafenib [260]. The same system was also made use for encapsulating a photosensitizer, m-tetra(hydroxyphenyl)chlorin (mTHPC) and Src kinase inhibitor dasatinib for breast cancer [261]. Aryal et.al had earlier reported lipid-polymer particles for doxorubicin and camptothecin combination for prostate cancer [262]. Combretastatin and doxorubicin in lipid-PLGA nanocells exhibited temporally sequenced release of the drugs [263]. To broaden the applicability of combinatorial nanoparticles, Zhang et al. conducted a pioneering work in co-encapsulating hydrophobic and hydrophilic drugs within a polymeric nanoparticle platform [264]. In the study, RNA aptamers were conjugated to the surface of PLGA-PEG polymeric micelles loaded with hydrophobic anticancer drug, docetaxel.

1.3.11 Nanotechnology to overcome limitations of EGCG

The drawbacks of EGCG in terms of its stability and bioavailability have been addressed by utilizing nanotechnology based approaches in drug delivery (Table 1.2). Depending on the type of nanoparticle, incorporated EGCG can be partially or completely sequestered in it, resulting in high stability. One of the first attempts to encapsulate EGCG was by Siddhiqi et.al wherein they used PLA-PEG nanoparticles to demonstrate enhanced bioavailability and efficacy in prostate cancer [265]. Nanoliposomes loaded with EGCG dramatically enhanced its stability both in 1X phosphate-buffered saline (PBS) and Eagle's minimum essential (EME) cell culture medium [266]. Barras et al. [267] demonstrated that free EGCG and EGCG-loaded SLNs in water exhibited 100% degradation within 4 h and over 4 weeks, respectively. In addition, free and nano-EGCG displayed burst and sustained release properties, respectively [268, 269]. Chitosan has been explored as a carrier for improving EGCG bioavailability owing to its positive
charge and muco-adhesive properties [270-272]. With regard to the biological efficacy of nano-EGCG, Hu et al reported that treating HepG2 cells with 26–37 μM of nano-EGCG resulted in higher cellular antioxidant activity compared to free EGCG at the same concentrations [272]. Siddiqui et al. [265] demonstrated that, compared to free EGCG, nano-EGCG (PLGA nanoparticles) exhibited more than 10-fold dose advantage in inducing apoptosis, decreasing viability and inhibiting colony formation of prostate cancer cells. The IC50 values of free and nano-EGCG are 43.6 and 3.74 μM, respectively. They also gave tumor xenograft mice either 100 μg of nano-EGCG or 1 mg of free EGCG three times per week through intraperitoneal injection and found that nano-EGCG, even at a dose 10-fold lower, significantly reduced prostate tumor size. Moreover, when incorporating target ligands on the surface of nanoparticles, to specifically target an antigen on prostate cancer cells, the EGCG nanoparticles can reduce the viability of prostate cancer cells to a significantly larger degree compared to EGCG nanoparticles without target ligands [272, 273].

Table 1.2: Type, characteristics, functions and application of EGCG nanoparticles
(Adapted from J Nutri Biochem, 2014)

<table>
<thead>
<tr>
<th>NP type</th>
<th>NP characteristics</th>
<th>Experiment model/dose/route</th>
<th>Functions</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitosan–caseinophosphopeptide NPs</td>
<td>SZ: around 143 nm</td>
<td>HepG2 cells; dose: 0.125 mg/ml; duration: 2 h</td>
<td>↑ Sustained release ↑ Cellular uptake ↑ Antioxidant activity</td>
<td>Antioxidant</td>
</tr>
<tr>
<td></td>
<td>ZP: 31 mV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EE: 70.5%–81.7%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chitosan–caseinophosphopeptide NPs</td>
<td>SZ: around 150 nm</td>
<td>Caco-2 cells</td>
<td>↑ EGCG intestinal permeability and absorption through Caco-2 cells</td>
<td>Enhance bioavailability</td>
</tr>
<tr>
<td></td>
<td>ZP: 32 mV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chitosan NPs</td>
<td>SZ: around 440 nm</td>
<td>Male Swiss outbred mice; dose: 0.76 mg/kg bw of free or nano-EGCG; route: oral gavage; blood collection: PK</td>
<td>↑ EGCG stability ↑ EGCG bioavailability</td>
<td>Enhance bioavailability</td>
</tr>
<tr>
<td></td>
<td>ZP: 25 mV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nanoparticle Type</td>
<td>Particle Size and Zeta Potential</td>
<td>Cell Type/Condition</td>
<td>Effect</td>
<td>Disease/Condition</td>
</tr>
<tr>
<td>-------------------</td>
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</tr>
<tr>
<td><strong>PLGA–PEG NPs</strong></td>
<td>SZ: around 80 nm, EE: around 8%</td>
<td>PSMA-positive prostate cancer (LNCaP) cells; dose: 30 μM of free or nano-EGCG; duration: 48 and 72 h</td>
<td>↑ Sustained release, ↓ Viability of LNCaP cells, No effect on viability of normal cells</td>
<td>Prostate cancer</td>
</tr>
<tr>
<td><strong>Gum Arabic and maltodextrin NPs</strong></td>
<td>SZ: around 100 nm, ZP: −12 mV, EE: &gt;80%</td>
<td>Human prostate carcinoma cells; dose: 0–10 μM.</td>
<td>Retain EGCG anticancer activity</td>
<td>Prostate cancer</td>
</tr>
<tr>
<td><strong>Chitosan NPs</strong></td>
<td>SZ: 440 nm, ZP: 25 mV</td>
<td>Excised jejunum from male, Swiss outbred mice</td>
<td>↑ EGCG stability, ↑ EGCG intestinal absorption</td>
<td>Enhance bioavailability</td>
</tr>
<tr>
<td><strong>Nanolipidic EGCG NPs</strong></td>
<td>SZ: 30–80 nm</td>
<td>Male Sprague-Dawley rats; dose: 100 mg/kg bw; route: oral gavage; blood collection; PK</td>
<td>↑ EGCG bioavailability by more than 2-fold, ↓ Brain beta-amyloid plaque formation</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td><strong>NLCs</strong></td>
<td>SZ: 30 to 260 nm, EE: 95%</td>
<td>N/A</td>
<td>↑ EGCG stability</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Poly(lactide–polyethylene glycol) (PLA–PEG) NPs</strong></td>
<td>SZ: 285 nm, ZP: −7.92 mV</td>
<td>Prostate cancer (PC3) cells; dose: 1–80 μM of free or nano-EGCG; duration: 24 and 48 h, Tumor xenograft mice; dose: 1 mg and 100 μg of free or nano-EGCG, respectively; route: intraperitoneal injection 3 times per week</td>
<td>↑ Apoptosis, ↓ Cell viability, ↓ Colony formation, ↓ Tumor size, ↓ Angiogenesis</td>
<td>Prostate cancer</td>
</tr>
</tbody>
</table>

EE, encapsulation efficiency; N/A, not applicable; NP, Nanoparticle; PSMA, prostate-specific membrane antigen; SZ, size; ZP, zeta potential; bw, body weight; PK, Pharmacokinetics; ↑, increase; ↓, decrease.
1.3.12 Nanotechnology to overcome limitations of Ptx

Owing to the poor aqueous solubility of Ptx, it is currently formulated in a vehicle composed of 1:1 blend of Cremophor EL (polyethoxylated castor oil) and ethanol which is diluted 5-20-fold in normal saline or dextrose solution (5%) for administration. However, various health issues related to the drug vehicle have been reported. One of the substantial problems associated with this formulation is that the Ethanol:Cremophor vehicle required to solubilize Ptx is toxic [274]. Although it has been used to administer other drugs such as cyclosporine [275] and teniposide [276], the amount of Cremophor necessary to deliver the required doses of Ptx is significantly higher than that administered with any other marketed drug [277]. Another limitation of Ptx is its chemical instability. According to one study, Ptx solutions in the range of pH 4–8 were stable for 72 h [278]. On the other hand, a rapid degradation occurred at pH 11 (the small bowel pH) and numerous degradation products were observed within 1.5 h, and its degradation was virtually complete in 72 h. In addition to the possibility of metabolic interactions, Ptx has a high serum protein binding capacity (90–95%) [279-281]. Generally, a co-administration of agents which can potentially displace bound Ptx, is done. However the toxicity due to co-administration of the drugs demand compromises to be made in terms of the anticancer activity of Ptx.

Current approaches to improve such limitations of Ptx are focused mainly on the development of formulations that are devoid of Cremophor EL®, investigation of the possibility of a large-scale preparation and a request for a longer-term stability. These different approaches have shown some promising possibilities to replace Taxol by a less irritable preparation such as its: (i) micelle formulations [282] (ii) water-soluble prodrug preparations [283] (iii) enzyme-activatable prodrug preparations conjugated with antibodies or albumin [284, 285] (iv) parenteral emulsions [286] (v) microspheres [287] (vi) cyclodextrins [288] and (vi) nanocrystals [289]. Only Abraxane® (albumin nanoparticle-based Ptx preparation approved by US FDA) [290] and Lipusu® (liposomal Ptx approved by State FDA of China) [291] have entered the field of clinical applications.
1.4 Aim and hypothesis of the thesis

Although both the anti-cancer molecules viz., Ptx and EGCG have been extensively explored in their respective nanoforms to understand various aspects of their physico-chemical characteristics, biological activities/limitations, pharmacokinetics and pharmacodynamics, they have hardly been investigated in combination to address issues in cancer. The concept of combining Ptx and EGCG in a single nanosystem at defined ratios would be promising owing to a possible interplay between the two drugs eventuating in improved chemotherapy.

**AIM**

To create an equilibrium between the opposing effects of Ptx and EGCG on NF-κB signaling in cancer such as to overcome the adversities prevalent in patients receiving Ptx therapy and also augment/sensitize Ptx therapy with EGCG.

**HYPOTHESIS**

A nanocarrier mediated delivery of Ptx & EGCG would:
- prevent paclitaxel-induced NF-kB activation and downstream signaling
- bring about synergistic/additive anti-cancer effects
- alleviate the limitations of individual drugs

1.5 Specific objectives of the thesis

The following are the specific objectives of this thesis work:
- Design and develop a polymer-protein core/shell nanocarrier to optimally encapsulate Ptx and EGCG with good entrapment efficiencies for the dual drugs and sustained release profiles.
- Toxicological (Hemo-, cyto- and immuno-compatibility) analysis of the nanocarrier to qualify it for intravenous administration.
- Biodistribution and Pharmacokinetics of the nanocarrier and drugs in vivo.
- Biological evaluation of EGFR targeted and non-targeted dual drug loaded nanocarrier in breast cancer cells -
  - In vitro efficacy analysis, evaluation of NF-κB activation and downstream genes (MMP9, VEGFA, BIRC5 and p21) specific for metastasis, angiogenesis, survival and apoptosis.
- Isolation and culturing of breast cancer cells derived from tumor tissues of breast cancer patients.
- Immunohistochemistry and cytotoxicity analysis of bare drugs, individual nanodrugs, non-targeted and targeted (EGFR and HER2) combination nanomedicine on breast cancer patient samples.