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Applications of Nanosystems to Anticancer Drug Therapy (Part II. Dendrimers, Micelles, Lipid-based Nanosystems)

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Abstract: The great efforts of many researchers have brought down some of the barriers that exist to turn a good *in vitro* compound into a potential *in vivo* drug. The advent of pharmaceutical nanotechnology has allowed an arsenal of drugs with poor stability, low solubility, high off-target toxicity and other disadvantageous features, to be accessible as pharmaceutical products that could be administered to a patient. Nanotechnology was introduced in drug delivery very long ago, but has flourished with unprecedented intensity during the last twenty years and now a diversity of nano-based preparations are at clinical stage of development or already available in the market. Undoubtedly, nanotechnology plays a key role in future pharmaceutical development and pharmacotherapy. In the first part of this review, we have already discussed recent (2008-2012) patents on linear polymer-based nanosystems (nanogels, nanospheres and nanocapsules) applications to cancer therapy. Here, we have expanded such analysis to branched polymers (dendrimers), self-assembling nanomicelles and lipid-based nanocarriers.

Keywords: Anticancer drug therapy, dendrimers, lipid-based nanosystems, liposomes, micelles, nanostructured lipid carriers, patents, solid lipid nanocarriers.

1. INTRODUCTION

Multiple dose regimes are the most frequent drug-based therapeutic intervention. Leaving aside topical medications, conventional drug delivery systems rely on establishing a dynamic equilibrium or, more precisely, a pseudo-equilibrium between the free drug plasmatic concentration and the free drug concentrations in all the other body tissues (let us remember that living organisms are open systems; thus, a true equilibrium is seldom achieved due to the permanent mass exchange with the environment). After a number of doses are administered, a steady state is reached, during which plasmatic concentration will fluctuate –as long as the treatment goes on- between practically fixed maximal and minimal steady state concentrations. Since only the free, unbound drug can interact with its molecular target, the free drug levels at the vicinity of the site of action generally determine the extent of the pharmacological response [1]. A non-trivial implication of the former approach is that, to attain effective concentrations of an active ingredient in its biophase or site of action, the patient is subjected to systemic exposure to the drug, which often leads to off-target undesirable side effects. In other words, conventional drug delivery systems are characterized by non-specific distribution. Patients receiving anticancer treatment constitute a very illustrative example of the consequences of the previous setting: the well-recognized adverse reactions to chemotherapy emerge from interactions between the drug molecules and non-cancerous, healthy cells. These side-effects could then be ameliorated or avoided if targeted drug delivery systems were used.

On the other hand, a number of active ingredients cannot be fully exploited due to biopharmaceutical/pharmacokinetic issues. For example, a given drug, due to its physicochemical properties, might be non compatible with certain routes of administration. Frequently, drugs with scarce aqueous solubility cannot be formulated as intravenous solution. Drugs with poor gastrointestinal absorption, high first pass metabolism or low chemical stability in the gastro-intestinal media often preclude oral administration. Active ingredients with short half-life present difficulties to built up and sustain effective levels (reducing the duration of the pharmacological effect or requiring large doses just to compensate biotransformation). Finally, interaction of the free drug with efflux transporters from the ABC superfamily (e.g. P-glycoprotein, Multi-Drug Resistance Proteins) results in a reduced bioavailability and is linked to multi-drug resistance issues in a number of disorders such as epilepsy and cancer [2-4].

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So far we have mentioned disposition and safety issues related to: unfavorable physicochemical properties (poor solubility, poor permeability); unwanted interactions with the biological systems (metabolic enzymes, transporters) and; high off-target drug levels due to non-targeted distribution. These problems, which occur regularly with traditional small-molecule therapies, are even more frequent in the case of emerging therapies, particularly, biotherapies (gene therapy, therapeutic proteins), which owing to the intrinsic nature of complex macromolecules are far more susceptible to enzymatic cleavage, inactivation, poor permeability, slow distribution and immunogenicity [5-9]. As a result, the development of adequate delivery vectors is a key issue in the field of advanced biotherapeutics. While traditional delivery systems only deal with release and absorption of the therapeutic agent, (with no direct involvement on the modulation of distribution and elimination processes), advanced delivery systems should be able to retain their integrity throughout the drug distribution events, while permeating through different epithelia and endothelia, selectively releasing the drug in the proximity of the drug target. An ideal drug delivery device should, therefore: a) compensate unfavorable physicochemical properties of the active ingredient; b) encapsulate, entrap or adsorb drug molecules; c) conceal the drug from enzymatic cleavage, rapid metabolization and recognition by efflux transporters; d) extravasate; e) direct the drug to its therapeutic target; in the case of intracellular targets, promote cell uptake; f) once in the vicinity of the target (and not before), release the drug load in a controlled manner; g) present no toxicity nor accumulation within the body, preferentially, be biodegradable. Some years ago, a device which gathered such a wide range of features would have been unconceivable. Today, burgeoning advances on nanobiotechnology have brought us nearer and nearer to our seemingly utopical delivery system. We have recently discussed recent patents on linear polymer-based nanosystems applications to cancer therapy [10]. Here, we have extended such analysis to based on other materials, namely: branched polymers (dendrimers), self-assembling nanomicelles and lipid-based nanosystems. We have focused on patents published between 2008 and the present which describe embodiments related to cancer therapeutics.

2. DENDRIMERS

Dendrimers attracted much attention after they were first investigated by Tomalia 20 years ago [11], and they have become a growing research area in recent years. The name "dendrimer" comes from the greek *dendron*, which means tree. They differ from traditional polymers in that they have a multi-branched, 3D architecture with very low polydispersity and high functionality. They possess perfect nanoarchitectures comprising three different parts: a) a focal core; b) building blocks with several interior layers composed of repeating units or folds and; c) multiple peripheral functional groups [12].

Figure **1** shows a scheme of a dendrimer structure. The dendritic structure is characterised by 'layers' between each branching point: each layer of concentric branching units constitutes one complete generation (G) in the dendrimer series and it is identified with a specific generation number (G2, G3….G7, etc.). The core is sometimes denoted generation 'zero' (G0) [13, 14].

The high level of control over the architecture of dendrimers, their size, shape, branching length, density, and surface functionality, make these compounds ideal carriers

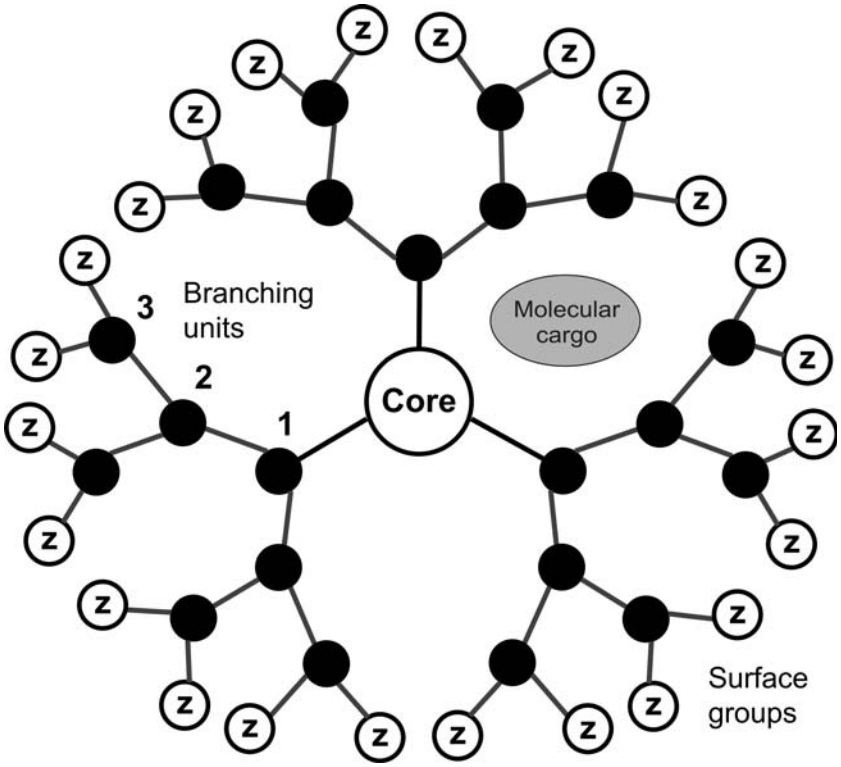

Fig. (1). Schematic representation of a dendrimer structure.

in biomedical applications such as imaging, drug delivery, gene and cancer therapy, and many others [15-17]. Poly (amidoamine) (PAMAM) dendrimers were the first synthesized and commercialized dendrimer family, and they are commercially available with the trade mark Starburst® dendrimers [18, 19].

In the first part of this review [10], the role of drug delivery as a critical aspect of drug therapies has been extensively discussed. Proper selection of the delivery system can control bioavailability and the concentration profile, and reduce undesirable side effects. Dendrimers represent an exciting opportunity for chemists to produce macromolecular structures with a specifically tailored function. They are the same size as serum proteins and hence are capable of directly entering into the tumor microvasculature by Enhanced Permeability and Retention (EPR) effect [20, 21]. Furthermore, the high density of surface groups allows attachment of targeting groups as well as groups that modify the behavior or toxicity of dendrimers, while smaller drug moieties can be encapsulated in the inner core [22].

Studies of biomedical applications of dendrimers are becoming more and more frequent, not only as nanoparticulate drug delivery systems but also as nonviral gene vectors [23, 24]. Using EGFP-C2 as a marker gene, it has been shown that PAMAM dendrimers, which have positively charged amine groups on their surface, can deliver the gene to various organs in the body after intravenous (IV) injection, achieving high expression levels in the lungs, liver, kidney, and spleen, with minimum or no cytotoxicity [25].

In spite of these promising results, care must be taken before proposing the systemic administration of dendrimeric systems: it is worth considering their biodistribution in the body and assessing the risk of unacceptable toxicity or immunogenicity that these artificial materials could cause [26]. Attaching cationic or anionic species to the dendrimer surface can influence the biocompatibility and disposition of these nanoparticles. Several studies demonstrated that the toxicity of dendrimers is not only generation (size)-dependent (the smaller being less toxic) but also that cationic dendrimers are more cytotoxic than their anionic counterparts [26-30]. Amine-terminated (PAMAM) dendrimers have recently been shown to activate platelets and cause a fatal, disseminated intravascular coagulation (DIC)-like condition in mice and rats [31-33]. A very recent work of Jones *et al.* demonstrate that, upon addition to blood, cationic G7 PAMAM dendrimers induce fibrinogen aggregation, which may contribute to the *in vivo* DIC-like phenomenon [34].

Overall, dendrimers are particularly well-suited for the delivery of anticancer drugs and imaging agents because of their high water solubility, monodisperse size, and uniform composition, which will lead to consistent batch-to-batch anticancer activity of dendrimers-based drug delivery systems [14]. Cellular uptake of these systems proved to be significantly higher than linear polymeric carriers such as N-(2-hydroxypropyl)methacrylamide (HPMA) [35, 36] and polyethylene glycol (PEG) [37, 38], which can be attributed to dendrimer's nano-size and compact spherical geometry in solution.

2.1. Optimizing Breast Cancer Therapy with Lymphatically Targeted Dendrimers

Active targeting of polymer-drug conjugates to cancer cells is commonly achieved by conjugation of tumor-specific targeting ligands (i.e. vitamins, carbohydrate residues, peptides, antibodies), which selectively bind to receptors that are expressed on the surface of cancer cells triggering receptor-mediated endocytosis and internalization of the whole conjugate. On the other hand, passive targeting relies on the EPR effect to improve tumor accumulation of the drug, but in tumors that are not highly vascularized, the EPR effect is greatly reduced and untargeted nanocarriers have fewer advantages. Accordingly, passive targeting is not effective in the treatment of tumors with low vascularity, such as cancerous cells that can be found in the lymphatic system [39].

In a 2012 patent, Forrest *et al.* [40] developed a chemotherapeutic composition for treating breast cancer (BC) that has the advantage of avoiding side effects by delivering chemotherapy directly to the tumor tissue in early cancers. It simultaneously reduces the risk of relapse, since it is preferentially directed into the lymphatics where it destroys the "seeds" that can cause recurrence after surgery. This invention avoids high off-target levels of the free drug (and thus, toxic side-effects) and surgery-related pain.

Neoadjuvant systemic chemotherapy is standard care for locally advanced BC, but after treatment, cancer typically spreads first via the lymphatics with little stroma invasion before becoming a systemic disease [41]. Surgical treatment for early stage BC involves resection of the primary tumor along with the draining sentinel lymph node and further lymphatic resection if warranted. However, this procedure may miss nanoscopic metastases in the lymph nodes. Localized radiation to the breast and lymphatics along with systemic chemotherapy reduce the risk of relapse, but these treatments cause extensive damage to healthy tissues [42, 43].

The invention consists in 10-80 nm nanoconjugates of a dendrimeric nanocarrier and a chemotherapeutic agent. Cancerous cells that are not vascularized, such as those found within the lymphatic system, are treated or inhibited by selectively accumulating the active agent in the loco-regional lymphatics of the tumor via a subcutaneous (SC) injection at the site of the tumor (e.g. injection into the upper mammary fat pad of female subjects for treatment of BC), allowing the drug to be delivered along the lymphatic pathway where tumors are most likely to initially metastasize.

The dendrimer generation can be selected to optimize the ratio of lymphatic to capillary uptake and dendrimer nanoconjugates with cisplatin, epirubicin and docetaxel are included in the patent as embodiments. Dendrimers may be also conjugated with specific targeting agents such as epidermal growth factor (EGF), since activated EGF receptor (EGFR) is endocytosed, and it is highly over-expressed in aggressive BC with poor prognosis. Although EGFR is expressed at lower levels in other tissues, passive localization of the nanocarrier to the lymphatics will minimize nonspecific interactions. The results presented in the work by Forrest *et al.* show that a locally injected nanoconjugate with cisplatin has similar cytotoxicity to free drug in cell cultures

but with better pharmacokinetic characteristics than IV cisplatin in rats: the nanoconjugate increased the plasma area under the curve (AUC) by 2.7-fold and the ipsilateral lymph node AUC by 3.8-fold compared to normal cisplatin, with a reduced peak plasma level (Cmax) which is beneficial for reducing systemic toxicity. On the other hand, pathology studies on rats receiving the nanoconjugate treatment showed normal appearance of brain and lymph nodes, with less necrosis and inflammation in the kidneys and liver compared to IV cisplatin at all the tested dose range.

2.2. Dendrimers for Radiotherapy Applications

Another targeted dendrimer-based antitumor formulation is presented in the 2012 patent application from Babich *et al.* [44]. Figure **2** shows a schematic representation of the invention, where a dendrimer is conjugated with a metal radionuclide to provide a complex for imaging tissues or for the radio-therapeutic treatment of cancer tissue.

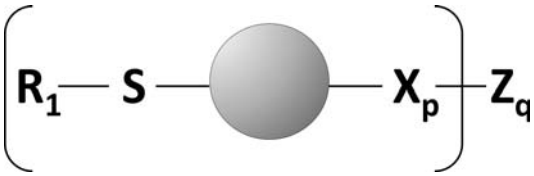

Fig. (2). Schematic representation of the invention: the sphere represents the dendrimer core of generation n (n = 1-10). Each generation is associated with a predetermined number (p) of surface groups (X: -COOR', - NR'R"), which may be conjugated to a prostate specific membrane antigen targeting moiety (Z), in a given proportion (q). S is sulfur and R_1 is a metal chelator. Adapted from Babich *et al.* [44].

Radioactive molecules that selectively bind to specific tumor cell surface proteins provide an attractive route for imaging and treating tumors under non-invasive conditions [45, 46]. The patent of Babich *et al.* deals with radiolabeled ligands of the Prostate Specific Membrane Antigen (PSMA) protein, often over-expressed on prostate cancer cells and the vasculature of other types of solid tumors. The invention is directed to the synthesis of chelator-dendrimer conjugates of PSMA targeting moeities which, after being complexed with an adequate metal radionuclide, may be administered by different routes as a non-invasive cancer treatment or imaging method.

2.3. Improving Anticancer Therapy: Specific Drug Targeting Plus Specific Drug Delivering

Given the dendrimeric characteristic structure, a great variety of different molecules may be attached to their terminal groups, which leads to a countless number of possible combinations between them. The most commonly developed dendrimer-conjugated systems include a therapeutic agent and a targeting moiety; however, other kind of chemical groups may also be part of these nanoconjugates.

Photodynamic cancer therapy involves the systemic administration of photosensitizers to solid tumor tissues followed by local illumination with light of a specific wavelength, leading to photochemical destruction of cancer cells via generation of singlet oxygen or superoxide from molecular oxygen. For example, the local surface plasmon effect has been used to develop metallic nanoparticles [47, 48], even though inorganic nanoparticles arise particular safety concerns. A patent by Albrecht *et al.* [49] introduces a dendrimer-based complex where therapeutic molecules, targeting moieties and photosensitizes are covalently attached to the end-groups. Upon exposure to radiation of a suitable wavelength, the photosensitizers are activated to break up the dendrimer structure and release the therapeutic molecules. Activation of the photosensitizers at a desired time produces radicals such as singlet oxygen, which release the active molecules by breaking the chains to which they are bonded. Because singlet oxygen has a very limited range of action, it can be activated so that only the dendrimer bonds are broken, avoiding affecting other molecules or cells.

The invention allows for predictable release of the drug: there is no risk of accidental premature release due to the strength of the covalent bonding, and instantaneous release can be achieved, even with relatively harmless low intensity light. Also, because the photosensitizers are activated by specific wavelengths, the risk of premature release by unintended irradiation by ambient light can be eliminated. Examples of useful photosensitizers include chlorophylls, porphyrins, pheophorbides, chlorins, porphycenes, texaphyrines and phthalocyanines, among others.

In addition, this dendrimer-based invention may have more than one type of therapeutic molecule attached to the dendrimer (i.e. folate antagonists, purine and pyrimidine antagonists, platinum compounds, nitrosoureas, anticancer antibiotics, antimitotics). To ensure site-specific targeting, a suitable addressing molecule can be coupled to the complex such as, for example, herceptin, a site-specific monoclonal antibody that targets the HER-2 protein, usually over-expressed in BC [50], or folic acid, since the folate receptor is over-expressed in more than 90% of ovarian carcinomas [51].

The 2012 patent of Baker Jr. *et al.* [52] represents another clear example of the versatility and diversity that dendrimer-based nanosystems may offer. The authors propose the synthesis of dendrimer-linker conjugates that also comprise one or more therapeutic, targeting or imaging components, as well as a trigger agent. The trigger agent may be conjugated to the dendrimer and to the linker (Fig. (**3A**)) or only to the linker, in which case the linker is further conjugated to the dendrimer and to the therapeutic compound (Fig. (**3B**)). In both cases, a labile bond links the trigger and the linker.

The dendrimer conjugates shown in Fig. (**3**) are not limited to any particular dendrimer. In the presented examples, the authors use PAMAM dendrimers (G3, G5 or G7) linked through a covalent bond to targeting agents (*e.g.* folic acid) and/or imaging agents (e.g. fluorescein isothiocyanate, FITC). Those dendrimer-based systems were further conjugated to: (A) a trigger molecule, conjugated to a linker, conjugated to a therapeutic agent; or (B) a linker, simultaneously conjugated to a trigger and a therapeutic molecule.

Regarding the trigger agent, it may be sensitive to (and therefore cleaved by) a tumor associated enzyme (e.g. glucuronidase, β-lactamase or proteases as cathepsin, plasmin) or it may be sensitive to hypoxia (e.g. indolequinone), a feature of several disease states including cancer. The authors state

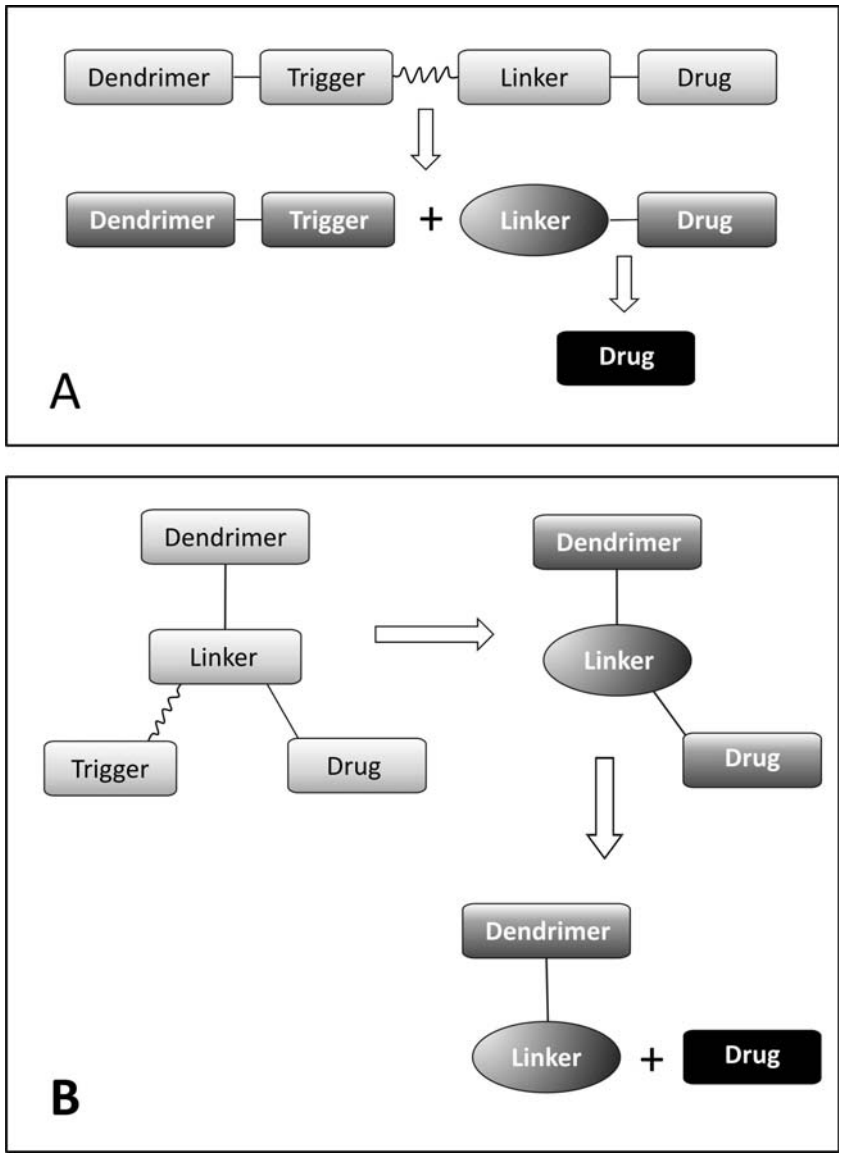

Fig. (3). Schemes of the two arrays descripted in Baker *et al.* patent. (**A**): Dendrimer-trigger-linker-drug array; (**B**): Dendrimer-[(trigger)-linker-(drug)] array. Adapted from Baker Jr. *et al* [52].

that the anticancer drugs may be doxorubicin (Dox), paclitaxel (PTX), docetaxel (DTX), 5-fluorouracil and/or 9-aminocamptothecin, among others. The linker agent is the most diverse, it could be an elimination linker (1,4 or 1,6 elimination), a cyclization based linker, a branched self-elimination linker or a heteroaromatic nitrogen containing one.

Is beyond the scope of this review to describe in detail all chemical possibilities that the authors describe in their patent, but we want to emphasize the multiplicity of options offered by dendrimers as targeted and/or triggered drug delivery systems. Indeed, the group of Baker Jr. *et al.* has a vast experience in the field and own several patents related to dendrimer nanoconjugated systems [52-55].

3. POLYMERIC MICELLES

Block copolymer micelles (BCM) are self-assembled nano-sized aggregates of amphiphilic copolymers consisting of hydrophilic and hydrophobic monomer units with the length of the hydrophilic block exceeding to some extent that of the hydrophobic one. If the length of the hydrophilic block is too high, copolymers exist in water as unimers (individual molecules), while molecules with very long hydrophobic block prefer to form structures with nonmicellar morphology, such as rods and lamellae [56].

They show high stability both *in vitro* and *in vivo* and good biocompatibility, they can solubilize a broad variety of poorly soluble drugs in their inner core and, due to their hydrophilic shell and generally small size, they exhibit prolonged *in vivo* circulation times and can accumulate in tumor tissues by the EPR effect, as well as evade scavenging by the mononuclear phagocyte system (MPS) [57, 58]. The size of the micelles can be controlled within the diameter range of 20-100 nm, to ensure that they do not pass through normal vessel walls and thus reducing the incidence of the adverse effects of the drugs [59]. The hydrophobic micelle core, which acts as a drug reservoir, is surrounded by a hydrophilic corona that provides a protective interface between the

core and the aqueous external environment. Alterations in the composition of the constituent copolymers can influence important performance-related parameters including micelle size, core-drug compatibility, drug loading capacity, drug release kinetics, and stability, thus permitting the manipulation of the pharmacokinetic profile and tissue distribution of the encapsulated drug [60, 61].

Incorporation of drugs within biocompatible and/or biodegradable BCM has been shown to reduce systemic toxicity while increasing drug solubility and site-specific tumor accumulation [62, 63]. Moreover, specific polymeric micelles having stimuli-responsive amphiphilic block copolymers, targeting ligand molecules, or monoclonal antibody molecules are also manufactured [57].

As a result of the growing interest in this promising drug delivery platform over the past two decades, BCM-based drug formulations are gaining increasing attention and are continuously in pre-clinical development and clinical evaluation. In 2008, Matsumura reviewed the status of several micellar drug delivery vehicles in clinical or pre-clinical stages [59], and a 2009 survey of the literature revealed more than 5000 publications on BCM for drug delivery [64]. In this context, the patents field is not an exception: an increasing number of inventions related to BCM for anticancer drug delivery are found in the last years.

3.1. Nanomicelles as Safer Pharmaceutical Excipients for Combination Therapy

Single drug formulations provide only limited success in anticancer therapy. Novel combinations of molecularly targeted agents with chemotherapy have gained increasing attention in research that aims at overcoming drug resistance and attaining highly effective cancer regimens [65]. One of the most important requirements of combination therapy is a simple and efficacious drug delivery system, since most chemotherapeutics currently in use are poorly water soluble. Combining two or three drugs in a formulation presents additional challenges in clinical practice because of compatibility and stability issues.

On the other hand, safer and more effective treatments with a drug combination rely on the development of biocompatible delivery systems capable of solubilizing the drugs without using harsh surfactants or excipients.

Taxanes (PTX and DTX) are microtubule stabilizing agents recognized as effective chemotherapeutics for a wide variety of solid tumors [66, 67]. Their clinical application is however limited due to poor aqueous solubility and oral bioavailability. To date, only two commercial PTX formulations have been developed. The first formulation developed uses 1:1 mixture of Cremophor EL® and ethanol to increase the solubility of intravenously administered PTX (Taxol®, traditional PTX formulation) [68]. However, Cremophor may have serious adverse side effects including severe hypersensitivity reactions, neurotoxicity, nephrotoxicity, and hypotensive vasodilatation [69-73]. The second formulation, ABRAXANE®, is an injectable suspension of albumin-bound PTX-nanoparticles [74-76]. Unfortunately, neuropathy toxicity has been shown to be remarkably increased when compared to the traditional PTX formulation [77, 78]. Similar issues have been observed with the available DTX preparation, Taxotere®, which is a concentrated injectable nonaqueous solution containing DTX in a vehicle composed of polysorbate 80 diluted with dehydrated ethyl alcohol in water for injection. Several toxic side effects have resulted from the administration of Taxotere® formulations [79]. All patients treated with Taxotere® are required to be premedicated with oral corticosteroids, to reduce the incidence and severity of adverse reactions [80]. Different strategies have been pursued to produce safer and better-tolerated taxane formulations than the current ones. Alternative formulations of PTX and DTX that avoid the use of Cremophor EL (used for PTX administration) and polysorbate 80 have been proposed [79].

A very interesting drug delivery system based on nanomicelles for the delivery of taxcane is disclosed in the 2011 patent application by Kang *et al*. [81], with the innovative characteristic of combining an anticancer drug with a P-glycoprotein (Pgp) inhibitor, which makes it a viable option to treat some resistant types of cancer cells. The polymeric micelle composition contains an amphiphilic double block copolymer and a taxane and the Pgp inhibitor cyclosporine A (CsA) as its active ingredients. It accumulates in cancer tissue at high concentrations. The proposed system has two main additional advantages: first, it exhibits superior anticancer effects for the cancer cells that have exhibited resistance due to over-expression of Pgp; second, it does not cause hypersensitive reactions because it does not include a solubilizer. What is more, our belief is that local inhibition of Pgp may be useful to avoid the safety issues linked to off-target Pgp inhibition, which seems to be responsible for some of the serious side-effects that have resulted in the stoppage of clinical trials of first, second and even third generation Pgp-modulators [82-84].

The authors proposed two versions of the nano-micelle based system: a first one, wherein the active ingredients are encapsulated together in the same complex micelle formed from an amphiphilic diblock copolymer, and a second one wherein the taxane and CsA are encapsulated in different micelles that are mixed before their administration. In the patent application, the authors include examples with both PTX and DTX and mPEG-PLA as the micelle copolymer. With this preparation method, the particle size of the complex micelles is between 40 and 50 nm, and between 17 and 30 nm in the case of the single micelles in the mixture.

The patent application also includes the results of several studies performed on the micelle compositions, such as their retention in the bloodstream and delivery of the drug, inhibitory effect on Pgp and anticancer activity. This last study was performed *in vivo* in 4 groups of athymic nude mice injected with human resistant colon cancer cells (DLD-1) that received the CsA-containing polymer micelle composition, the PTX-containing polymer micelle composition and the mixed composition respectively, through the tail vein. The fourth group was a control. The group treated with the CsA-containing composition had no anticancer activity, moreover, the tumor volume increased when compared to the control group. In contrast, the groups treated with the PTX-containing composition or the mixed polymer micelle composition showed improved inhibitory activity against cancer

growth compared with the control group; the group treated with the mixed composition showed the best results.

Another invention that offers the possibility of combined cancer therapy without the need of using potentially toxic pharmaceutical excipients is described in two 2012 patents by Glen Kwon [85]. In both documents, the author aims at developing a pharmaceutical system to combine chemotherapy and signal transduction inhibition.

Heat Shock Protein 90 (Hsp90) is an important target for cancer therapy due to its key role in regulating proteins that are involved in tumor cell proliferation. A geldanamycin derivative, the 17-allylamino-17-demethoxygeldanamycin (17-AAG) is a first-in-class inhibitor of Hsp90, inhibiting its function as a chaperone protein for the proper folding of oncogenic signal transduction proteins, such as Akt, ErbB2, Raf-1, and mutant EGFR [86]. The major obstacle for delivery of 17-AAG is its limited aqueous solubility, which has resulted in the use of complicated formulations with excipients such as Cremophor EL®, DMSO, and/or ethanol. As stated before, these substances are known for inducing hypersensitivity reactions and anaphylaxis, and require patient pre-treatment with antihistamines and steroids [87].

On the other hand, the PI3K/Akt/mTOR pathway is the most deregulated pathway leading to cancer. However, signal transduction inhibitors that target mTOR (e.g. rapamycin) have not yet been effective to treat cancer, and the PI3-Akt-TOR pathway has proven more complex than it was earlier believed [88]. In murine tumor models and in early clinical trials, PTX has been shown to act synergistically with rapamycin, and with 17-AAG [89]. Additionally, rapamycin and 17-AAG can act in a synergistic manner on breast cancer cells, presumably due to inhibition of mTOR by rapamycin and inhibition of the oncogenic kinase Akt by 17-AAG [90]. This dual drug action is of interest because clinical experience with rapamycin and its analogues suggests that Akt activation by a feedback mechanism appears to be a major cause of failure in the treatment with mTOR inhibitors [70, 91]. Figure **4** schematically shows the combined effect of Rapamycin and inhibition of Hsp90 on the PI3K/Akt/mTOR pathway.

The author proposes a 3-in-1 polymeric micelle nanocontainer for PTX, rapamycin, and 17-AAG, prepared from the biocompatible poly(ethyleneglycol)-block-poly(lactic acid) (PEG-b-PLA) copolymer, in order to increase the water solubility of the drugs in one nano-sized aqueous vehicle. In addition of being a non-toxic carrier, the micelles do not possess a foul odor, which is a problem with many formulations currently in clinical trials. Micelle encapsulation may also reduce the occurrence of side effects (e.g. hepatotoxic-

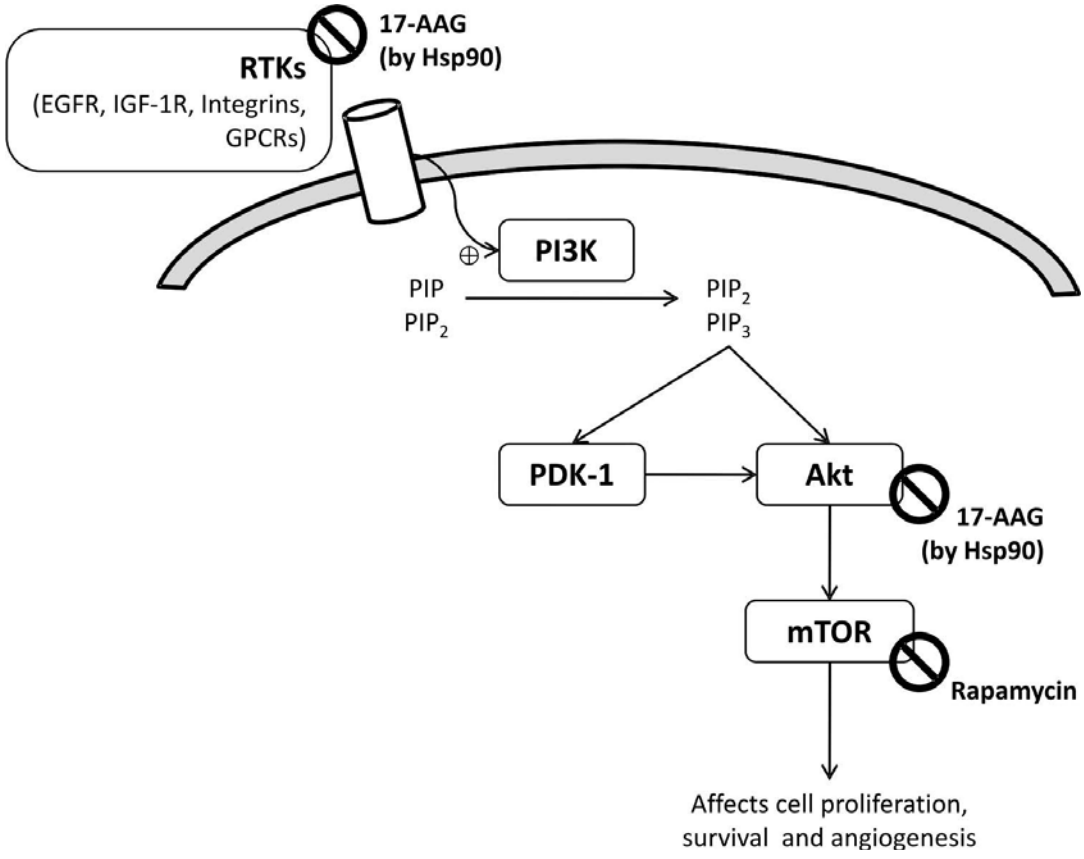

Fig. (4). Scheme of the combined effect of Rapamycin and inhibition of Hsp90 on the PI3K/Akt/mTOR pathway. Receptor tyrosine kinases (RTKs) such as IGF-IR and EGFR, integrins, and G-protein coupled receptors (GPCRs) can stimulate PI3K. PI3K phosphorylates phosphoinositides (PI) on the 3D position, and these 3P-phosphoinositides activates both Akt and PDK-1. Akt propagates its signal to affect transcription, apoptosis, and cell cycle progression. Akt activates mTOR, which affects cell proliferation, survival and angiogenesis. It is inhibited by rapamycin. Hsp90 functions as a chaperone protein for the proper folding of oncogenic signal transduction proteins, such as Akt and RTKs, which are therefore indirectly inhibited by the Hsp90 inhibitor 17-AAG.

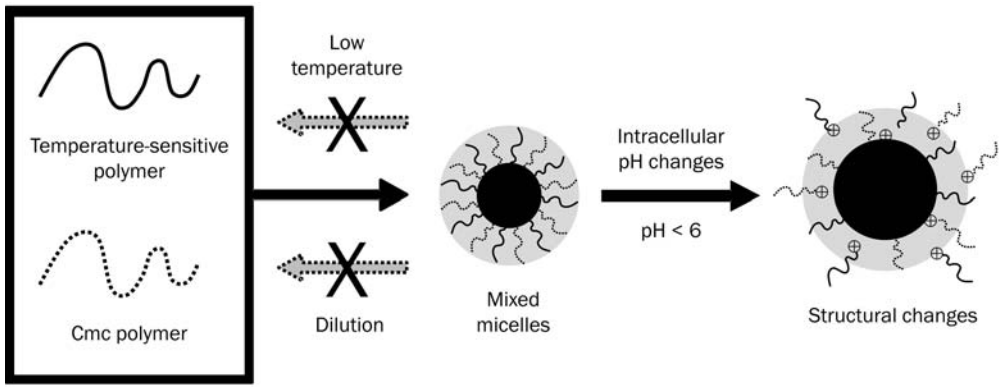

Fig. (5). Schematic representation of the concept for designing the mPEG-b-PLA and mPEG-b-P(NnPAAm-co-VIm) diblock copolymers micelles, and the speculated structural changes that they undergo during intracellular drug delivery. Adapted from Hsiue *et al.* [99] and Lo *et al.* [100].

ity, neutropenia, neuropathy) by maintaining the agents within the micelles until they are delivered to a target area of the body.

As in the previously reviewed patent of Kang *et al.*, the drugs can be incorporated together into individual micelles (multiple drug micelles, MDM), or individually into PEG-PLA micelles (single drug micelles, SDM). SDM of different drugs can then be combined to provide a single drug micelle drug combination (SDMDC) composition in an aqueous vehicle (e.g. saline or aqueous carbohydrate solution), to provide a therapeutic drug delivery formulation for IV or IP administration.

Another advantage of the invention is that SDM can be prepared and combined prior to administration, or they can be sequentially administered to the patient. Such sequential administration allows for synergistic anticancer activity, such as the administration of PTX before 17-AAG, or for tumor priming, whereby the administration of a first dose can kill tumor cells, reduce tumor cell density, and/or allow for greater uptake of a second administered dose of SDM (or alternatively two drug MDM or SDMDC).

Preparation of the formulations can be carried out on a large scale. The formulation provides ease of sterilization due to the small size of the micelles, ease of drug administration as an aqueous vehicle, low toxicity due to the proven safety of the copolymer, avoidance of noxious vehicles that are required in the clinic for the individual drugs, and synergistic anti-tumor efficacy.

3.2. Stimulus-Responsive Polymeric Micelles for Anticancer Drug Delivery

Micelles for anticancer drug delivery could be roughly divided into two categories based on the drug loading method: polymer-drug conjugates and micelles encapsulating the drug by physical hydrophobic interactions. The micelles of the latter category usually have stability drawbacks, since they cannot maintain their integral structure after IV injection due to dramatic dilution and interaction with surfactant proteins within the blood [92, 93], thus leading to premature disassembly and drug loss. Several strategies have been proposed to overcome such stability problem. For example, micellar structure has been strengthened by crosslinking the core and/or shell regions [94-96] and by mixing a crystalline copolymer and a copolymer with lower critical micellar concentration (CMC) to prevent dissociation from micelles [97, 98].

A 2012 patent by Hsiue *et al.* [99] represents another step in that direction: the authors introduce a new class of polymeric micelles that includes a copolymer of two copolymers: a first one sensitive to temperature (TS copolymer), in order to achieve a lower critical solution temperature (LCST); and a second one, not sensitive to temperature, able to form micelles above its CMC, that will be termed "CMC copolymer". By using that polymer combination, the authors aim at producing micelles with complementary effects in adjusting external temperature shift (storage vs. body temperature) and concentration change (dilution after IV injection) [100].

Both CMC and TS diblock copolymers include hydrophilic (mPEG in both cases) and hydrophobic polymeric segments. While the hydrophilic segment is always mPEG, the hydrophobic one is PLA in the case of the CMC polymer, and a combination of N-n-propylacrylamide (NnPAAm) as temperature-sensitive monomer with vinylimidazole (VIm) as pH-/ionic strength sensitive monomer, in the case of the TS copolymer. Figure **5** shows a schematic representation of the mPEG-b-PLA and mPEG-b-P(NnPAAm-co-VIm) diblock copolymers micelles.

The effective dissociation constant (pKa) of VIm is reported to be 6.0 [101]. It is known that during endocytosis a significant drop in the pH value takes place from the physiological value (7.4-7.2) to pH 6.5-5.0 in the endosomes, and to around pH 4.5 in primary and secondary lysosomes [102, 103]. Through intracellular pH changes, VIms are protonized so to increase the LCST of mPEG-b-P(NnPAAm-co-VIm): the micelles have a LCST lower than 37°C at pH 7-8, and greater than 37ºC at pH 6 or less. Consequently, electrostatic repulsive forces arise to dissociate the mixed micelle structure and release the incorporated drug.

The authors presented results of the drug release and cytotoxicity assays on Dox-mixed micelles, which show that this system provides promising applications in intracellular drug delivery. In conclusion, the authors have combined the physicochemical properties of a TS copolymer and a CMC copolymer to greatly improve micellar stability and extend their applications in controlled drug delivery.

3.3. Nanomicelles in Theranostics

In a patent application of 2009, Doris *et al.* disclosed polymerized micelles resulting from the polymerization of amphiphilic molecules of type PDA-NTA (PDA: polydiacetylenic and NTA: nitrilotriacetic), which can be loaded with hydrophobic active substances and used as nanovectors [104]. In a 2012 patent application of the same group [105], three types of micelles were synthesized and studied: the PDA-NTA and two PDA-based micelles with PEG coating, referred as PDA-PEG350 and PDA-PEG2000 micelles, according to the PEG length. PDA-NTA micelles result from the self-assembly of a single amphiphilic monomer [106], while PDA-PEG350 and PDA-PEG2000 micelles are composed of a mixture of two amphiphilic monomers (their chemical structures and detailed synthesis procedure can be found in the patent application).

Important differences were observed between the different micelle types. PEG coatings are known to enhance blood residence time [107-113] and their positive effect is illustrated by the results presented by the authors. Short and long term kinetic studies with the micelles conjugated with NIR emitting FluoProbes® 730 (FP730, diagnostic agent) showed a rapidly fluorescence decrease with NTA-coated micelles, while longer times were obtained for the PEG-coated ones, and among them, PEG2000-coated micelles retain their fluorescence more than twice the time of PDA-PEG350.

In relation to *in vivo* tumor targeting of the FP730 conjugated micelles, the behavior of PDA-NTA and PDA-PEG350 micelles was quite comparable, with little tumor uptake and contrast after 24 hours. On the contrary, PDA-PEG2000 micelles showed strong and persistent tumor uptake after 24 hours with maximum contrast after 48 hours, which remained constant for more than a week [114]. Therefore, the passive drug delivery properties of PDA-PEG2000 micelles were further explored [115]. The micelles were easily and efficiently loaded with PTX with a technique suitable for virtually any therapeutic molecule, since it requires neither extended contact with water nor prolonged heating (see further details in the original document), and showed good *in vitro* cytotoxicity on MDA-MB-231 cells (breast tumor cancer cells isolated from pleural effusions [116]). In addition, PDA-PEG2000 micelles behaved ideally in multiple injection conditions. During one month, they were administered to mice bearing MDA-MB-231 tumors by intraperitoneal (IP) injection on a semi weekly basis. Tumor uptake by EPR effect was observed as soon as 24 hours after the first injection and the accumulation was maintained and strengthened by repeated doses to reach a maximum after the sixth injection, which was constant throughout the remainder of the experiment. None of the involved animals died during the experiment and body weights of the treated mice remained steady, indicating that PTX-loaded PDA-PEG2000 micelles were well tolerated.

Therefore, this invention provides apparently non toxic micelles that advantageously exhibit satisfactory blood residence time, tumor uptake and imaging contrast. The combination of imaging and drug-loading properties in a single object points out the PDA-PEG2000 micelles as the most promising nanoparticle presented by the authors and appear as a potential tool for theragnosis, able to achieve simultaneous diagnosis and treatment, thus rendering the overall medical process less invasive and easier to carry out. As a further advantage, the authors stated that contrary to a large number of nanoparticles, the synthesis of the proposed micelles is controllable, reproducible, and economical [105].

4. LIPID-BASED NANOSYSTEMS

Absence of toxicity either *in vivo* or in the environment (as a byproduct) is one of the most important features that nanocarriers intended for drug delivery applications should possess. In this sense, lipid-based nanoparticles are probably the least toxic for clinical applications [117]. The hydrophobic constituents of lipid-based systems provide a suitable environment for entrapment of hydrophobic drugs, which represent about 40% of newly developed drugs [118]. Lipid-based drug delivery systems have been particularly recognized as innovative formulation approaches capable of enhancing lipophilic drug absorption and thus clinical efficacy [58].

4.1. Liposomes

Liposomes (LP) are defined as artificial vesicles composed of one or more closed, concentric phospholipid (or related lipids) bilayer membranes surrounding an aqueous core [108, 109, 119-121]. Classical or conventional LP are formed spontaneously by dispersion of amphiphilic lipids (and usually cholesterol) in aqueous media, which, upon hydration, self-assemble to form bilayers surrounding an aqueous interior [122-124]. Their size ranges from 20 to 1000 nm and even more [108, 109]. Both lipophilic and hydrophilic drugs can be entrapped into LP because of their biphasic character [109, 110, 121, 124, 125].

The phospholipids employed to produce LP can be either synthetic or natural [108]. As they are generally formed from naturally occurring phospholipids, cholesterol, sphingolipids, long chain fatty acids and others, they are readily biodegradable [123, 126]. The stability of the membrane bilayer as well as the retention of incorporated drugs depend on lipid composition and cholesterol content of the liposomal membranes [110].

LP are usually characterized in terms of their size, the number of concentric bilayers (lamellae), and the composition and physical properties of the lipids used [121, 127, 128]. Since there are a wide variety of phospholipids, it is possible to change the LP size, charge, and surface properties by adding new ingredients to the lipid mixture [108]. Thus they can be designed to provide control over properties such as elimination half lives, permeability, biodistribution and targeting specificity [122, 129].

LP have been used to encapsulate and deliver chemotherapeutics for more than three decades: to the moment, the most clinically successful LP have been small (up to 100 nm) unilamellar vesicles [130]. Doxorubicin hydrochloride (Dox-HCl) liposomal injection (Caelyx® in Europe, Doxil® in the USA), which received marketing approval in 1995, was the first nanoscale delivery system to receive clinical approval in cancer therapy for acquired immune deficiency syndrome (AIDS)-related Kaposi's sarcoma [131, 132]. Some other LP are already on the market, e.g. DaunoXome®

(Daunorubicin citrate in LP of Diatos, France) for advanced AIDS-related Kaposi's sarcoma, and AmBisome® (Amphotericin B in LP of Gilead Sciences, USA) for fungal infections [119]. Currently, virtually all traditional anticancer drugs have been encapsulated in LP using different technologies and many of them have entered clinical trials, indicating that this is a rapidly developing field [122].

4.2. LP Containing Curcumin for Pancreatic Cancer Treatment

A recent patent by Kurzrock *et al*. [133] describes the development of a LP formulation encapsulating curcumin or curcumin analogues. Curcumin, a diphenolic compound extracted from the rhizome of turmeric (*Curcuma longa*), is a prominent candidate for treating many diseases, among them cancer [134, 135]. Curcumin and some curcumin derivatives have shown antioxidant, anti-inflammatory, and *in vitro* antitumor activity [136]. Among signaling pathways affected by curcumin are key survival pathways regulated by the transcription factor nuclear factor-kB (NF-kB) and Akt, as well as cytoprotective pathways dependent on Nrf2 [137]. In resting cells, NF-kB is sequestered in the cytoplasm by the inhibitory proteins of the I-kB family. Following stimulation of cells by inflammatory cytokines, and bacterial (e.g. lipopolysaccharides) and viral products, the inhibitor of I-kB) is phosphorylated by kinases, leading to its degradation through the ubiquitin-proteasome pathway. Thus, in the absence of I-kB, NF-kB dimmers are released, translocated to the nucleus, and subsequently bound to their cognate elements on target genes. Abnormal activation of NF-kB has been shown to be involved in the survival, development, and progression of tumors. It is therefore an important pharmacological target due to its involvement in cancer, inflammatory and autoimmune diseases [138]. Curcumin has been shown to suppress the expression of epidermal growth receptor and estrogen receptors, which are cancer-associated growth factors [139], and it sensitizes tumor cells to first-line chemotherapies and radiation [140-142]. What is more, curcumin and its analogs seem to downregulate ATP Binding Cassette (ABC) transporters such as Pgp, Breast Cancer Resistance Protein (BCRP) and Multidrug Resistance Protein 1 (MRP-1) [143-145], an interesting property having in mind that overexpression of such transporters constitutes one of the main mechanisms of drug resistance in cancer [146-148].

Curcumin bioavailability after oral administration is poor and inhibitory concentrations cannot be achieved by the oral route [149-151]. IV administration of free curcumin has also been found ineffective to built up significant concentrations of curcumin in any tissue, due to rapid systemic biotransformation and excretion [152]. Encapsulating curcumin within nanocarriers has been proposed as an alternative to solve the previously mentioned issues. Various types of nanoparticles, especially LP, have been tested for the delivery of an active form of curcumin to tumors [134, 153].

The authors of the patent under discussion propose a series of formulations of conventional and PEGylated-LP. They also test their effects on the proliferation and survival of pancreatic, breast and melanoma cancer cells through the MTT assay. Free curcumin and non-labeled LP were used as controls. The results showed that LP curcumin has equivalent or greater anti-proliferative and apoptotic effects than free curcumin. The authors also studied the ability of the pancreatic cell line to recover after exposure to LP curcumin and there was a concentration-dependent loss of ability to recover, indicating that the effects of LP curcumin are irreversible. Since the surface charge and lipid composition of the LP are known to influence the membrane fusion and ABC transporters modulation [130, 154, 155] the authors also tested different combinations of neutral, anionic and cationic lipids.

4.3. Multifunctional LP

Assessing the *in vivo* distribution of LP is a key point to understand and predict their efficacy and side-effects [156]. Radiolabeled LP have potential applications in diagnostic imaging and radionuclide therapy [157-159]. Several radionuclides can be used to label LP for monitoring their *in vivo* behavior in a non-invasively manner [160, 161]. ^{188}Rhenium (^{188}Re) and ^{186}Re are two diagnostic and therapeutic radionuclides which have excellent physical properties [157]. ^{188}Re is a 15% γ-emitter with high-energy β emission [162, 163], hence being appropriate for the treatment of larger tumors, since its long maximum path-lengths allow the irradiation of several layers of tumor cells [164] and it has short physical half-life [165-167].

The combination of chemotherapeutic drugs with radiation has been shown to improve survival and regional control of various types of cancer compared with radiotherapy alone [168, 169]. LP can provide several advantages for bimodality radiochemotherapy applications, among them passive and active targeting [158]. For example, Ogihara-Umeda *et al.* have reported a higher accumulation of small-sized (80 nm) radionuclide encapsulating-LP in tumors compared with that of the free form [170].

To achieve labeling LP, radioisotopes can be attached to the surface, intercalated into the double membrane, or encapsulated within the inner space of the LP [157, 171].

A 2011 patent from Chiu *et al.* [172] provides a one pot process, in which PEGylated-LP react with a radionuclide labeled solution, a chemotherapeutic drug (Dox) and a targeting ligand (DSPE-PEG-Bombesin), at appropriate temperature (4°C - 100°C), to form tumor targeting radiochemotherapeutics ^{188}Re-Dox-Liposome-Bombesin. The targeting moiety Bombesin (BBN) is a 14-aminoacid peptide overexpressed in a diversity of tumors including breast and prostate cancer [173-175].

The cytotoxic activity of the loaded LP was assessed on PC-3 human prostate cancer cell line; the results demonstrated that ^{188}Re-Dox-Liposome-BBN has a superior cytotoxic activity compared with ^{188}Re-N,N-bis(2-mercaptoethyl)- N′, N′-diethylenediamine (BMEDA) complex, ^{188}Re-Liposome-BBN, Dox-Liposome-BBN, or normal saline (as control). Finally, imaging was acquired at different times after IV injection of ^{188}Re-Liposome-BBN. The images revealed a high uptake in tumors at 1 and 24 h after administration. These results show that the multifunctional ^{188}Re-labeled nanoliposomes are useful for imaging, delivery and targeting in cancer diagnosis and therapy.

One of the shortcomings of radiotherapy is that after cessation of treatment, recurrence of the tumor can occur. Recurrence of the tumor has been partly attributed to the presence of radioresistant hypoxic cells [176], and the enhancement of radiation doses to damage the hypoxic tumor tissue causes more destruction of healthy tissue. Radiosensitizers are chemical agents that have the capacity to increase the lethal effects of radiation (preferentially sensitizing hypoxic tumor cells) when are administered in conjunction with radiation. There are a variety of radiosensitizers that act by more than one mechanism. In particular, inert metal radiosensitizers such as gold nanoparticles (GNP) synergistically increase the anti-cancer activity of anti-cancer agents and of radiotherapy due to the increased of the number of DNA double strand breaks (DSB) and/or single strand breaks (SSB) [177].

In a 2012 patent from Sanche *et al.* [178], a combination of an anti-cancer agent which binds to DNA (an alkylating agent, preferably a platinum compound such as cisplatin) and a metal radiosensitizer (GNP) were encapsulated in LP, allowing to potentiate the radiotherapy of cancer since both bind to DNA and synergistically increase the amount of DSB induced by the ionizing radiation.

In one embodiment, cisplatin was chemically linked to supercoiled pGEM-3Zf plasmid DNA to produce a cisplatin-DNA complex. GNP, which electrostatically binds to pure DNA, were added to this complex to obtain cisplatin-GNP-DNA. In order to analyze the synergistic effect of metal nanoparticles and cisplatin in the production of DSB and SSB on DNA, various complexes of DNA were prepared with different molar ratios. Dry films (1-mm-thick gold foil) of pure DNA and the complexes GNP-DNA, cisplatin-DNA and cisplatin-GNP-DNA were exposed to the 60 keV electron beam of a transmission electron microscope (TEM). After a given electron fluence, the samples were retrieved from the TEM and the DNA damage analyzed by electrophoresis. The dependence of the yields of SSB and DSB as well as the loss of supercoiled DNA were measured as functions of exposure. Table **1** shows the results obtained for different DNA film preparations. Both GNP and cisplatin binding to DNA increase the production of SSB and DSB, but the highest yields were obtained with both species bound to DNA.

In another embodiment of the invention, the authors developed LP encapsulating GNP and cisplatin (LipoGold®). LipoGold® were coated with PEG and the formulation comprised dipalmitoylphosphatidylcholine (DPPC), 3β-[N-(N′,N′-dimethylaminoethane)-carbamoyl]-cholesterol (DC-Chol), Dioleoyl Phosphatidylethanolamine (DOPE) and DPPC-PEG2000. The diameter of LipoGold® was less than 400 nm (preferably between 100 nm and 150 nm).

LipoGold® was administered *in vivo* to a rat cancer model. F98 glioma tumors were implanted in Fischer rat brains. Ten days after F98 glioma cells implantation, LipoGold® was infused in the internal carotid artery. The efficacy of LipoGold® was compared to control animals and other platinum compounds (free oxaliplatin and cisplatin, Lipoplatin®-of cisplatin- and Lipoxal®-of oxaliplatin-) using the same surgical procedures. Twenty four hours after chemotherapeutic treatments the rats were irradiated. Lipoplatin®, Lipoxal® and LipoGold® did not produced any apparent toxicity on the animal treated following drug administration. Conversely, free platinum compounds produced high toxicity and animals died before sham animals. The drug uptake was measured in the tumor, adjacent healthy tissues and different organs such as the kidneys, liver and blood. Finally, the synergistic effect of the combination of LipoGold® and radiation was evaluated by combining different doses of LipoGold® with radiation.

4.4. Changing the Pharmacokinetics of Bisphosphonates

Bisphosphonates (BP) are the most potent inhibitors of bone resorption and represent the treatment of choice for different diseases, such as osteoporosis, Paget's disease and bone metastases. In particular, zoledronic acid (ZOL) is the most frequently BP used for the treatment of the complications derived from bone metastases [179-181]. Numerous studies have demonstrated that ZOL also induces apoptosis and inhibits growth of a variety of cancer cell types *in vitro* including prostate [182], breast [183], melanoma [184], osteosarcoma [185, 186], and myeloma tumor cells [187-189]. ZOL suppresses prenylation of small GTPases (e.g. Ras proteins) that regulate the proliferation, invasive properties and pro-angiogenic activity of human tumor cells [190-195]. Nevertheless, the drug presents an unfavorable pharmacokinetic profile, characterized by a short plasma half-life due to its rapid clearance from circulation and rapid and preferential accumulation in bone, which limit the use of ZOL as an antitumor agent in extraskeletal tissues [196-200] and make the direct anti-cancer activity difficult to demonstrate *in vivo*.

Table 1. The Yields (Y in 10^{-15} Electron^{-1} molecule^{-1}) for the Formation of SSB, DSB and Loss of Supercoiled DNA Induced by 60 keV Electrons in 2900nm Thick Films of DNA of Different Compositions Deposited on a Gold Foil. The Quoted Errors Represent the Maximum Deviations of Three Identical Measurements. Extracted from Sanche *et al.* [178].

Samples	Y of SSB	Y of DSB	Y of Loss of Supercoiled
Pure DNA	3.72 ± 0.3	0.77 ± 0.1	-5.46 ± 0.54
GNP:DNA = 1:1	8.65 ± 0.9	1.79 ± 0.2	-10.5 ± 1.1
Cisplatin:DNA = 2:1	9.49 ± 0.91	1.95 ± 0.2	-12.3 ± 1.2
Cisplatin:GNP:DNA = 2:1:1	11.1 ± 1.2	7.68 ± 1.0	-20.1 ± 2.0
GNP:DNA = 1:10	5.38 ± 0.56	1.11 ± 0.3	-6.8 ± 1.0
Cisplatin:GNP:DNA = 20:1:10	10.2 ± 1.1	3.93 ± 0.5	-14.9 ± 1.6

direct anti-cancer activity difficult to demonstrate *in vivo*. On the other hand, the use of high ZOL doses is certainly questionable owing to the risks of side effects, such as osteonecrosis of the jaws [179, 201].

In the light of these considerations, a new formulation capable of modifying ZOL pharmacokinetics and distribution, inducing a lower drug accumulation into the bone and a longer half-life in blood, might be useful to take advantage of its anti-apoptotic and anti-proliferative effects in peripheral tumors. Nanotechnology constitutes a powerful tool to improve the pharmacokinetic profiles of drugs [202, 203]. The major drug clearance mechanisms of the body act primarily on free circulating drug; thus, encapsulating drugs constitutes a valid strategy to hide the active ingredient from drug-metabolizing enzymes and to avoid renal extractions as well (note that the size of most nanoparticles precludes their efficient renal clearance) [204, 205].

Therefore, the level of exposure to a certain encapsulated drug may be tailored by controlling both the drug release and the nanovehicle degradation kinetics. What is more, targeted nanovehicles can prevent unwanted off-target interactions. Beside ZOL, other BP such as pamidronate and alendronate have already been encapsulated into conventional LP to use their ability to accumulate in the RES and for their macrophage-depleting properties [206, 207].

In a 2009 patent from Abbruzzese *et al.* [208], the authors showed that PEGylated-LP allow the use of ZOL for the treatment of several tumors. Compared with free ZOL, LP encapsulating ZOL (LipoZOL) induced a stronger *in vitro* inhibition of growth on different cell lines such as PC3, DU145 and LNCaP of prostate cancer; MCF7, MDA-MB468 and CG5 of breast cancer; Caki2 and 769P of kidney cancer; M14 and M14+ of melanoma; KB of head/neck cancer; H1355 of lung cancer; BXPC3 of pancreas cancer; and RPMI, KMS, DOX and OPM2 of myeloma. LipoZOL also showed improved inhibition of tumor growth and overall survival in murine models of human prostate cancer and multiple myeloma, in comparison with free ZOL. Moreover, a strong inhibition of vasculogenic events without evidence of necrosis in the tumor xenografts from prostate cancer was recorded after treatment with LipoZOL [202]. The formulation strategy presented some drawbacks such as limited physical stability, low encapsulation efficiency and drug loss following rehydratation after lyophilization.

In a more recent patent of the same inventors [209], the group developed nanocomplexes consisting of ZOL-containing self-assembly PEGylated nanoparticles based on ZOL complexes with calcium phosphate nanoparticles (CaPZ nanoparticles, an inorganic nanovector), and cationic LP consisting of a lipid mixture comprising phosphodiglycerids and sphingolipids. The general process for preparing the nanocomplex comprises the following steps: a) mixing a suspension containing the inorganic nanovectors with a solution containing ZOL, and b) mixing a suspension of LP with the suspension obtained from step a). PEGylation was achieved by two different strategies: CaPZ nanoparticles were mixed with PEGylated-LP (pre-PLCaPZ nanoparticles); or alternatively, CaPZ nanoparticles were first mixed with cationic LP and then PEGylated by post-insertion method (post-PLCaPZ nanoparticles). The amount of ZOL loaded in post-PLCaPZ nanoparticles was 5 times lower than that found in pre-PLCaPZ nanoparticles. Pre-PLCaPZ nanoparticles showed the best technological characteristics, with a narrow size distribution and a high ZOL loading.

The effect of increasing concentrations of ZOL encapsulated in pre- and post-PLCaPZ nanoparticles on growth inhibition of different human cancer cell lines was studied by MTT assay. Evaluation of apoptosis was performed by TUNEL technique. In all cases, when using ZOL-encapsulating nanoparticles, a potentiating effect in all tested cell lines was found, if compared with the free ZOL. The cytotoxicity of post-PLCaPZ nanoparticles was significantly higher than that observed for pre-PLCaPZ nanoparticles.

In vivo studies on PC-3 human prostate cancer xenograft model in mice were performed with ZOL free or complexed with pre-PLCaPZ nanoparticles. Antitumor efficacy was assessed through the percent of tumor weight inhibition and the tumor growth delay. The results show that ZOL complexed with pre-PLCaPZ nanoparticles produces the major efficacy on inhibition of tumor growth.

Images of the nanocomplexes were acquired by cold field electron gun scanning electron microscopy. For this purposes PC3M-luc2 (a luciferase expressing cell line which was stably transfected with the Iuc2 firefly luciferase gene) was used. Mice were injected intraperitoneally with D-luciferin, and imaged in the supine position 10-15 min after luciferin injection. After 4 months from tumor cell injection, no tumor was evident neither through palpability nor through luminescence analysis in those animals treated with PLCaPZ nanoparticles Fig. (**6**). In these mice, a progressive reduction of the luminescence associated to the tumor cells was observed with a complete regression of the luminescence at 56 days from the tumor cell injection.

In another embodiment of the invention, the authors proposed the preparation of nanocomplexes wherein the LP comprises a ligand for receptors overexpressed by cancer cells, in particular, human-transferrin.

The nanocomplexes showed an antitumor effect higher than that observed with LipoZOL previously developed. In this regard, the authors recently published a study [203] comparing the technological and anti-cancer features of either ZOL-containing self-assembly PEGylated nanoparticles or Lipo-ZOL. ZOL-containing nanoparticles showed superior technological characteristics in terms of mean diameter, size distribution, and ZOL encapsulation efficiency, compared to Lipo-ZOL. Moreover, the anticancer activity of nanoparticles in nude mice xenografted with prostate cancer PC3 cells was higher than that one induced by Lipo-ZOL. In addition, nanoparticles induced the complete remission of tumor xenografts and an increase of survival time higher than that one observed with Lipo-ZOL. Nanoparticles (but not Lipo-ZOL) caused a statistically significant reduction of the tumor-associated macrophages in tumor xenografts. The effects of the nanoparticles were also higher in terms of neoangiogenesis inhibition. These results suggest the development of ZOL-encapsulating nanoparticles may be promising in the treatment of human cancer.

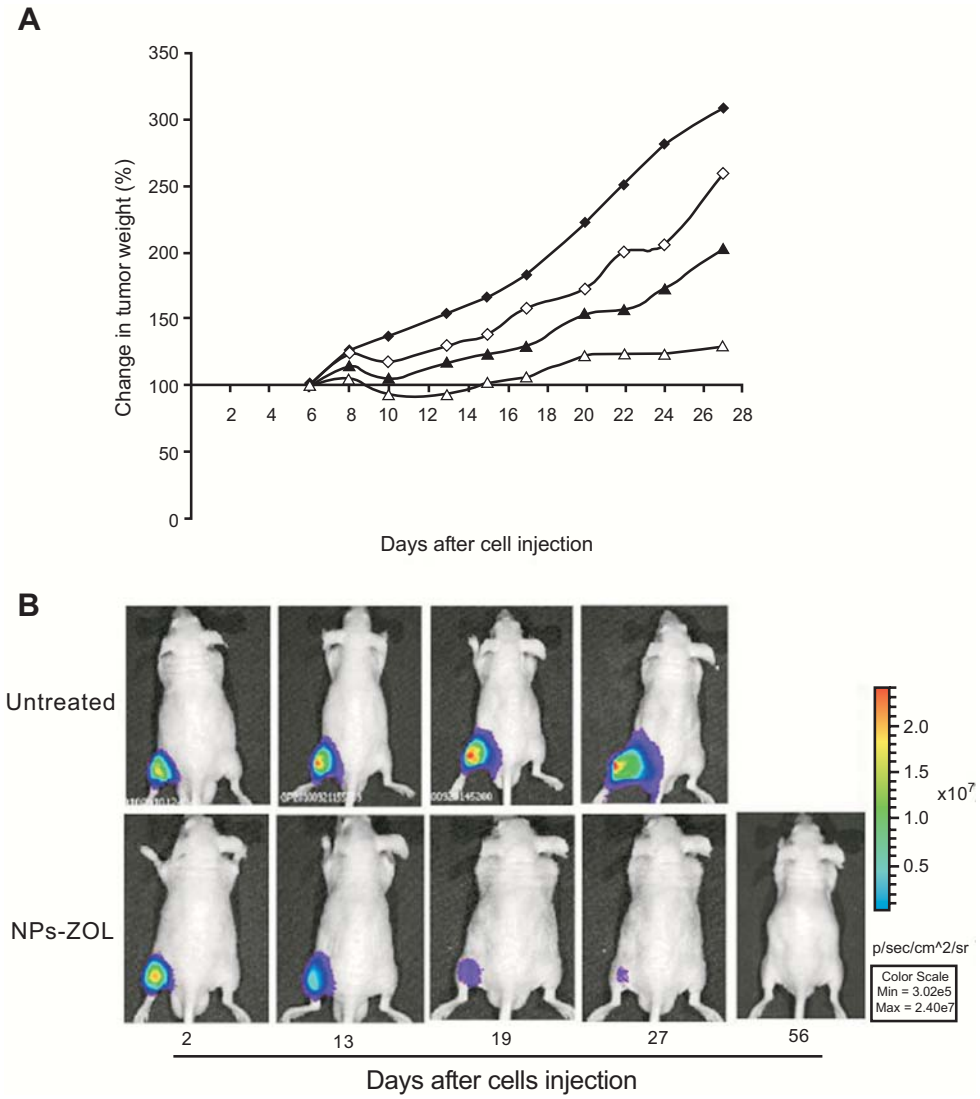

Fig. (6). A) Encapsulation in nanoparticles increases antitumoral efficacy of ZOL against human prostate PC3M-luc2 cell line xenografts. Mice were injected i.m. with PC3M-luc2 cell line cells and starting from day 6 treated as follows: (♦) untreated; (◊) free ZOL; (▲) Lipo-ZOL; (Δ) PLCaPZ nanoparticles. **B**) Example of luminescence associated to injected tumour cells in an untreated mouse (upper panel) and in a mouse treated with PLCaPZ nanoparticles (NPs-ZOL, lower panel) achieving a complete regression of the tumour after 56 days from the initial tumour cell injection. Extracted from Marra *et al.* [203].

4.5. Thermosensitive LP

The LP anticancer drug formulations with good *in vivo* stability and tumor accumulation characteristics not always show improvement in the therapeutic activity of the encapsulated drug [210-213]. This may be because the most stable LP formulations can retain the encapsulated drug so well that the drug is not released from them. Several types of targeted LP have been explored [110, 214, 215], however, most have failed to show any remarkable therapeutic efficacy because of several reasons such as antigenicity of the ligands, inability of the drug to reach the appropriate cellular compartment in the active (free) form and the presence of histological barriers between the LP and its cellular binding site. Some solutions that are being explored to deal with such issues include the use of small and highly stable, low-immunogenic peptides as targeting ligands [214].

Alternatively, stimuli-responsive LP that release their cargo in the appropriate place (stimuli-triggered or smart drug release) have been conceived. Various triggering mechanisms have been described in the literature, including those that rely on changes in local microenvironment such as decreased pH [216, 217] and the presence of specific enzymes [218], as well as the use of externally applied triggers such as light [219], ultrasound [220] and heat [221, 222]. Among the various triggers for liposomal content release, the use of heat has been actively pursued because of several advantages:

1) Increased tumor blood flow and microvascular permeability in the locally heated tumor, increase the extravasation and the accumulation of LP [223-225].

2) Thermal therapy has been used as an adjuvant therapy to surgery, radiation and chemotherapy, and recently, hy-

perthermia has been shown to be directly cytotoxic to tumor cells [226, 227].

3) Supra-additive cytotoxic effects may be achieved when hyperthermia is used in combination with several chemotherapeutic agents which can be delivered via LP encapsulation [228-230].

In the light of these therapeutic benefits, the combination of hyperthermia and liposomal delivery of chemotherapeutics to treat solid tumors has attracted much research activity. Temperature sensitive LP or thermosensitive LP (TLP) were developed by Yatvin *et al.* [231], Weinstein *et al.* [232] and others [233, 234] for improved distribution to targeted sites [235]. These TLP are formed by a mixture of synthetic phospholipids that have a gel to liquid phase transition temperature (Tc) few degrees above the physiological temperature, a range easily obtainable by clinically local hyperthermia (41-42°C) to allow triggered drug release. DPPC, which has a Tc of 41.5°C, has been the lipid of choice as the primary constituent of TLP. In early studies, other lipids such as dipalmitoylphosphatidylglycerol (DPPG) and distearoylphosphatidylcholine (DSPC) have also been added in various ratios to modify the drug release temperature to the desired range [231, 232, 236].

The *in vivo* stability has been improved by incorporating PEG-conjugated lipids (PEG-lipids) [237-239]. However, these formulations have problems with the rate and the extent of release of the encapsulated drug upon heating. In order to solve this issue, lysolipids have been included as a third component based on the premise that these lipids destabilizes regions in the membrane, facilitating catastrophic failure of the membrane's permeability barrier allowing rapid release of contents [240].

A recent patent of Mei *et al.* [241] describes the development of TLP for the delivery of anticancer agents, in particular DTX and carboplatin. The authors also claim that any active agent may be included, for example, therapeutic and/or imaging agents. TLP formulation comprises a phosphatidylcholine, such as DPPC; a phosphatidylglycerol, such as distearoylphosphatidylglycerol (DSPG); a lysolipid, such as monostearoylphosphatidylcholine (MSPC); and a PEG-lipid, such as DSPE-PEG2000.

The *in vivo* drug distribution of the TLP containing DTX was studied in mice. One leg was heated, treated, and heated again. The data showed that the TLP delivered more than twice DTX to the heated leg than to the non-heated leg. In free DTX injection and non-TLP containing DTX groups, the drug concentration was similar to that in the heated and non-heated tissue. The authors also evaluated the *in vivo* efficacy of DTX delivery of TLP in mice bearing. Lewis lung tumors. TLP resulted in greater tumor inhibition (98.27%) than free DTX (77.91%).

A recent patent from Li *et al.* [242] provide a simple and sensitive novel liposomal formulation for drug targeting and release under mild hyperthermia (40-42°C). The liposomal formulation focuses on a minority component of Brij (preferably 1-8 mol%) combined with DPPC lipid. Brij molecules are single chain surfactants, commercially available with different PEG chain lengths and/or acyl chain structures. Brij compounds have been studied in colloidal formulations [243, 244], typically are nontoxic and common in pharmaceutical formulations [245], with safety being demonstrated in clinical trials [246]. The different TLP formulations can comprise a diagnostic agent (gadolinium) or an anticancer agent (Dox, gemcitabine or cisplatin) entrapped in the interior space of the LP, and the diameter varies from about 30nm to about 250nm with a T_m ranging between 40-42°C. Physicochemical properties of some Dox-TLP prepared are listed in Table **2**. The sensitivity to mild hyperthermia conditions of different TLP formulations was examined thoroughly. A variety of *in vitro* and *in vivo* analyses were performed to evaluate the temperature-dependent drug release, intracellular uptake of drug released, the cytotoxic activity, pharmacokinetic and biodistribution profiles, toxicity on healthy tissues and antitumor efficacy. For *in vitro* studies, mouse mammary carcinoma cell line EMT-6, ovarian carcinoma cell line A2780 and adriamycin- resistant cell line A2780-ADR were used. For *in vivo* studies, tumor-free mice and tumors models of mice bearing subcutaneous (s.c.) implants of EMT-6 cells, PAN02 murine pancreatic cells and LL2 murine lung cancer cells into both lower legs were tested. One leg tumor was heated and the other leg tumor was used as the unheated control.

One of the most advanced thermal sensitive liposomal formulation is composed of DPPC/MSPC/DSPE-PEG (90/10/4, molar ratio) and described as lysolipid-temperature-sensitive-LP (LTSL) [222, 227, 247-252], and is currently in Phase III clinical trials for liver cancer and Phase II for recurrent breast cancer on chest wall. When heated to 42°C, the LTSL

Table 2. Physicochemical Properties of Some Dox-TLP Prepared. Extracted from Li *et al.* [242].

Liposomal Formulation	Lipid Composition (Molar Ratio)	Diameter (nm)	T_m (°C)	Encapsulation Efficiency of Dox (%)
LTSL	DPPC/MSPC/DSPE-PEG2000=86/10/4	101.3 ± 0.5	41.55	100.1 ± 2.5
DPPC-LP	DPPC	96.5 ± 1.5	40.74	60.5 ± 5.2
Brij76-LP	DPPC/Brij76=96/4	120.5 ± 3.5	40.79	68.6 ± 3.2
Brij78-LP (HaT formulation)	DPPC/Brij78=96/4	103.3 ± 4.3	41.93	97.5 ± 3.5
Brij98-LP	DPPC/Brij98=96/4	111.3 ± 7.2	40.24	1.15 ± 0.41
Brij700-LP	DPPC/Brij700=96/4	115.3 ± 7.1	40.76	71.5 ± 5.1

released 100% Dox in 2-3 min [249, 251-254], leading to a ~15-fold increase in drug delivery to the heated tumor [227] and eradication of the s.c. inoculated human xenograft tumor in a mouse model [222, 227]. Direct comparisons of drug release in response to hyperthermia conditions were drawn between the proposed and LTSL formulations, to determine if the incorporation of the Brij-surfactant in the membrane would influence release profiles. In concordance with the data reported, the LTSL formulation released 100% of Dox within 3 min at 42°C, but at 40°C only reached full release after 10min. In contrast, the novel and optimal liposomal formulation, called Hyperthermia-activated cytoToxic (HaT) formulation, released 100% of Dox within 3 min at 40°C and no release could be detected at 30 and 37°C. Besides, HaT formulation showed further increased membrane permeability upon mild hyperthermia compared to the LTSL formulation, the Dox released from the HaT was taken up into the cells efficiently, and the cytotoxicity was similar to that free Dox, demonstrating the best thermal sensitivity among all formulations screened. Similarly, 100% of gemcitabine and 80-100% of cisplatin were released from the HaT formulation within 2 min at 40°C, while cisplatin was released from LTSL in 5 min at 42°C, in concordance with data reported [254]. Additionally, The HaT-LP have good stability with no diameter change in one month of storage at 4°C and very little drug leakage (< 3%). These results confirm that the HaT formulation is an improvement over LTSL.

The TLP were prepared using two different methods: the post- insertion method [255] and the conventional thin film method [252]. The post-insertion method was employed to incorporate Brij 78 only onto the outer leaflet of the LP. Nevertheless, the resulting formulation (DPPC-LP post-inserted with 8 mol% Brij 78) displayed similar release kinetics compared to the LP prepared with the thin film hydration method (DPPC/Brij 78 = 96/4). Additionally, the active agent is loaded into the TLP via two different methods: a citric acid pH gradient and a copper ion gradient.

It is well known that achieving homogeneous temperature distribution in tumors using currently available heating technologies has been a challenge [256-258]. Therefore, designing a novel carrier that exhibits an improved thermal sensitivity (a more rapid kinetic of release at the lower range of hyperthermia, i.e. 39-40°C) is expected to enhance drug delivery with heterogeneously heated tumor environments. In the mouse pharmacokinetic studies, HaT and LTSL formulations could retain the drug in the blood of the mice that were under no hyperthermia. After hyperthermia at the tumor (42°C) the drug release was significantly more complete for HaT than for LTSL, and this observation was further confirmed by a measured 1.4-fold increase in tumor Dox content for the HaT formulation. The improved pharmacokinetics and biodistribution results for the HaT group were likely due to the heterogeneous thermal distribution in the heated tumor, which favored rapid release from HaT formulation at the lower range of the hyperthermic temperatures (i.e. 40°C). These results were also supported by the measurement of significantly enhanced antitumor effect and the good performance of HaT on toxicity studies, suggesting that this novel formulation can compensate for the hurdles presented by tumor heterogeneity and may be useful to further improve the tumor delivery compared to the current LTSL formulation. Furthermore, complete remission of the distal tumor (15-25 days later) in mice that experience complete regression of the primary tumor, potentially suggest additional immune stimulation by HaT-Dox. In the same way, pharmacokinetic, biodistribution, antitumor efficacy and toxicity studies for HaT-Gemcitabine into tumor-bearing mice showed similar results. In addition, HaT liposome labelled with a targeting ligand like Arg-Gly-Asp (RGD) peptide that binds to certain classes of integrins [259] showed increased retention in the tumor microvaculature, increased dose released within the locally heated tumor and increased action against the tumor cells compared to free HaT formulation.

On the one hand, the identity of Brij78 as a surfactant and a possible mediator of membrane lysis was studied, but a hemolysis assay confirmed that the HaT displayed similar blood compatibility compared to the LTSL formulation, suggesting Brij78 was tightly associated with the LP and therefore, did not induce membrane lysis. On the other hand, it has been reported that Brij78 affects Pgp function, and accordingly, cells exposed to the Brij78-containing nanoemulsion formulation lost their multidrug resistance phenotype [260]. The results confirmed that the HaT formulation had the effect of enhancing Dox action on the resistant clone (A2780-ADR).

The good thermosensitive properties conferred by the polyethoxylated surfactants are restricted to a specific set of compositions. The authors also studied the characteristics of Dox-loaded Brij 78 LP with different phospholipids, but only the DPPC/Brij78 liposome (HaT-formulation) have all the advantageous characteristics of high drug loading efficiency, low leakage at 37°C and high release at 42°C.

Finally, gadolinium has been previously studied as an indicator of drug release from the liposomal formulations *in vitro* and *in vivo* by MRI [253, 261, 262]. The drug delivery and the MRI signal attenuation was found to correlate well [235, 261, 263]. The authors investigated the release of the encapsulated content from the HaT formulation at different temperatures by MRI: the results are consistent with that of the drug release profile. Therefore, the HaT-Gd formulation might be used for monitoring the drug release/delivery in the tumor using non-invasive MRI.

In conclusion, the rapid drug release at the lower temperature (40°C) offered the HaT formulation an advantage over LTST in delivering an increased amount of the drug to the heterogeneously heated tumor. In addition, the replacement of DSPC-PEG and MSPC with Brij78 not only conferred both stealth and thermosensitivity properties, but it also assisted in overcoming drug resistance and represents a more simple formulation. *In vivo* efficacy studies demonstrated that this novel formulation significantly improved drug delivery to the heated tumor and is more effective in regressing the solid tumor compared to free drug and the LTSL formulation.

4.6. Novel LP Composition to Improve Drug Delivery

The presence of titratable ammonium, such as unsubstituted ammonium ion (NH_4^+), as well as primary and secondary straight chain alkylammonium ions into the inner space of the LP, is known to provide enhanced encapsulation of

weak amphiphilic bases via a mechanism of "transmembrane gradient-driven" loading [264, 265]. However, these ammonia compounds possess hydrogen atoms that easily enter into reactions of nucleophilic substitution, and they can react chemically with the liposome-entrapped entities (e.g., drugs), therefore impairing the chemical integrity of such entities during or after the liposome loading process. Thus, the entrapped substituted ammonium compound should be chemically inert. A 2012 patent from Hong *et al*. [266] relates to LP compositions that include one or more substituted ammonium and/or polyanions within its inner space. The authors proposed that LP compositions with tertiary and quaternary ammonium that do not have substitutable hydrogen or a sterically hindered primary or secondary ammonium, may represent an improvement over the formulations available. The liposome-entrapped substituted ammonium compounds selected are low or non-toxic and can be in any suitable form (e.g., polyanion salt). The substituting organic groups (e.g. hydrocarbons) are of the size and physicochemical properties sufficient to ensure little or substantially no distribution of de ammonium compound into the bilayer portion of LP, minimizing the risk of destabilization of the LP.

LP with different lipid matrix composition were studied for their effect onto many parameters such as loading efficiency capacity, stability during storage, *in vivo* and *in vitro* drug stability and release, *in vitro* uptake and cytotoxicity against cancer cells, antitumor activity, toxicity and blood pharmacokinetics against multiple cancer xenografts in nude mice. The cells tested for above discussed purposes were human breast carcinoma BT-474, estrogen-dependent ductal adenocarcinoma that over-expresses C-ErbB2 (HER2) receptor, human colon carcinoma (HT-29), MDA-MB-468 EGFR-over-expressing human breast cancer cells, MCF-7 human breast cancer cells with low EGFR expression, MDA-MB-453 cancer cells, HER2-overexpressing human non-small cell lung carcinoma cells CaLu-3, HER2-overexpressing human breast carcinoma cells SKBr-3, C-26 syngeneic murine colon cancer tumors and U87 human brain cancer cells. The drugs evaluated include irinotecan (CPT-11), topotecan, Dox, 6-(3-Aminopropyl) Ellipticine (6-APE), vincristine, vinblastine, vinorelbine, 2-(2-(*N,N*-diethylamino)ethyl) ellipticinium (2-DAE). The targeting immune-LP were prepared by attachment of EGFR-specific Fab′ antibody fragments [267] and by conjugating the anti-HER2 single chain human Fv antibody fragment to LP surface [268, 269].

The authors discovered that polyanionised polyhydroxylated compounds with only strong acid dissociation provide liposomal encapsulation with better drug retention than compounds having weakly acidic dissociation. In general, polyanionization inside the LP was usually at a level that facilitates the delivery and release of the drug entrapped inside the LP at the site of action, but decreases the release of the entrapped drug prematurely. The degree of polyanionization inside the LP can be used to regulate the release characteristics (release rate and kinetics) of the encapsulated drug. On the other hand, the authors found that substituted ammonia with higher pKa values, that is, formed by more strongly basic amines, were more effective than those formed from weaker amines in stabilizing the drug inside LP. For example, both inositol hexaphosphate and sucrose octasulfate salts of triethylammonium (pKa = 10.75) were notably more effective than corresponding salts of triethanolammonium (pKa = 7.76) in stabilizing CPT-11 within the LP *in vivo*. In conclusion, the LP of the present invention provided a combination of the high efficacy of the entrapped therapeutic agent with high stability during storage. In general, the LP of the invention showed higher stability against the premature release (leakage) of the entrapped drug under *in vivo* conditions. In particular, LP loaded with vinca alkaloid drugs or camptothecin derivatives, showed remarkable stability against drug leakage *in vivo*. In addition of the extended blood life, the LP showed sustained release characteristics, and increased antitumor activity in the studied tumor model without an appreciable increase in toxicity. The cell-targeting ability and the intracellular delivery of drugs were also confirmed.

4.7. Improving the Delivery of Short Hairpin RNA for Cancer Treatment

The emerging class of RNA interference (RNAi) therapeutics is a novel approach to treat human diseases. Considering the fact that the RNAi is a natural cellular mechanism for regulating gene expression, these molecules have been gaining attention as useful tools for treatment of tumors [270-275]. Briefly, long double stranded ribonucleic acid (dsRNA) molecules are processed to small interfering RNA (siRNA) via the cytoplasmic enzyme Dicer. The siRNA is then bound to the RNA-induced silencing complex (RISC) such that the sense ("passenger") strand is removed and the antisense ("guide") strand is retained in the complex. The RISC complex is then able to bind its complementary mRNA and enable cleavage of the mRNA by the endonuclease argonaute-2 (hAgo2), ultimately leading to degradation of the target mRNA and reduction in protein expression [276-280]. In recent years, a variety of RNAi molecules that can inhibit tumor growth have been developed [281-284]. However, a key challenge toward reaching the potential of this technology is the safe and efficient delivery of siRNA to target tissues. The physical chemical properties of siRNAs (namely size ~13 kDa, polyanionic charge, and hydrophilicity) preclude passive diffusion across most cell membranes. Furthermore, intravenous injection of naked unmodified siRNA results in rapid renal clearance, degradation by RNAses and potential stimulation of an immune response via recognition by Toll-like receptors (TLRs) [285, 286]. For systemic administration, novel delivery systems are required to confer "drug-like" pharmacokinetic and pharmacodynamic properties, such as increased circulation time, distribution to target tissues, and effective cytoplasmic delivery to RISC. In order to solve those problems, a variety of RNAi delivery methods are currently under development [270, 287-289]. Various lipid-based delivery systems have been developed for *in vivo* delivery application of siRNA [289]. For example, methods for delivering RNAi molecules to tumor cells using complexes prepared by mixing an RNAi molecule with LP have been developed [286, 290, 291]. Although, a lot of significant advancements have been made in the field of *in vivo* siRNA delivery, there are still challenges to be overcome [289].

A 2013 patent from Ishida *et al.* [292] reported a method for delivering a short hairpin RNA (shRNA) capable of inhibiting expression of thymidylate synthase (TS) by RNAi action, bounded to the surface of a PEG-modified cationic

LP. The present inventors have previously reported [293] the sequence of this RNAi molecule and demonstrated that it is capable of inhibit TS expression (a folate-dependent enzyme that plays a pivotal role in DNA replication/repair and cancer cell proliferation, and represents a valid target for the treatment of several tumor types) [294]. The degree of TS expression inhibition caused by shRNA decreases by approximately 40% to 60% upon transfection with the use of a PEG-modified cationic LP.

A relationship between the TS expression level and the sensitivity of a 5-FU has been reported [295, 296]. Among cancer patients, 5-FU antitumor agents are remarkably effective for cancer patients with relatively low TS expression levels, while on the other hand, cancer patients with relatively high TS expression levels have resistance to 5-FU antitumor agents. Administration of the antitumor agent of the present invention enables suppression of TS production in tumor tissue, allowing an increase in the sensitivity of a 5-FU. In addition, the authors claim that the accumulation of PEG-modified cationic LP into the tumors increases when is administered in combination with a 5-FU antitumor agent. This are in concordance with the data reported that active alteration of the tumor microenvironment caused by the treatment with vaso-active agents or anticancer agents may improve delivery of anticancer agents associated with nanocarriers [297-301].

The authors used cancer cells that exhibit high TS expression levels to tests the invention, such as human colorectal cancer cell lines DLD-1, DLD-1/5FU (a 5-FU-resistant DLD-1 cell line) and a human mesothelioma cell line. The results showed that shRNA and siRNA can significantly inhibit TS expression in DLD-1 and DLD-1/FU cells. Besides, proliferation inhibitory effects of the lipoplexes were studied with or without 5-FU agent demostrating higher effects in the presence of 5-FU. The authors also studied the antitumor effects of PEG-modified Lipoplex containing shRNA targeting TS with or without 5-FU co-administration onto DLD-1 cancer-bearing mouse, and PEG-modified Lipoplex containing shRNA targeting or non-targeting TS with or without pemetrexed sodium hydrate onto MSTO-211H cancer-bearing mouse. Carcinostatic activity was examined based on changes in tumor volume and body weight. The groups treated with chemoterapeutic agent or the PEG-modified Lipoplex preparation alone exhibited lower tumor growth inhibitory effects in comparison with the groups treated with the combination of both.

In the light of these results, the authors proved that, when shRNA capable of inhibiting TS expression is electrostatically bound to the surface of a PEG-modified cationic LP, it can be readily delivered to cancer cells and hence, proliferation of TS-expressing tumor can be inhibited. In addition, when a PEG-modified Lipoplex is used in combination with a conventional chemotherapeutic agent for treating tumors, preferably an antitumor agent having TS inhibitory action (like 5-FU or pemetrexed sodium hydrate), sensitivity of cancer cells against the chemotherapeutic agent can be enhanced.

4.8. Active Targeting of LP to Tumor Vasculature

Angiogenesis is a required step for the expansion during tumorogenesis and constitutes a rate-limiting step for tumor progression [302]. Thus, tumor blood vessels are prime targets for the inhibition of tumor growth. Because tumor vasculature expresses unique markers that distinguish it from normal vasculature [303], targeting based on these markers is a promising strategy for cancer treatment. Many of these specific markers are proteins associated with tumor-induced angiogenesis [304]. The cell adhesion receptors, integrins $\alpha v\beta 3$ and $\alpha v\beta 5$, are over-expressed in tumor vasculature [305]. Indeed, one of the RGD-peptides identified by *in vivo* phage-display for tumor targeting recognizes $\alpha v\beta 5$ [306]. Peptides specific for these integrins have been used as ligands for targeted delivery of anti-cancer and anti-angiogenic agents [307]. What is more, blood vessels are composed of nonmalignant endothelial cells that are genetically stable, and develop little or no drug resistance [308].

The development of phage-displayed peptide libraries over the past decade has ushered in the opportunity to identify small peptides that are effective for targeting purposes. A recent patent from Wu *et al.* [309] presents polynucleotides, peptides, and variants thereof identified by *in vivo* phage display that can specifically target neovasculature. One peptide in particular, SP5-52, recognized the neovasculature of multiple human tumors in SCID mice (eight xenografts tested), but did not target normal blood vessels of the lung, heart and brain. This peptide also binds to VEGF-stimulated human vascular endothelial cells (HUVECs) and to blood vessels of human lung cancer biopsy specimens. The results showed that SP5-52 has the highest binding affinity to the tumor tissues from all different types of human cancer xenografts evaluated. The authors claim that this phenomenon suggests that vasculature in solid tumors may express a universal receptor that can be recognized by the SP5-52 peptide.

SP5-52 peptide-linked LP carrying Dox (SP5-52-Lipo-Dox) were prepared. The LP are composed of DSPC, cholesterol and PEG-DSPE. L-peptide was coupled to NHS-PEG-DSPE (*N*-hydroxysuccinimido-carboxyl-polyethylene glycol-derived distearoylphosphatidylethanolamine) to produce peptidyl-PEG-DSPE. Peptidyl-PEG-DSPE was transferred to pre-formed LP after co-incubation at temperature above the transition temperature of lipid bilayer [310]. The LP have diameters between 65 nm and 75 nm and the number of peptide molecules per liposome varies from about 300 to 500. The LP were tested onto human lung (CL1-5) and oral (SAS) cancer xenografts established in SCID mice. SP5-52-Lipo-Dox enhanced the efficacy of the drug against human cancer xenografts in SCID mice in comparison with Lipo-Dox alone. This targeting liposome significantly increased the survival rate of these two human cancer animal models. The tumor vasculature was destroyed and markedly decreased by SP5-52-Lipo-Dox treatment. These studies indicate that the SP5-52-peptide can specifically bind to vasculature in multiple tumors, and is a good candidate for targeted drug delivery to solid tumors.

4.9. Solid Lipid Nanoparticles

LP have some important drawbacks: they are manufactured through processes that involve organic solvents, they are unstable in biological fluids and more generally in aqueous solutions (they cannot be commercialized as such), and they present poor batch-to-batch reproducibility and difficul-

ties in sterilization [311, 312]. Therefore, there is a need to develop alternative nanocarriers based on lipid components. It is hoped that these drug carriers will allow higher control over drug release and delivery of therapeutics which may not efficiently load into LP. Between the variety of lipid carriers, Nanostructured Lipid Carriers (NLC) and Solid Lipid Nanoparticles (SLN) have been developed and used as parenteral drug delivery systems, especially in cancer chemotherapy [313, 314]. SLN were developed in the middle of the 1990s, by replacing the liquid lipid (oil) of an oil-in-water nanoemulsion by a solid lipid or a blend of solid lipids [315]. The use of solid lipids instead of oils is a very attractive idea to achieve controlled drug release, since the drug molecule mobility in a solid matrix should be intuitively lower compared with an oily phase [118]. SLN have a diameter approximately between 50 and 1000 nm [316]. Moreover, large-scale manufacturing of SLN is possible (while other systems such as polymeric nanoparticles have faced scaling-up issues), and solvent use can be avoided using high-pressure homogenization with extant machinery [5, 117, 118]. A couple of limitations to the use of SLN come from their limited loading capacity because of the formation of a highly ordered, perfect lipid crystal matrix [317]. After preparation, at least some of the particles crystallize in higher energy modifications that, during storage, evolve to low energy, more ordered modifications that lead to drug expulsion [117]. These drawbacks have been solved by second generation lipid nanoparticles, NLC, in which the solid lipids are blended with oils. Even though the blends still present solid state at body temperature, the solid lipid matrix is less ordered (and even amorphous) compared with SLN, thus admitting higher drug loads and minimizing drug expulsion [117, 118, 316, 318]. Depending on the method of production and the lipid blend composition, different types of NLCs are obtained: imperfect, amorphous and multiple type. In the imperfect type, lipid crystallization is altered by small amounts of oils. In the amorphous type, the lipid matrix is solid but not crystalline (amorphous state): this can be achieved by mixing specific lipids, for example, hydroxyoctacosanyl hydroxystearate with isopropyl myristate. In the multiple type, the solid lipid matrix contains tiny oil compartments: they are obtained by mixing a solid lipid with a higher amount of oil. The basic idea is that by providing a less-ordered structure to the lipid matrix, the payload for active compounds is increased and expulsion of the compound during storage is avoided [319].

4.10. Improving the Platinum Species Pharmacokinetics

Platinum compounds are effective anticancer drugs used to treat solid tumors, particularly against ovarian and lung cancer. Nevertheless, after IV administration, platinum species tend to bind irreversibly to plasma proteins. Besides, the fraction of free drug in plasma seems to undergo a rapid degradation to form inactive species. Other issues linked to platinum compounds treatment are systemic toxicity (nephrotoxicity, bone marrow suppression) and intrinsic or acquired resistance. These issues limit the free drug levels and hamper platinum compounds efficacy in clinical trials [320, 321]. One strategy that can be used to overcome the side effects and occurrence of resistance to platinum drugs is to encapsulate them in polymer formulations or in lipid-based systems [322, 323].

A patent from Gasco *et al.* [322] presents the development of SLN of hydrophilic platinum complexes wherein the platinum metal atom is chelated by suitable ligands, particularly anionic ligands and ligands containing amino groups. In particular the authors proposed the formulation of the platinum dinitrate salt described in a previous patent and presented in Fig. (**7**) [324]. The SLN of platinum compounds are obtained from warm microemulsions using the procedure and technology described in US5250236 patent [325]. The SLN loaded with the drug are spherical, with an average diameter between 70 and 200 nm, and are suitable for IV and oral administration. When incorporated within SLN, the platinum compound is stable in plasma and does not interact with plasma proteins. The loaded SLN are well tolerated when administered to CD1 mice and show an improved therapeutic index when compared to aqueous solutions of the same compound.

Another patent from Boulikas [326] discloses a method for encapsulating cisplatin, cisplatin derivatives and other positively charged antineoplastic drugs (e.g. Dox) into LP having different composition in their inner and outer layers. Combination of platinum compounds and Dox or p53 gene therapy are also considered within the invention. According to the disclosed method, the positively charged active ingredient (e.g. cisplatin) is complexed with a phosphatidyl glycerol lipid (PGL) derivative. Variations in the molar ratio between the drug and the PGL derivative change the net charge of the complex thus allowing targeting different tissues. What is more, complexing cisplatin compounds with PGL enhances its aqueous solubility and encapsulation efficiency. The complex is mixed with an at least 20% solution of an organic solvent to form micelles, which are later encapsulated within vesicle-forming lipids. The PGL derivative may be partially replaced by negatively charged peptides with fusogenic properties, such as the fusion peptide of hemagglutinin from influenza virus (a large table disclosing possible fusogenic moieties is disclosed in the patent). One of the examples presented in the patent shows that the injection of

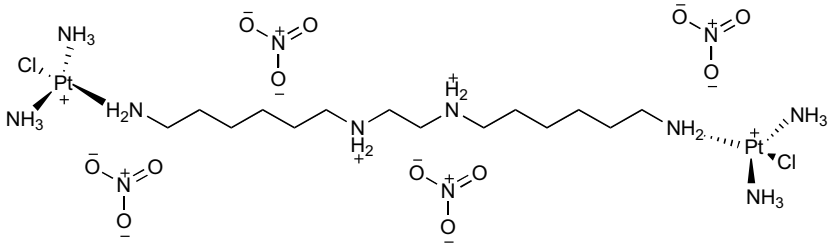

Fig. (7). Chemical structure of the platinum complex.

encapsulated cisplatin induces tumor regression on SCID mice with MCF-7 tumors.

4.11. Lipid Nano-formulations for Improving the Delivery of Statins and Tocotrienol for Anticancer Therapy

Statins are inhibitors of the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol synthesis [327]. The inhibition of this key enzyme in the mevalonate pathway (constitutively active in malignant cells) and, subsequently the isoprenylation of Ras family proteins [328], lead to suppression of cell proliferation and induction of apoptosis [329-331]. For this reason, the anticancer potential of statins has been extensively studied [332-335]. Statins are an example of a promising cancer chemotherapeutic agent that display antiproliferative and apoptotic activity against many types of cancer cell lines [328, 336-338] and in various *in vivo* models [339, 340], whose clinical applicability has been limited due to high-dose toxicity [332].

On the other hand, much evidence has accumulated over the past few years, which demonstrates that dietary supplementation with specific members of the vitamin E family, particularly tocotrienols, can provide significant protection at multiple stages of mammary carcinogenesis [341-345]. What is more, tocotrienols displays anticancer effects, but their limited absorption and distribution throughout the body has made it difficult to obtain and sustain therapeutic levels in the blood and target tissues [332]. Tocotrienol has also been shown to induce post-transcriptional down-regulation of HMG-CoA reductase, resulting in a significant decrease in cholesterol synthesis [345]. Since statins and tocotrienols suppress HMG-CoA reductase activity through independent mechanisms, it was thought that combined low-dose treatment with these agents may produce additive or even synergistic anticancer effects, while avoiding high-dose side effects, and low bioavailability issues [332].

The recent patent from Nazzal *et al.* [346] is related to the development of SLN, NLC and nanoemulsions (NE) which can contain, depending on the case: a therapeutic amount of tocotrienol (tocotrienol-rich-fraction, TRF) and/or a therapeutic amount of a statin (simvastatin), non-tocotrienol lipids and a surface active agent (polaxamer 188). TRF of palm oil is an oily mixture of tocopherols and tocotrienols, in which tocotrienols constitute 70-90% of the blend. First, the formulations were evaluated without simvastatin. The structures of SLN (based only on solid lipids), NLC (based on an amount of TRF added to solid lipids) and TRF-NE (for which no solid lipids were used) were characterized by thermal analysis using differential scanning calorimetry, whereas the TRF localization, entrapment and/or affinity to the solid lipids were tested by Proton Nuclear Magnetic Resonance studies (^{1}H-NMR). The results revealed that the size, polydispersity, melting endotherm, and fusion enthalpy of the nanoparticles decreased with an increase in TRF loading. TRF spectra in NLC however, were distinctly different from those observed in TRF-NE and SLN. TRF molecules are preferentially entrapped within the cores of NLC yielding stable spherical-nanoparticles.

To evaluate the effect of NLC composition on TRF entrapment, four high melting point lipids that vary in their chemistry, namely glycerol tristearate, glyceryl behenate, glycerol palmitostearate and cetyl palmitate, were selected as the matrix forming lipids. The long term stability of the TRF-NLC with respect to their size was also evaluated and the results showed that NLC are sufficiently stable (both physically and chemically) after 6 months of storage at room temperature.

In another embodiment of the invention, the authors evaluated the SLN and NCL again, but now incorporating simvastatin. For SLN, the lipid phase used was glyceryl behenate; and NLC was achieved by substituting a portion of glyceryl behenate with either TRF or α-tocopherol. The already described results indicated that the presence of TRF and α-tocopherol were correlated with disorder in the crystalline structure of glyceryl behenate. Various formulations were screened and the physiochemical properties of the simvastatin-TRF-nanoparticles, such as particle size, surface morphology, drug entrapment efficiency, *in vitro* drug release, and stability, were assessed. The cellular antiproliferative effect against the highly malignant neoplastic +SA mammary epithelial cells was evaluated through the MTT assay.

The results confirmed the existence of simvastatin as a molecular dispersion in the TRF-glyceryl behenate or α-tocopherol-glyceryl behenate blend and proved long term stability and high simvastatin entrapment efficiency. High entrapment efficiency of simvastatin in the NLC could be attributed to the presence of the liquid nanocompartments formed by TRF (or α-tocopherol), which were entrapped within the solid matrix of the NLC. The size of the nanoparticles decreased significantly when 50% of glyceryl behenate was substituted with TRF (or α-tocopherol). Lower particle size could be attributed to the efficient packing of the disrupted crystalline structure of glyceryl behenate when blended with TRF (or α-tocopherol). The entrapment of simvastatin into NLC did not change the size of the nanoparticles. Simvastatin nanoparticles had particle sizes of about 100 nm, which should be ideal for future *in vivo* administration. They have spherical or ellipsoidal shapes and monodispersity, and no simvastatin nanocrystals were observed. For the cellular antiproliferative effect, TRF or α-tocopherol NLC with or without simvastatin were studied and the results reflected the *in vitro* synergistic effect of TRF and simvastatin against tumor cells (Table **3**).

Table 3. Antiproliferative Effect (IC_{50} Values) for the TRF or α-Tocopherol-NLC with or without Simvastatin. Extracted from Nazzal *et al.* [346].

Formulation	IC_{50} (μM)
Simvastatin-TRF-NLC	0.52 ± 0.02
Simvastatin-α-tocopherol-NLC	0.76 ± 0.05
TRF-NLC	1.50 ± 0.12
α-Tocopherol-NLC	17.70 ± 0.74

In the last embodiments of the invention, the authors used the melt emulsification technique for the preparation of

Table 4. Antiproliferative Effect (IC_{50} Values) for TRF/BSA, TRF-50-NLC, and TRF-10-NLC (Mean ± SEM, n = 6). Extracted from Nazzal *et al.* [346].

Formulation	Secondary Lipid	IC_{50} (μM)
TRF/BSA	N/A	2.73 ± 0.11
TRF-50-NLC	Cetyl palmitate	2.12 ± 0.21
	Glyceryl behenate	2.08 ± 0.003
	Glyceryl palmitostearate	2.15 ± 0.007
	Glyceryl tristearate	1.51 ± 0.05
TRF-10-NLC	Cetyl palmitate	1.25 ± 0.13
	Glyceryl behenate	1.22 ± 0.05
	Glyceryl palmitostearate	1.67 ± 0.14
	Glyceryl tristearate	1.46 ± 0.08

the SLN [347]. The impact of process parameters such as sonication time, power and pulsar rate and lipidic composition on the size and polydispersity of SLN was evaluated. Different SLN were prepared using one of the four lipids used before as their lipid core. Once established the optimal formulation and parameters, TRF was incorporated into SLN to form NLC (substituting 10% or 50% of the lipid phase). The nanoparticles presented good long-term stability.

Their cellular anti-proliferative effect against neoplastic +SA mammary epithelial cells was evaluated. For this purpose, the activity of TRF-NLC stock formulations were assessed and compared to SLN and a solution of TRF in bovine serum albumin (BSA).

The 50% growth inhibition (IC_{50}) values for TRF/BSA and TRF-NLC are presented in Table **4**. Overall, IC_{50} values for the TRF-NLC were lower than the IC_{50} value for the TRF/BSA solution. TRF-10-NLC had significantly lower IC_{50} values than those observed for the TRF-50-NLC of the same lipid type.

In conclusion, the use of NLC as TRF and simvastatin delivery vectors enhanced their antiproliferative effect on +SA mammary epithelial cells. The administration of those drugs (either alone or in combination) through this lipid-based nanosystem may provide a solution to overcome adverse effects related to simvastatin high doses and bioavailability issues due to TRF poor absorption.

Anti-proliferation data shown in Fig. (**8**) and the IC_{50} values shown in Table **3** demonstrated that entrapment of TRF within NLC increased their potency. IC_{50} values for TRF-50-NLC were higher than the IC_{50} values for TRF-10-NLC.

4.12. Hydrophobic Nanoparticles Formulations with Mucoadhesive Properties for Enhancing the Cellular Uptake of Anticancer Drugs

A 2012 patent from Dash *et al.* [348] describes the development of nanoparticles formed of a hydrophobic core that contains an anticancer agent (such as PTX or gemcitabine) and a hydrophilic surface layer surrounding such core. The nanoparticles can include other biologically active materials which can be delivered together with the cancer therapeutic agent (e.g. dexamethasone, DEX). The hydrophobic core is composed of a glyceryl mono fatty acid ester, while the hydrophilic surface layer is made of chitosan. Placing the chitosan on the surface layer of the glyceryl mono fatty acid ester, creates a positively charged surface layer in order to make each nanoparticle more mucoadhesive to negatively charged mucin at the cancer cells. In this sense, it is believed that the nanoparticles having mucoadhesive properties increase the effect of the therapeutic agent on cancer cells in the sample through the bioadhesion to transmembrane mucin glycoproteins that are over-expressed on almost all human adenocarcinomas, increasing cellular uptake [349, 350].

Chitosan/glyceryl monooleate (GMO) nanoparticles were characterized by determining the mean particle size, size distribution, mean zeta potential, nanoparticle yield, the percent drug loading, the encapsulation efficiency, the physical state of the drug in the formulation, the *in vitro* drug release profiles of different formulations in the presence or absence of a surfactant (Tween-80), and the *in vitro* bioadhesion, cellular uptake and cytotoxicity (by MTT assay) profile in MDA-MB-231 human breast cancer cells. Finally, the safety and efficacy of the localized PTX-nanoparticules were compared to the conventional PTX formulation in an *in vivo* model of MDA-MB-231 human breast cancer cells.

The results showed that chitosan/GMO can form polycationic nano-sized particles (400-700 nm) with the therapeutic agent entrapped, absorbed, or chemically coupled in the biopolymeric matrices. In addition, the formulation demonstrates high yields and entrapment efficiencies along with sustained release characteristics. This formulation can be stored in a lyophilized powder that is easily re-suspended in an aqueous matrix, and the drug incorporated within the nanoparticles exists in a non-crystalline state. The *in vitro* drug release profiles showed common characteristics of initial burst-release, followed by a slow near zero-order rate of release over the experimental period. The chitosan/GMO nanoparticles showed evidence of significant mucoadhesive properties and increased cellular association (when com-

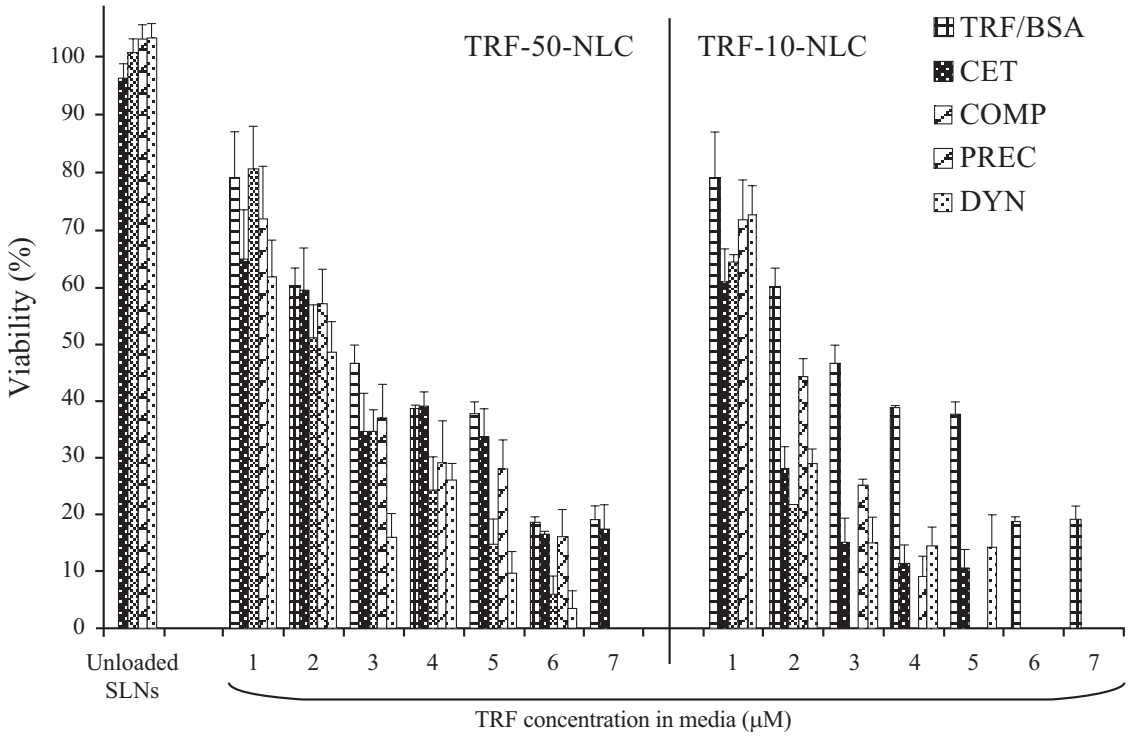

Fig. (8). Anti-proliferative effects of unloaded SLN as compared to TRF-NLC at 10% and 50% TRF load of the total lipid phase, and TRF/BSA solution (as reference) on neoplastic +SA mamary epithelial cells. Cells were initially plated at a density of 5×10^4 cells/well (6 wells/group) in 24-well plates and exposed to formulation-supplemented media for a 4-day treatment period. Vertical bars indicate the mean cell count with lines indicating the standard error of the mean. CET: cetyl palmitate, COMP: glyceryl behenate, PREC: glycerol palmitostearate, DYN: glycerol tristearate. Reprinted with permission from Nazzal *et al.* [346].

pared to a solution of free PTX) in MDA-MB-231 cells. The increased cellular association correlates with a significant increase in cell cytotoxicity. The mucoadhesive properties of chitosan have been also shown effective in the delivery of various molecules in adenocarcinomas both *in vitro* and *in vivo* [351-353]. Shikata *et al.* demonstrated increased cellular internalization of drug loaded chitosan nanoparticles in squamous cell carcinomas (SCC-VII) and melanoma cells (B16F10) when compared to drug solutions alone [352]. These advantages may allow lower doses of PTX to achieve an efficacious therapeutic window, thus minimizing the adverse side effects.

In the *in vivo* model of MDA-MB-231 human breast cancer cells, PTX nanoparticle formulation was compared to a control (no treatment), PTX standard clinical IV solution and a placebo (blank nanoparticle formulation). After a single intratumoral bolus dose of the PTX-nanoparticles, a significant decrease (50%) in tumor diameter was observed on day 15 when compared to control, placebo, and intravenously administered PTX. Systemic administration was also evaluated and similar results were obtained. In another embodiment of the invention, the authors prepared and characterized a GEM-nanoparticle formulation consisting of chitosan and GMO. They evaluated the physiochemical properties, particle size, surface charge, GEM encapsulation, *in vitro* release, and *in vitro* cellular association, safety and efficacy of the nanoparticle in an *in vitro* pancreatic cancer model (MiaPaCa-2 and BxPC-3). The results obtained are in the same line that those obtained for PTX formulations.

The authors also assessed the cellular uptake and subcellular localization of the developed nanoparticle drug delivery systems composed only of chitosan and GMO. The study showed that the chitosan/GMO nanoparticle formulation clearly colocalized in the nuclear compartment, which might make them appropriate for gene delivery.

4.13. Improving the Stability of Docetaxel-SLN Formulation

Phospholipid-based LP formulations for PTX and DTX have been developed to solve the already mentioned vehicle-related safety issues (see section 3.1) [80, 354, 355]. The main advantage of these formulations is the elimination of toxicity related to the Cremophor EL or polysorbate 80, and a reduction in the toxicity of the taxane itself, as demonstrated in several animal tumor models [80, 354, 356].

Using the technology disclosed in US5439686 patent [357], as we mentioned before, ABRAXANE®, a highly useful formulation for drug delivery of PTX has been developed. In that case, the nanoparticles produced were amorphous. When the method described in US5439686 patent was applied to produce a DTX nanoparticle dispersion, the particles began to precipitate within 1 hour of the preparation due to Ostwald ripening [358]. Thus the method, owing to the physical instability, is not useful for the preparation of DTX nanoparticles dispersed in aqueous medium. The growth of particles in dispersion can result in sedimentation of the particles during storage. It is particularly important that the particle size in a dispersion of a pharmacologically

active compound remains constant because a change in particle size is likely to affect the bioavailability, and hence, the efficacy of the compound. Furthermore, if the dispersion is required for IV administration, growth of the particles in the dispersion may render the dispersion unsuitable for this purpose.

A patent from Singh [359] shows the development of a stable dispersion of SLN in an aqueous medium without appreciable Ostwald ripening effect, for the delivery of DTX. Stable dispersions of solid particles of DTX in an aqueous medium can be prepared using an oil-in-water emulsion process that comprises the following steps: DTX and Ostwald ripening inhibitors (e.g. a mixture of cholesterol and cholesteryl stearate or hexadecyl hexadecanoate) are dissolved in a suitable solvent (e.g. chloroform). In the next stage, in order to make solid nanoparticles, a protein (e.g. human serum albumin) is added into the aqueous phase to act as a stabilizing agent or an emulsifier. In the last stage, an emulsion is formed by homogenization under high pressure and high shear forces.

The SLN formulation of the invention showed to be less prone to Ostwald ripening due to the presence of the Ostwald ripening inhibitors, and more stable in solution than the formulations disclosed in the prior art. The authors evaluated the efficacy of SLN formulations with varying Ostwald ripening inhibitor compositions, particle size, and DTX to protein ratio on various systems such as human cell lines and animal models. The SLN formulation of the invention also showed to be less toxic than the DTX administered in its free form.

CURRENT & FUTURE DEVELOPMENTS

Nanotechnology has come a long way since it was first conceived more than 50 years ago by physicist Richard Feynman. Feynman's visions of the enormous possibilities available in the molecular world spawned the discipline of nanotechnology, which has now become one of the most promising sciences with many fields of application, likely to have a profound impact on our economy and society, perhaps comparable to that of information technology or molecular biology.

Nanotechnology is a broad, highly interdisciplinary, still evolving field, and its relevance is evidenced by the fact that most countries invest a significant and increasing budget in nanoscience. Furthermore, among all the existing applications of nanothecnology, cancer research is one of the hottest fields, with current efforts focused on how to use nanotechnology to radically change the ability of medicine to diagnose, understand and treat cancer. In 2004, the US National Cancer Institute (NCI) launched the Alliance for Nanotechnology in Cancer, a $144 million cancer nanotechnology initiative to advance a number of promising nanotechnologies for the diagnosis, treatment and prevention of cancer. After five years, the success of the program led NCI to announce in 2010 an investment of approximately $30 million per year for the next five years for the second phase of the Alliance's research and training initiatives [360]. Through this review, in its two parts, we have attempted to present patents that represent clear examples of the new developments that are being carried out to tailor physicochemical properties of chemotherapeutic agents and develop safer treatments that specifically target malignant cells. Experts believe that nanotechnology will transform the very foundations of the diagnosis, treatment and, most importantly, prevention of this deadly disease.

It would be wrong to think that the future of pharmaceutical nanotechnology will only mean more and better pharmaceutical systems or therapeutics agents. We believe that the real future will involve much more than that: it will require to change some fundamental concepts and to build new paradigms. If we are able to develop pharmaceutical systems selectively delivered and released in its site of action, and specific in their molecular target, we will have to deeply review several pharmacokinetic and pharmacological principles and models, like how to measure the drug levels and what is considered as drug dose, as well as which will be the procedures to evaluate the safety of pharmaceutical nanosystems.

It can be seen, therefore, that we are in the beginning of an era that, undoubtedly, will be very productive and creative in offering new opportunities for a more rational fight against disease and human suffering.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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REFERENCES

[1] Smith DA, Di L, Kerns EH. The effect of plasma protein binding on *in vivo* efficacy: Misconceptions in drug discovery. Nat Rev Drug Discov 2010; 9(12): 929-39.

[2] Szakacs G, Paterson JK, Ludwig JA, Booth-Genthe C, Gottesman MM. Targeting multidrug resistance in cancer. Nat Rev Drug Discov 2006; 5(3): 219-34.

[3] Sharom FJ. ABC multidrug transporters: Structure, function and role in chemoresistance. Pharmacogenomics 2008; 9(1): 105-27.

[4] Talevi A, Bruno-Blanch LE. Efflux-Transporters at the Blood-Brain Barrier: Therapeutic Opportunities. In: Montenegro PA, Juárez SM, Eds. The Bood-Brain Barrier: New Research. ed. New York, Nova Publishers; 2012, pp. 117-44.

[5] Muller RH, Keck CM. Challenges and solutions for the delivery of biotech drugs: A review of drug nanocrystal technology and lipid nanoparticles. J Biotechnol 2004; 113(1-3): 151-70.

[6] Nishikawa M, Takakura Y, Hashida M. Theoretical considerations involving the pharmacokinetics of plasmid DNA. Adv Drug Deliv Rev 2005; 57(5): 675-88.

[7] Torchilin V. Intracellular delivery of protein and peptide therapeutics. Drug Discov Today Tech 2008; 5(2-3): e95-e103.

[8] Parra-Guillen ZP, Gonzalez-Aseguinolaza G, Berraondo P, Troconiz IF. Gene therapy: A pharmacokinetic/pharmacodynamic modelling overview. Pharm Res 2010; 27(8): 1487-97.

[9] Vugmeyster Y, Xu X, Theil FP, Khawli LA, Leach MW. Pharmacokinetics and toxicology of therapeutic proteins: Advances and challenges. World J Biol Chem 2012; 3(4): 73-92.

[10] Talevi A, Gantner ME, Ruiz ME. Applications of nanosystems to anticancer drug therapy (Part I. Nanogels, nanospheres, nanocapsules) Recent Pat Anticancer Drug Discov 2013; 9(1): 83-98.

[11] Tomalia DA, Baker H, Dewald J, Hall M, Kallos G, Martin S, *et al*. A new class of polymers: Starburst-dendritic macromolecules. Polym J 1985; 17(1): 117-132.

[12] Tomalia DA. Birth of a new macromolecular architecture: Dendrimers as quantized building blocks for nanoscale synthetic polymer chemistry. Prog Polym Sci 2005; 30(3-4): 294-324.

[13] Grayson SM, Fréchet JMJ. Convergent dendrons and dendrimers: From synthesis to applications. Chem Rev 2001; 101(12): 3819-68.

[14] Medina SH, El-Sayed MEH. Dendrimers as carriers for delivery of chemotherapeutic agents. Chem Rev 2009; 109(7): 3141-57.

[15] Na M, Yiyun C, Tongwen X, Yang D, Xiaomin W, Zhenwei L, *et al*. Dendrimers as potential drug carriers. Part II. Prolonged delivery of ketoprofen by *in vitro* and *in vivo* studies. Eur J Med Chem 2006; 41(5): 670-4.

[16] Reuter JD, Myc A, Hayes MM, Gan Z, Roy R, Qin D, *et al*. Inhibition of viral adhesion and infection by sialic-acid-conjugated dendritic polymers. Bioconjugate Chem 1999; 10(2): 271-8.

[17] Vandamme TF, Brobeck L. Poly(amidoamine) dendrimers as ophthalmic vehicles for ocular delivery of pilocarpine nitrate and tropicamide. J Control Release 2005; 102(1): 23-38.

[18] Cheng Y, Xu Z, Ma M, Xu T. Dendrimers as drug carriers: Applications in different routes of drug administration. J Pharm Sci 2008; 97(1): 123-43.

[19] Esfand R, Tomalia DA. Poly(amidoamine) (PAMAM) dendrimers: From biomimicry to drug delivery and biomedical applications. Drug Discov Today 2001; 6(8): 427-36.

[20] Tekade RK, Kumar PV, Jain NK. Dendrimers in oncology: An expanding horizon. Chem Rev 2008; 109(1): 49-87.

[21] Boas U, Heegaard PMH. Dendrimers in drug research. Chem Soc Rev 2004; 33(1): 43-63.

[22] Svenson S, Tomalia DA. Dendrimers in biomedical applications-reflections on the field. Adv Drug Delivery Rev 2012; doi: 10.1016/j.addr.2012.09.030.

[23] Liu M, Kono K, Fréchet JMJ. Water-soluble dendritic unimolecular micelles: Their potential as drug delivery agents. J Control Release 2000; 65(1-2): 121-31.

[24] Kuo J-hS, Liou M-j, Chiu H-c. Evaluating the gene-expression profiles of HeLa cancer cells treated with activated and nonactivated poly(amidoamine) dendrimers, and their DNA complexes. Mol Pharm 2010; 7(3): 805-14.

[25] Hui Zhong, He Z-G, Zheng Li, Li G-Y, Shen S-R, Li X-L. Studies on polyamidoamine dendrimers as efficient gene delivery vector. J Biomater Appl 2008; 22(6): 527-44.

[26] Duncan R, Izzo L. Dendrimer biocompatibility and toxicity. Adv Drug Delivery Rev 2005; 57(15): 2215-37.

[27] King Heiden TC, Dengler E, Kao WJ, Heideman W, Peterson RE. Developmental toxicity of low generation PAMAM dendrimers in zebrafish. Toxicol Appl Pharmacol 2007; 225(1): 70-9.

[28] Tomalia DA, Reyna LA, Svenson S. Dendrimers as multi-purpose nanodevices for oncology drug delivery and diagnostic imaging. Biochem Soc Trans 2007; 35(1): 61-7.

[29] Thassu D, Deleers M, Pathak Y. Nanoparticulate Drug Delivery Systems. 1st ed. Informa Healthcare: New York 2007.

[30] Jevprasesphant R, Penny J, Attwood D, McKeown NB, D'Emanuele A. Engineering of dendrimer surfaces to enhance transepithelial transport and reduce cytotoxicity. Pharm Res 2003; 20(10): 1543-50.

[31] Heiden TC, Dengler E, Kao WJ, Heideman W, Peterson RE. Developmental toxicity of low generation PAMAM dendrimers in zebrafish. Toxicol Appl Pharmacol 2007; 225(1): 70-9.

[32] Dobrovolskaia MA, Patri AK, Simak J, Hall JB, Semberova J, De Paoli Lacerda SH, *et al*. Nanoparticle size and surface charge determine effects of PAMAM dendrimers on human platelets *in vitro*. Mol Pharm 2012; 9(3): 382-93.

[33] Jones CF, Campbell RA, Franks Z, Gibson CC, Thiagarajan G, Vieira-de-Abreu A, *et al*. Cationic PAMAM dendrimers disrupt key platelet functions. Mol Pharm 2012; 9(6): 1599-611.

[34] Jones CF, Campbell RA, Brooks AE, Assemi S, Tadjiki S, Thiagarajan G, *et al*. Cationic PAMAM dendrimers aggressively initiate blood clot formation. ACS Nano 2012; 6(11): 9900-910.

[35] Jelinkova M, Strohalm J, Etrych T, Ulbrich K, Rihova B. Starlike vs. classic macromolecular prodrugs: Two different antibody-targeted HPMA copolymers of doxorubicin studied *in vitro* and *in vivo* as potential anticancer drugs. Pharm Res 2003; 20(10): 1558-64.

[36] Kopecek J, Kopeckova P, Minko T, Lu Z. HPMA copolymer-anticancer drug conjugates: Design, activity, and mechanism of action. Eur J Pharm Biopharm 2000; 50(1): 61-81.

[37] Khandare JJ, Jayant S, Singh A, Chandna P, Wang Y, Vorsa N, *et al*. Dendrimer versus linear conjugate: Influence of polymeric architecture on the delivery and anticancer effect of paclitaxel. Bioconjugate Chem 2006; 17(6): 1464-72.

[38] Greenwald RB. PEG drugs: An overview. J Control Release 2001; 74(1-3): 159-71.

[39] Cai S, Xie Y, Davies NM, Cohen MS, Forrest ML. Pharmacokinetics and disposition of a localized lymphatic polymeric hyaluronan conjugate of cisplatin in rodents. J Pharm Sci 2010; 99(6): 2664-71.

[40] Forrest, L., Cohen, M., Cai, S. Intralymphatic chemotherapy drug carriers. US8088412 (**2012**) & EP2242514A4 (**2013**).

[41] Oussoren C, Storm G. Liposomes to target the lymphatics by subcutaneous administration. Adv Drug Delivery Rev 2001; 50(1-2): 143-56.

[42] Denham JW, Hamilton CS, Christie D, O'Brien M, Bonaventura A, Stewart JF, *et al*. Simultaneous adjuvant radiation therapy and chemotherapy in high-risk breast cancer-toxicity and dose modification: A transtasman radiation oncology group multi-institution study. Int J Radiat Oncol Biol Phys 1995; 31(2): 305-13.

[43] Coles CE, Moody AM, Wilson CB, Burnet NG. Reduction of radiotherapy-induced late complications in early breast cancer: The role of intensity-modulated radiation therapy and partial breast irradiation: Part I - normal tissue complications. Clin Oncol 2005; 17(1): 16-24.

[44] Babich, J.W., Joyal, J.L., Zimmerman, C. PSMA-targeted dendrimers. WO2012078534 (**2012**) & EP2648766A1 (**2013**).

[45] Yu MK, Park J, Jon S. Targeting strategies for multifunctional nanoparticles in cancer imaging and therapy. Theranostics 2012; 2(1): 3-44.

[46] Okarvi SM. Peptide-based radiopharmaceuticals and cytotoxic conjugates: Potential tools against cancer. Cancer Treat Rev 2008; 34(1): 13-26.

[47] Kalele S, Gosavi SW, Urban J, Kulkarni SK. Nanoshell particles: Synthesis, properties and applications. Curr Sci 2006; 91(8): 1038-52.

[48] Petryayeva E, Krull UJ. Localized surface plasmon resonance: Nanostructures, bioassays and biosensing--a review. Anal Chim Acta 2011; 706(1): 8-24.

[49] Albrecht, V., Wiehe, A., Roeder, B., Neuberger, W. Photo-triggered release of active substances from dendrimer-photosensitizer complexes. US8153111 (**2012**).

[50] Ross JS, Fletcher JA, Linette GP, Stec J, Clark E, Ayers M, *et al*. The HER-2/neu gene and protein in breast cancer 2003: Biomarker and target of therapy. Oncologist 2003; 8(4): 307-25.

[51] Sudimack J, Lee RJ. Targeted drug delivery via the folate receptor. Adv Drug Delivery Rev 2000; 41(2): 147-62.

[52] Baker, Jr., J.R., Cheng, X.-M., Thomas, T.P., Huang, B.M. Dendrimer conjugates. US8252834 (**2012**) & US20090287005 (**2009**).

[53] Baker, Jr., J.R., Banaszak Holl, M.M., Cheng, X.-M., Huang, B.M., McNemy, D., Mullen, D.G., Thomas, T.P. Dendrimer based modular platforms. US20100158850 (**2010**).

[54] Baker, Jr., J.R., Shukla, R., Thomas, T.P. Dendrimer based compositions and methods of using the same. US20090088376 (**2009**).

[55] Baker, Jr., J.R., Cheng, X.-M., Van Der Spek, A.F.L., Huang, B.M., Thomas, T.P. Dendrimer conjugates. US20100160299 (**2010**) & US8445528 (**2013**).

[56] Torchilin VP. Polymeric Micelles as Pharmaceutical Carriers. In: Uchegbu IF, Eds. Polymers in Drug Delivery. 1st ed. Boca Raton, CRC Press; 2006, pp. 111-30.

[57] Jones MC, Leroux JC. Polymeric micelles - a new generation of colloidal drug carriers. Eur J Pharm Biopharm 1999; 48(2): 101-11.

[58] Gad SC. Pharmaceutical Manufacturing Handbook: Production and Processes. 1st ed. Wiley-Interscience: New Jersey 2008.

[59] Matsumura Y. Polymeric micellar delivery systems in oncology. Jpn J Clin Oncol 2008; 38(12): 793-802.

[60] Aliabadi HM, Shahin M, Brocks DR, Lavasanifar A. Disposition of drugs in block copolymer micelle delivery systems: From discovery to recovery. Clin Pharmacokinet 2008; 47(10): 619-34.

[61] Alexis F, Pridgen E, Molnar LK, Farokhzad OC. Factors affecting the clearance and biodistribution of polymeric nanoparticles. Mol Pharm 2008; 5(4): 505-15.

[62] Nishiyama N, Kataoka K. Current state, achievements, and future prospects of polymeric micelles as nanocarriers for drug and gene delivery. Pharmacol Ther 2006; 112(3): 630-48.

[63] Liu J, Lee H, Allen C. Formulation of drugs in block copolymer micelles: Drug loading and release. Curr Pharm Des 2006; 12(36): 4685-701.

[64] Mikhail AS, Allen C. Block copolymer micelles for delivery of cancer therapy: Transport at the whole body, tissue and cellular levels. J Control Release 2009; 138(3): 214-23.

[65] Bagnyukova TV, Serebriiskii IG, Zhou Y, Hopper-Borge EA, Golemis EA, Astsaturov I. Chemotherapy and signaling: How can targeted therapies supercharge cytotoxic agents? Cancer Biol Ther 2010; 10(9): 839-53.

[66] Ajani JA, Fairweather J, Dumas P, Patt YZ, Pazdur R, Mansfield PF. Phase II study of Taxol in patients with advanced gastric carcinoma. Cancer J Sci Am 1998; 4(4): 269-74.

[67] Rogers BB. Taxol: A promising new drug of the '90s. Oncol Nurs Forum 1993; 20(10): 1483-9.

[68] Tarr BD, Yalkowsky SH. A new parenteral vehicle for the administration of some poorly water soluble anti-cancer drugs. J Parenter Sci Technol 1987; 41(1): 31-3.

[69] ten Tije AJ, Verweij J, Loos WJ, Sparreboom A. Pharmacological effects of formulation vehicles: Implications for cancer chemotherapy. Clin Pharmacokinet 2003; 42(7): 665-85.

[70] Shin H-C, Cho H, Lai TC, Kozak KR, Kolesar JM, Kwon GS. Pharmacokinetic study of 3-in-1 poly(ethylene glycol)-block-poly(D, L-lactic acid) micelles carrying paclitaxel, 17-allylamino-17-demethoxygeldanamycin, and rapamycin. J Control Release 2012; 163(1): 93-9.

[71] Chao TC, Chu Z, Tseng LM, Chiou TJ, Hsieh RK, Wang WS, *et al*. Paclitaxel in a novel formulation containing less Cremophor EL as first-line therapy for advanced breast cancer: A phase II trial. Invest New Drugs 2005; 23(2): 171-7.

[72] Gelderblom H, Verweij J, Nooter K, Sparreboom A. Cremophor EL: The drawbacks and advantages of vehicle selection for drug formulation. Eur J Cancer 2001; 37(13): 1590-8.

[73] Friedland D, Gorman G, Treat J. Hypersensitivity reactions from taxol and etoposide. J Natl Cancer Inst 1993; 85(24): 2036.

[74] Ibrahim NK, Desai N, Legha S, Soon-Shiong P, Theriault RL, Rivera E, *et al*. Phase I and pharmacokinetic study of ABI-007, a Cremophor-free, protein-stabilized, nanoparticle formulation of paclitaxel. Clin Cancer Res 2002; 8(5): 1038-44.

[75] Gradishar WJ. Albumin-bound paclitaxel: A next-generation taxane. Expert Opin Pharmacother 2006; 7(8): 1041-53.

[76] Stinchcombe TE. Nanoparticle albumin-bound paclitaxel: A novel Cremphor-EL-free formulation of paclitaxel. Nanomedicine (Lond) 2007; 2(4): 415-23.

[77] Socinski M. Update on nanoparticle albumin-bound paclitaxel. Clin Adv Hematol Oncol 2006; 4(10): 745-6.

[78] Villano JL, Mehta D, Radhakrishnan L. Abraxane induced life-threatening toxicities with metastatic breast cancer and hepatic insufficiency. Invest New Drugs 2006; 24(5): 455-6.

[79] Hennenfent KL, Govindan R. Novel formulations of taxanes: A review. Old wine in a new bottle? Ann Oncol 2006; 17(5): 735-49.

[80] van Oosterom AT, Schrijvers D, Schriivers D. Docetaxel (Taxotere), a review of preclinical and clinical experience. Part II: Clinical experience. Anticancer Drugs 1995; 6(3): 356-68.

[81] Kang, H.-W., Seo, M.H., Kim, B.O. Polymer micelle composition for treatment fo resistant cancer cells. EP2359860 (**2011**).

[82] Deeken JF, Loscher W. The blood-brain barrier and cancer: Transporters, treatment, and Trojan horses. Clin Cancer Res 2007; 13(6): 1663-74.

[83] Lhomme C, Joly F, Walker JL, Lissoni AA, Nicoletto MO, Manikhas GM, *et al*. Phase III study of valspodar (PSC 833) combined with paclitaxel and carboplatin compared with paclitaxel and carboplatin alone in patients with stage IV or suboptimally debulked stage III epithelial ovarian cancer or primary peritoneal cancer. J Clin Oncol 2008; 26(16): 2674-82.

[84] Tiwari AK, Sodani K, Dai CL, Ashby CR, Jr., Chen ZS. Revisiting the ABCs of multidrug resistance in cancer chemotherapy. Curr Pharm Biotechnol 2011; 12(4): 570-94.

[85] Kwon, G.S. Micelle encapsulation of therapeutic agents. US8236329 (**2012**), US20120276171 (**2012**) & US8383136 (**2013**).

[86] Solit DB, Chiosis G. Development and application of Hsp90 inhibitors. Drug Discov Today 2008; 13(1-2): 38-43.

[87] Bagatell R, Gore L, Egorin MJ, Ho R, Heller G, Boucher N, *et al*. Phase I pharmacokinetic and pharmacodynamic study of 17-N-allylamino-17-demethoxygeldanamycin in pediatric patients with recurrent or refractory solid tumors: Aa pediatric oncology experimental therapeutics investigators consortium study. Clin Cancer Res 2007; 13(6): 1783-8.

[88] LoPiccolo J, Blumenthal GM, Bernstein WB, Dennis PA. Targeting the PI3K/Akt/mTOR pathway: Effective combinations and clinical considerations. Drug Resist Updat 2008; 11(1-2): 32-50.

[89] Nguyen DM, Lorang D, Chen GA, Stewart JHt, Tabibi E, Schrump DS. Enhancement of paclitaxel-mediated cytotoxicity in lung cancer cells by 17-allylamino geldanamycin: *In vitro* and *in vivo* analysis. Ann Thorac Surg 2001; 72(2): 371-8; discussion 378-9.

[90] Roforth MM, Tan C. Combination of rapamycin and 17-allylamino-17-demethoxygeldanamycin abrogates Akt activation and potentiates mTOR blockade in breast cancer cells. Anticancer Drugs 2008; 19(7): 681-8.

[91] Hasenstein JR, Shin HC, Kasmerchak K, Buehler D, Kwon GS, Kozak KR. Antitumor activity of triolimus: A novel multidrug-loaded micelle containing paclitaxel, rapamycin, and 17-AAG. Mol Cancer Ther 2012; 11(10): 2233-42.

[92] Burt HM, Zhang X, Toleikis P, Embree L, Hunter WL. Development of copolymers of poly(d,l-lactide) and methoxypolyethylene glycol as micellar carriers of paclitaxel. Colloids Surf B Biointerfaces 1999; 16(1-4): 161-71.

[93] Savic R, Azzam T, Eisenberg A, Maysinger D. Assessment of the integrity of poly(caprolactone)-b-poly(ethylene oxide) micelles under biological conditions: A fluorogenic-based approach. Langmuir 2006; 22(8): 3570-8.

[94] Iijima M, Nagasaki Y, Okada T, Kato M, Kataoka K. Core-polymerized reactive micelles from heterotelechelic amphiphilic block copolymers. Macromolecules 1999; 32(4): 1140-6.

[95] Thurmond KB, Kowalewski T, Wooley KL. Water-soluble knedel-like structures: The preparation of shell-cross-linked small particles. J Am Chem Soc 1996; 118(30): 7239-40.

[96] Rios-Doria J, Carie A, Costich T, Burke B, Skaff H, Panicucci R, *et al*. A versatile polymer micelle drug delivery system for encapsulation and *in vivo* stabilization of hydrophobic anticancer drugs. J Drug Deliv 2012; 2012: 951741.

[97] Kang N, Perron ME, Prud'homme RE, Zhang Y, Gaucher G, Leroux JC. Stereocomplex block copolymer micelles: Core-shell nanostructures with enhanced stability. Nano Lett 2005; 5(2): 315-9.

[98] Lo CL, Huang CK, Lin KM, Hsiue GH. Mixed micelles formed from graft and diblock copolymers for application in intracellular drug delivery. Biomaterials 2007; 28(6): 1225-35.

[99] Hsiue, G.H., Lo, C.L., Lin, S.J., Tsai, H.C. Stable micelles formed with diblock copolymers of critical micelle concentration copolymer and temperature-sensitive copolymer. US8299178 (**2012**).

[100] Lo C-L, Lin S-J, Tsai H-C, Chan W-H, Tsai C-H, Cheng C-HD, *et al*. Mixed micelle systems formed from critical micelle concentration and temperature-sensitive diblock copolymers for doxorubicin delivery. Biomaterials 2009; 30(23-24): 3961-70.

[101] Overberger CG, Kawakami Y. Esterolytic activity of imidazole-containing polymers. Synthesis and characterization of copoly[1-alkyl-4- or 5-vinylimidazole/4(5)-vinylimidazole] and its catalytic activity in the hydrolysis of p-nitrophenyl acetate. J Polym Sci Pol Chem 1978; 16(6): 1237-48.

[102] Haag R, Kratz F. Polymer therapeutics: Concepts and applications. Angew Chem Int Ed Engl 2006; 45(8): 1198-215.

[103] Duncan R. The dawning era of polymer therapeutics. Nat Rev Drug Discov 2003; 2(5): 347-60.

[104] Ogier, J., Doris, E., Lefoulon, F., Arnauld, T. Polymerized micelles. WO2009133325 (**2009**) & AU9241887BB (**2013**).

[105] Doris, E., Duconge, F., Gravel, E., Mackiewicz, N., Tavitian, B. Polymerized micelles for diagnosis. WO2012020337 (**2012**) & EP2603200A1 (**2013**).

[106] Ogier J, Arnauld T, Carrot G, Lhumeau A, Delbos J-M, Boursier C, *et al*. Enhanced drug loading in polymerized micellar cargo. Org Biomol Chem 2010; 8(17): 3902-7.

[107] Gref R, Domb A, Quellec P, Blunk T, Müller RH, Verbavatz JM, *et al*. The controlled intravenous delivery of drugs using PEG-coated sterically stabilized nanospheres. Adv Drug Delivery Rev 2012; 64(supplement): 316-26.

[108] Muehlmann LA, Joanitti GA, Silva JR, Longo JP, Azevedo RB. Liposomal photosensitizers: Potential platforms for anticancer photodynamic therapy. Braz J Med Biol Res 2011; 44(8): 729-37.

[109] Dhankhar R, Vyas SP, Jain AK, Arora S, Rath G, Goyal AK. Advances in novel drug delivery strategies for breast cancer therapy. Artif Cells Blood Substit Immobil Biotechnol 2010; 38(5): 230-49.

[110] Huwyler J, Drewe J, Krahenbuhl S. Tumor targeting using liposomal antineoplastic drugs. Int J Nanomedicine 2008; 3(1): 21-9.

[111] Torchilin VP. Recent advances with liposomes as pharmaceutical carriers. Nat Rev Drug Discov 2005; 4(2): 145-60.

[112] Kim CK, Lim SJ. Recent progress in drug delivery systems for anticancer agents. Arch Pharm Res 2002; 25(3): 229-39.

[113] Park JW. Liposome-based drug delivery in breast cancer treatment. Breast Cancer Res 2002; 4(3): 95-9.

[114] Mackiewicz N, Gravel E, Garofalakis A, Ogier J, John J, Dupont DM, *et al*. Tumor-targeted polydiacetylene micelles for *in vivo* imaging and drug delivery. Small 2011; 7(19): 2786-92.

[115] Gravel E, Ogier J, Arnauld T, Mackiewicz N, Duconge F, Doris E. Drug delivery and imaging with polydiacetylene micelles. Chemistry 2012; 18(2): 400-8.

[116] Cailleau R, Young R, Olive M, Reeves WJ, Jr. Breast tumor cell lines from pleural effusions. J Natl Cancer Inst 1974; 53(3): 661-74.

[117] Puri A, Loomis K, Smith B, Lee JH, Yavlovich A, Heldman E, *et al*. Lipid-based nanoparticles as pharmaceutical drug carriers: From concepts to clinic. Crit Rev Ther Drug Carrier Syst 2009; 26(6): 523-80.

[118] Martins S, Sarmento B, Ferreira DC, Souto EB. Lipid-based colloidal carriers for peptide and protein delivery--liposomes versus lipid nanoparticles. Int J Nanomedicine 2007; 2(4): 595-607.

[119] Sajja HK, East MP, Mao H, Wang YA, Nie S, Yang L. Development of multifunctional nanoparticles for targeted drug delivery and noninvasive imaging of therapeutic effect. Curr Drug Discov Technol 2009; 6(1): 43-51.

[120] Felber AE, Dufresne MH, Leroux JC. pH-sensitive vesicles, polymeric micelles, and nanospheres prepared with polycarboxylates. Adv Drug Deliv Rev 2012; 64(11): 979-92.

[121] de Leeuw J, de Vijlder HC, Bjerring P, Neumann HA. Liposomes in dermatology today. J Eur Acad Dermatol Venereol 2009; 23(5): 505-16.

[122] Slingerland M, Guchelaar HJ, Gelderblom H. Liposomal drug formulations in cancer therapy: 15 Years along the road. Drug Discov Today 2012; 17(3-4): 160-6.

[123] Samad A, Sultana Y, Aqil M. Liposomal drug delivery systems: An update review. Curr Drug Deliv 2007; 4(4): 297-305.

[124] Sapra P, Allen TM. Ligand-targeted liposomal anticancer drugs. Prog Lipid Res 2003; 42(5): 439-62.

[125] Torchilin VP. Fluorescence microscopy to follow the targeting of liposomes and micelles to cells and their intracellular fate. Adv Drug Deliv Rev 2005; 57(1): 95-109.

[126] Bangham AD, Standish MM, Watkins JC. Diffusion of univalent ions across the lamellae of swollen phospholipids. J Mol Biol 1965; 13(1): 238-52.

[127] Akbarzadeh A, Rezaei-Sadabady R, Davaran S, Joo SW, Zarghami N, Hanifehpour Y, *et al*. Liposome: Classification, preparation, and applications. Nanoscale Res Lett 2013; 8(1): 102.

[128] Meireles Batista C, Moraes Barros de Carvalho C, Santos Magalhães N. Lipossomas e suas aplicações terapêuticas: Estado da arte. Braz J Pharm Sci 2007; 43(2): 167-79.

[129] Allen TM, Cheng WW, Hare JI, Laginha KM. Pharmacokinetics and pharmacodynamics of lipidic nano-particles in cancer. Anticancer Agents Med Chem 2006; 6(6): 513-23.

[130] Mamot C, Drummond DC, Hong K, Kirpotin DB, Park JW. Liposome-based approaches to overcome anticancer drug resistance. Drug Resist Updat 2003; 6(5): 271-9.

[131] Bogner JR, Kronawitter U, Rolinski B, Truebenbach K, Goebel FD. Liposomal doxorubicin in the treatment of advanced AIDS-related Kaposi sarcoma. J Acquir Immune Defic Syndr 1994; 7(5): 463-8.

[132] Barenholz Y. Doxil(R)--the first FDA-approved nano-drug: Lessons learned. J Control Release 2012; 160(2): 117-34.

[133] Kurzrock, R., Li, L., Mehta, K., Aggarawal, B.B. Liposomal curcumin for treatment of cancer. US7968115 (**2011**).

[134] Yallapu MM, Jaggi M, Chauhan SC. Curcumin nanoformulations: A future nanomedicine for cancer. Drug Discov Today 2012; 17(1-2): 71-80.

[135] Maheshwari RK, Singh AK, Gaddipati J, Srimal RC. Multiple biological activities of curcumin: A short review. Life Sci 2006; 78(18): 2081-7.

[136] Araujo CC, Leon LL. Biological activities of *Curcuma longa* L. Mem Inst Oswaldo Cruz 2001; 96(5): 723-8.

[137] Hatcher H, Planalp R, Cho J, Torti FM, Torti SV. Curcumin: From ancient medicine to current clinical trials. Cell Mol Life Sci 2008; 65(11): 1631-52.

[138] Yu L, Mohamed AJ, Simonson OE, Vargas L, Blomberg KE, Bjorkstrand B, *et al*. Proteasome-dependent autoregulation of Bruton tyrosine kinase (Btk) promoter via NF-kappaB. Blood 2008; 111(9): 4617-26.

[139] Kunnumakkara AB, Anand P, Aggarwal BB. Curcumin inhibits proliferation, invasion, angiogenesis and metastasis of different cancers through interaction with multiple cell signaling proteins. Cancer Lett 2008; 269(2): 199-225.

[140] Landis-Piwowar KR, Milacic V, Chen D, Yang H, Zhao Y, Chan TH, *et al*. The proteasome as a potential target for novel anticancer drugs and chemosensitizers. Drug Resist Updat 2006; 9(6): 263-73.

[141] Page P, Yang LX. Novel chemoradiosensitizers for cancer therapy. Anticancer Res 2010; 30(9): 3675-82.

[142] Yallapu MM, Maher DM, Sundram V, Bell MC, Jaggi M, Chauhan SC. Curcumin induces chemo/radio-sensitization in ovarian cancer cells and curcumin nanoparticles inhibit ovarian cancer cell growth. J Ovarian Res 2010; 3: 11.

[143] Um Y, Cho S, Woo HB, Kim YK, Kim H, Ham J, *et al*. Synthesis of curcumin mimics with multidrug resistance reversal activities. Bioorg Med Chem 2008; 16(7): 3608-15.

[144] Ganta S, Amiji M. Coadministration of Paclitaxel and curcumin in nanoemulsion formulations to overcome multidrug resistance in tumor cells. Mol Pharm 2009; 6(3): 928-39.

[145] Ganta S, Devalapally H, Amiji M. Curcumin enhances oral bioavailability and anti-tumor therapeutic efficacy of paclitaxel upon administration in nanoemulsion formulation. J Pharm Sci 2010; 99(11): 4630-41.

[146] Leslie EM, Deeley RG, Cole SPC. Multidrug resistance proteins: Role of P-glycoprotein, MRP1, MRP2, and BCRP (ABCG2) in tissue defense. Toxicol Appl Pharmacol 2005; 204(3): 216-37.

[147] Marquez B, Van Bambeke F. ABC multidrug transporters: Target for modulation of drug pharmacokinetics and drug-drug interactions. Curr Drug Targets 2011; 12(5): 600-20.

[148] Lage H, Dietel M. Effect of the breast-cancer resistance protein on atypical multidrug resistance. Lancet Oncol 2000; 1(3): 169-75.

[149] Goel A, Kunnumakkara AB, Aggarwal BB. Curcumin as "Curecumin": From kitchen to clinic. Biochem Pharmacol 2008; 75(4): 787-809.

[150] Metzler M, Pfeiffer E, Schulz SI, Dempe JS. Curcumin uptake and metabolism. BioFactors 2013; 39(1): 14-20.

[151] Aggarwal BB, Sundaram C, Malani N, Ichikawa H. Curcumin: The Indian solid gold. Adv Exp Med Biol 2007; 595: 1-75.

[152] Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of curcumin: Problems and promises. Mol Pharm 2007; 4(6): 807-18.

[153] Muqbil I, Masood A, Sarkar FH, Mohammad RM, Azmi AS. Progress in nanotechnology based approaches to enhance the potential of chemopreventive agents. Cancers 2011; 3(1): 428-45.

[154] Lee KD, Hong K, Papahadjopoulos D. Recognition of liposomes by cells: *In vitro* binding and endocytosis mediated by specific lipid headgroups and surface charge density. Biochim Biophys Acta 1992; 1103(2): 185-97.

[155] Zhi D, Zhang S, Wang B, Zhao Y, Yang B, Yu S. Transfection efficiency of cationic lipids with different hydrophobic domains in gene delivery. Bioconjug Chem 2010; 21(4): 563-77.

[156] Harashima H, Ishida T, Kamiya H, Kiwada H. Pharmacokinetics of targeting with liposomes. Crit Rev Ther Drug Carrier Syst 2002; 19(3): 235-75.

[157] Bao A, Goins B, Klipper R, Negrete G, Phillips WT. Direct 99mTc labeling of pegylated liposomal doxorubicin (Doxil) for pharmacokinetic and non-invasive imaging studies. J Pharmacol Exp Ther 2004; 308(2): 419-25.

[158] Bao A, Goins B, Klipper R, Negrete G, Phillips WT. 186Re-liposome labeling using 186Re-SNS/S complexes: *In vitro* stability, imaging, and biodistribution in rats. J Nucl Med 2003; 44(12): 1992-9.

[159] Chen MH, Chang CH, Chang YJ, Chen LC, Yu CY, Wu YH, *et al*. MicroSPECT/CT imaging and pharmacokinetics of 188Re-(DXR)-liposome in human colorectal adenocarcinoma-bearing mice. Anticancer Res 2010; 30(1): 65-72.

[160] Harrington KJ, Lewanski CR, Stewart JS. Liposomes as vehicles for targeted therapy of cancer. Part 1: Preclinical development. Clin Oncol (R Coll Radiol) 2000; 12(1): 2-15.

[161] Harrington KJ, Lewanski CR, Stewart JS. Liposomes as vehicles for targeted therapy of cancer. Part 2: Clinical development. Clin Oncol (R Coll Radiol) 2000; 12(1): 16-24.

[162] Knapp FF, Jr. Rhenium-188--a generator-derived radioisotope for cancer therapy. Cancer Biother Radiopharm 1998; 13(5): 337-49.

[163] Torres LA, Coca MA, Batista JF, Casaco A, Lopez G, Garcia I, *et al*. Biodistribution and internal dosimetry of the 188Re-labelled humanized monoclonal antibody anti-epidemal growth factor receptor, nimotuzumab, in the locoregional treatment of malignant gliomas. Nucl Med Commun 2008; 29(1): 66-75.

[164] Miao Y, Quinn TP. Peptide-targeted radionuclide therapy for melanoma. Crit Rev Oncol Hematol 2008; 67(3): 213-28.

[165] Chang YJ, Chang CH, Chang TJ, Yu CY, Chen LC, Jan ML, *et al*. Biodistribution, pharmacokinetics and microSPECT/CT imaging of 188Re-bMEDA-liposome in a C26 murine colon carcinoma solid tumor animal model. Anticancer Res 2007; 27(4B): 2217-25.

[166] Iznaga-Escobar N. 188Re-direct labeling of monoclonal antibodies for radioimmunotherapy of solid tumors: Biodistribution, normal organ dosimetry, and toxicology. Nucl Med Biol 1998; 25(5): 441-7.

[167] Chen LC, Chang CH, Yu CY, Chang YJ, Hsu WC, Ho CL, *et al*. Biodistribution, pharmacokinetics and imaging of (188)Re-BMEDA-labeled pegylated liposomes after intraperitoneal injection in a C26 colon carcinoma ascites mouse model. Nucl Med Biol 2007; 34(4): 415-23.

[168] Choy H, Kim DW. Chemotherapy and irradiation interaction. Semin Oncol 2003; 30(4 Suppl 9): 3-10.

[169] Huber PE, Bischof M, Jenne J, Heiland S, Peschke P, Saffrich R, *et al*. Trimodal cancer treatment: Beneficial effects of combined antiangiogenesis, radiation, and chemotherapy. Cancer Res 2005; 65(9): 3643-55.

[170] Ogihara-Umeda I, Sasaki T, Kojima S, Nishigori H. Optimal radiolabeled liposomes for tumor imaging. J Nucl Med 1996; 37(2): 326-32.

[171] Hafeli U, Tiefenauer LX, Schbiger PA, Weder HG. A lipophilic complex with 186Re/188Re incorporated in liposomes suitable for radiotherapy. Int J Rad Appl Instrum B 1991; 18(5): 449-54.

[172] Chiu, S.P., Lee, T.W., Yu, C.Y., Ho, C.L., Hsu, W.C., Chang, Y.J. One pot processes of preparing multifunctional liposome drug for imaging, delivery and targeting in cancer diagnosis and therapy. US20110158903 (**2011**).

[173] Okarvi SM, Jammaz IA. Preparation and evaluation of bombesin peptide derivatives as potential tumor imaging agents: Effects of structure and composition of amino acid sequence on *in vitro* and *in vivo* characteristics. Nucl Med Biol 2012; 39(6): 795-804.

[174] Accardo A, Salsano G, Morisco A, Aurilio M, Parisi A, Maione F, *et al*. Peptide-modified liposomes for selective targeting of bombesin receptors overexpressed by cancer cells: A potential theranostic agent. Int J Nanomedicine 2012; 7: 2007-17.

[175] Gonzalez N, Moody TW, Igarashi H, Ito T, Jensen RT. Bombesin-related peptides and their receptors: Recent advances in their role in physiology and disease states. Curr Opin Endocrinol Diabetes Obes 2008; 15(1): 58-64.

[176] Overgaard J. Hypoxic radiosensitization: Adored and ignored. J Clin Oncol 2007; 25(26): 4066-74.

[177] Zheng Y, Hunting DJ, Ayotte P, Sanche L. Radiosensitization of DNA by gold nanoparticles irradiated with high-energy electrons. Radiat Res 2008; 169(1): 19-27.

[178] Sanche, L., Charest, G. Compositions comprising a radiosensitizer and an anti-cancer agent and methods of uses thereof. US20120093918 (**2012**).

[179] Caraglia M, Marra M, Naviglio S, Botti G, Addeo R, Abbruzzese A. Zoledronic acid: An unending tale for an antiresorptive agent. Expert Opin Pharmacother 2010; 11(1): 141-54.

[180] Hughes DE, Wright KR, Uy HL, Sasaki A, Yoneda T, Roodman GD, *et al*. Bisphosphonates promote apoptosis in murine osteoclasts *in vitro* and *in vivo*. J Bone Miner Res 1995; 10(10): 1478-87.

[181] Lipton A, Zheng M, Seaman J. Zoledronic acid delays the onset of skeletal-related events and progression of skeletal disease in patients with advanced renal cell carcinoma. Cancer 2003; 98(5): 962-9.

[182] Lee MV, Fong EM, Singer FR, Guenette RS. Bisphosphonate treatment inhibits the growth of prostate cancer cells. Cancer Res 2001; 61(6): 2602-8.

[183] Senaratne SG, Pirianov G, Mansi JL, Arnett TR, Colston KW. Bisphosphonates induce apoptosis in human breast cancer cell lines. Br J Cancer 2000; 82(8): 1459-68.

[184] Riebeling C, Forsea AM, Raisova M, Orfanos CE, Geilen CC. The bisphosphonate pamidronate induces apoptosis in human melanoma cells *in vitro*. Br J Cancer 2002; 87(3): 366-71.

[185] Mackie PS, Fisher JL, Zhou H, Choong PF. Bisphosphonates regulate cell growth and gene expression in the UMR 106-01 clonal rat osteosarcoma cell line. Br J Cancer 2001; 84(7): 951-8.

[186] Sonnemann J, Eckervogt V, Truckenbrod B, Boos J, Winkelmann W, van Valen F. The bisphosphonate pamidronate is a potent inhibitor of human osteosarcoma cell growth *in vitro*. Anticancer Drugs 2001; 12(5): 459-65.

[187] Shipman CM, Rogers MJ, Apperley JF, Russell RG, Croucher PI. Bisphosphonates induce apoptosis in human myeloma cell lines: A novel anti-tumour activity. Br J Haematol 1997; 98(3): 665-72.

[188] Aparicio A, Gardner A, Tu Y, Savage A, Berenson J, Lichtenstein A. *In vitro* cytoreductive effects on multiple myeloma cells induced by bisphosphonates. Leukemia 1998; 12(2): 220-9.

[189] Tassone P, Tagliaferri P, Rossi M, Calimeri T, Bulotta A, Abbruzzese A, *et al*. Challenging the current approaches to multiple myeloma-related bone disease: From bisphosphonates to target therapy. Curr Cancer Drug Targets 2009; 9(7): 854-70.

[190] Caraglia M, Budillon A, Tagliaferri P, Marra M, Abbruzzese A, Caponigro F. Isoprenylation of intracellular proteins as a new target for the therapy of human neoplasms: Preclinical and clinical implications. Curr Drug Targets 2005; 6(3): 301-23.

[191] Raikkonen J, Crockett JC, Rogers MJ, Monkkonen H, Auriola S, Monkkonen J. Zoledronic acid induces formation of a pro-apoptotic ATP analogue and isopentenyl pyrophosphate in osteoclasts *in vivo* and in MCF-7 cells *in vitro*. Br J Pharmacol 2009; 157(3): 427-35.

[192] Senaratne SG, Mansi JL, Colston KW. The bisphosphonate zoledronic acid impairs Ras membrane [correction of impairs membrane] localisation and induces cytochrome c release in breast cancer cells. Br J Cancer 2002; 86(9): 1479-86.

[193] Sewing L, Steinberg F, Schmidt H, Goke R. The bisphosphonate zoledronic acid inhibits the growth of HCT-116 colon carcinoma cells and induces tumor cell apoptosis. Apoptosis 2008; 13(6): 782-9.

[194] Boissier S, Ferreras M, Peyruchaud O, Magnetto S, Ebetino FH, Colombel M, *et al*. Bisphosphonates inhibit breast and prostate carcinoma cell invasion, an early event in the formation of bone metastases. Cancer Res 2000; 60(11): 2949-54.

[195] Wood J, Bonjean K, Ruetz S, Bellahcene A, Devy L, Foidart JM, *et al*. Novel antiangiogenic effects of the bisphosphonate compound zoledronic acid. J Pharmacol Exp Ther 2002; 302(3): 1055-61.

[196] Salzano G, Marra M, Porru M, Zappavigna S, Abbruzzese A, La Rotonda MI, *et al*. Self-assembly nanoparticles for the delivery of bisphosphonates into tumors. Int J Pharm 2011; 403(1-2): 292-7.

[197] Marra M, Abbruzzese A, Addeo R, Del Prete S, Tassone P, Tonini G, *et al*. Cutting the limits of aminobisphosphonates: New strategies for the potentiation of their anti-tumour effects. Curr Cancer Drug Targets 2009; 9(7): 791-800.

[198] Chen T, Berenson J, Vescio R, Swift R, Gilchick A, Goodin S, *et al*. Pharmacokinetics and pharmacodynamics of zoledronic acid in cancer patients with bone metastases. J Clin Pharmacol 2002; 42(11): 1228-36.

[199] Skerjanec A, Berenson J, Hsu C, Major P, Miller WH, Jr., Ravera C, *et al*. The pharmacokinetics and pharmacodynamics of zoledronic acid in cancer patients with varying degrees of renal function. J Clin Pharmacol 2003; 43(2): 154-62.

[200] Weiss HM, Pfaar U, Schweitzer A, Wiegand H, Skerjanec A, Schran H. Biodistribution and plasma protein binding of zoledronic acid. Drug Metab Dispos 2008; 36(10): 2043-9.

[201] Scheper MA, Badros A, Chaisuparat R, Cullen KJ, Meiller TF. Effect of zoledronic acid on oral fibroblasts and epithelial cells: A potential mechanism of bisphosphonate-associated osteonecrosis. Br J Haematol 2009; 144(5): 667-76.

[202] Marra M, Salzano G, Leonetti C, Tassone P, Scarsella M, Zappavigna S, *et al*. Nanotechnologies to use bisphosphonates as potent anticancer agents: The effects of zoledronic acid encapsulated into liposomes. Nanomedicine 2011; 7(6): 955-64.

[203] Marra M, Salzano G, Leonetti C, Porru M, Franco R, Zappavigna S, *et al*. New self-assembly nanoparticles and stealth liposomes for the delivery of zoledronic acid: A comparative study. Biotechnol Adv 2012; 30(1): 302-9.

[204] Longmire M, Choyke PL, Kobayashi H. Clearance properties of nano-sized particles and molecules as imaging agents: considerations and caveats. Nanomedicine (Lond) 2008; 3(5): 703-17.

[205] Choi HS, Liu W, Liu F, Nasr K, Misra P, Bawendi MG, *et al*. Design considerations for tumour-targeted nanoparticles. Nat Nanotechnol 2010; 5(1): 42-7.

[206] van Rooijen N, van Kesteren-Hendrikx E. Clodronate liposomes: Perspectives in research and therapeutics. J Liposome Res 2002; 12(1-2): 81-94.

[207] Danenberg HD, Golomb G, Groothuis A, Gao J, Epstein H, Swaminathan RV, *et al*. Liposomal alendronate inhibits systemic innate immunity and reduces in-stent neointimal hyperplasia in rabbits. Circulation 2003; 108(22): 2798-804.

[208] Abbruzzese, A., Caraglia, M., De Rosa, G., La Rotonda, M.I., Marra, M., Salzano, G., Tassone, P. Uso dell' acido zoledronico per la preparazione di formulazioni farmaceutiche per il trattamento del cancro della prostata e del mieloma multiplo. ITFI20090190 (**2009**).

[209] De Rosa, G., Caraglia, M., Tassone, P., La Rotonda, M.I., Abbruzzese, A., Salzano, G., Marra, M. Leonetti, C. Self-assembling nanoparticles for the release of bisphosphonates in the treatment of human cancers. WO2012042024 (**2012**) & EP2621539A1 (**2013**).

[210] Parr MJ, Masin D, Cullis PR, Bally MB. Accumulation of liposomal lipid and encapsulated doxorubicin in murine Lewis lung carcinoma: The lack of beneficial effects by coating liposomes with poly(ethylene glycol). J Pharmacol Exp Ther 1997; 280(3): 1319-27.

[211] Bandak S, Goren D, Horowitz A, Tzemach D, Gabizon A. Pharmacological studies of cisplatin encapsulated in long-circulating liposomes in mouse tumor models. Anticancer Drugs 1999; 10(10): 911-20.

[212] Zamboni WC, Gervais AC, Egorin MJ, Schellens JH, Zuhowski EG, Pluim D, *et al*. Systemic and tumor disposition of platinum after administration of cisplatin or STEALTH liposomal-cisplatin formulations (SPI-077 and SPI-077 B103) in a preclinical tumor model of melanoma. Cancer Chemother Pharmacol 2004; 53(4): 329-36.

[213] Harrington KJ, Lewanski CR, Northcote AD, Whittaker J, Wellbank H, Vile RG, *et al*. Phase I-II study of pegylated liposomal cisplatin (SPI-077) in patients with inoperable head and neck cancer. Ann Oncol 2001; 12(4): 493-6.

[214] Li C, Wang Y, Zhang X, Deng L, Zhang Y, Chen Z. Tumor-targeted liposomal drug delivery mediated by a diseleno bond-stabilized cyclic peptide. Int J Nanomedicine 2013; 8: 1051-62.

[215] Sharma G, Modgil A, Layek B, Arora K, Sun C, Law B, *et al*. Cell penetrating peptide tethered bi-ligand liposomes for delivery to brain *in vivo*: Biodistribution and transfection. J Control Release 2013; 167(1): 1-10.

[216] Mo R, Sun Q, Li N, Zhang C. Intracellular delivery and antitumor effects of pH-sensitive liposomes based on zwitterionic oligopeptide lipids. Biomaterials 2013; 34(11): 2773-86.

[217] Wehunt MP, Winschel CA, Khan AK, Guo TL, Abdrakhmanova GR, Sidorov V. Controlled drug-release system based on pH-sensitive chloride-triggerable liposomes. J Liposome Res 2013; 23(1): 37-46.

[218] Andresen TL, Davidsen J, Begtrup M, Mouritsen OG, Jorgensen K. Enzymatic release of antitumor ether lipids by specific phospholipase A2 activation of liposome-forming prodrugs. J Med Chem 2004; 47(7): 1694-703.

[219] Shum P, Kim JM, Thompson DH. Phototriggering of liposomal drug delivery systems. Adv Drug Deliv Rev 2001; 53(3): 273-84.

[220] Unger EC, McCreery TP, Sweitzer RH, Caldwell VE, Wu Y. Acoustically active lipospheres containing paclitaxel: A new therapeutic ultrasound contrast agent. Invest Radiol 1998; 33(12): 886-92.

[221] Needham D, Dewhirst MW. The development and testing of a new temperature-sensitive drug delivery system for the treatment of solid tumors. Adv Drug Deliv Rev 2001; 53(3): 285-305.

[222] Needham D, Anyarambhatla G, Kong G, Dewhirst MW. A new temperature-sensitive liposome for use with mild hyperthermia: Characterization and testing in a human tumor xenograft model. Cancer Res 2000; 60(5): 1197-201.

[223] Kong G, Braun RD, Dewhirst MW. Hyperthermia enables tumor-specific nanoparticle delivery: Effect of particle size. Cancer Res 2000; 60(16): 4440-5.

[224] Kong G, Braun RD, Dewhirst MW. Characterization of the effect of hyperthermia on nanoparticle extravasation from tumor vasculature. Cancer Res 2001; 61(7): 3027-32.

[225] Gaber MH, Wu NZ, Hong K, Huang SK, Dewhirst MW, Papahadjopoulos D. Thermosensitive liposomes: Extravasation and release of contents in tumor microvascular networks. Int J Radiat Oncol Biol Phys 1996; 36(5): 1177-87.

[226] Dewhirst MW, Prosnitz L, Thrall D, Prescott D, Clegg S, Charles C, *et al*. Hyperthermic treatment of malignant diseases: Current status and a view toward the future. Semin Oncol 1997; 24(6): 616-25.

[227] Kong G, Anyarambhatla G, Petros WP, Braun RD, Colvin OM, Needham D, *et al*. Efficacy of liposomes and hyperthermia in a human tumor xenograft model: Importance of triggered drug release. Cancer Res 2000; 60(24): 6950-7.

[228] Hahn GM, Braun J, Har-Kedar I. Thermochemotherapy: Synergism between hyperthermia (42-43 degrees) and adriamycin (of bleomycin) in mammalian cell inactivation. Proc Natl Acad Sci USA 1975; 72(3): 937-40.

[229] Herman TS. Temperature dependence of adriamycin, cis-diamminedichloroplatinum, bleomycin, and 1,3-bis(2-chloroethyl)-1-nitrosourea cytotoxicity *in vitro*. Cancer Res 1983; 43(2): 517-20.

[230] Wiedemann GJ, Robins HI, Katschinski DM, Mentzel M, D'Oleire F, Kutz M, *et al*. Systemic hyperthermia and ICE chemotherapy for sarcoma patients: Rationale and clinical status. Anticancer Res 1997; 17(4B): 2899-902.

[231] Yatvin MB, Weinstein JN, Dennis WH, Blumenthal R. Design of liposomes for enhanced local release of drugs by hyperthermia. Science 1978; 202(4374): 1290-3.

[232] Weinstein JN, Magin RL, Cysyk RL, Zaharko DS. Treatment of solid L1210 murine tumors with local hyperthermia and temperature-sensitive liposomes containing methotrexate. Cancer Res 1980; 40(5): 1388-95.

[233] Tacker JR, Anderson RU. Delivery of antitumor drug to bladder cancer by use of phase transition liposomes and hyperthermia. J Urol 1982; 127(6): 1211-4.

[234] Zou YY, Ueno M, Yamagishi M, Horikoshi I, Yamashita I, Tazawa K, *et al*. Targeting behavior of hepatic artery injected temperature sensitive liposomal adriamycin on tumor-bearing rats. Sel Cancer Ther 1990; 6(3): 119-27.

[235] Chiu GN, Abraham SA, Ickenstein LM, Ng R, Karlsson G, Edwards K, *et al*. Encapsulation of doxorubicin into thermosensitive liposomes via complexation with the transition metal manganese. J Control Release 2005; 104(2): 271-88.

[236] Weinstein JN, Magin RL, Yatvin MB, Zaharko DS. Liposomes and local hyperthermia: Selective delivery of methotrexate to heated tumors. Science 1979; 204(4389): 188-91.

[237] Maruyama K, Unezaki S, Takahashi N, Iwatsuru M. Enhanced delivery of doxorubicin to tumor by long-circulating thermosensitive liposomes and local hyperthermia. Biochim Biophys Acta 1993; 1149(2): 209-16.

[238] Unezaki S, Maruyama K, Takahashi N, Koyama M, Yuda T, Suginaka A, *et al*. Enhanced delivery and antitumor activity of doxorubicin using long-circulating thermosensitive liposomes containing amphipathic polyethylene glycol in combination with local hyperthermia. Pharm Res 1994; 11(8): 1180-5.

[239] Dos Santos N, Mayer LD, Abraham SA, Gallagher RC, Cox KA, Tardi PG, *et al*. Improved retention of idarubicin after intravenous injection obtained for cholesterol-free liposomes. Biochim Biophys Acta 2002; 1561(2): 188-201.

[240] Anyarambhatla GR, Needham D. Enhancement of the phase transition permeability of DPPC liposomes by incorporation of MPPC: A new temperature-sensitive liposome for use with mild hyperthermia. J Liposome Res 1999; 9(4): 491-506.

[241] Xingguo, M., Qingwei, J., Weiping, Y. Novel thermosensitive liposomes containing therapeutic agents. US20110200665 (**2011**) & EP2217209A4 (**2013**).

[242] Li, S.-D., Tagami, T., Ernsting, M.J. Thermosensitive liposomes. WO2012055020 (**2012**).

[243] Pardakhty A, Varshosaz J, Rouholamini A. *In vitro* study of polyoxyethylene alkyl ether niosomes for delivery of insulin. Int J Pharm 2007; 328(2): 130-41.

[244] Liu K, Sun J, Wang Y, He Y, Gao K, He Z. Preparation and characterization of 10-hydroxycamptothecin loaded nanostructured lipid carriers. Drug Dev Ind Pharm 2008; 34(5): 465-71.

[245] Pietkiewicz J, Zielinska K, Saczko J, Kulbacka J, Majkowski M, Wilk KA. New approach to hydrophobic cyanine-type photosensitizer delivery using polymeric oil-cored nanocarriers: hemolytic activity, *in vitro* cytotoxicity and localization in cancer cells. Eur J Pharm Sci 2010; 39(5): 322-35.

[246] Davis SS, Burnham WR, Wilson P, O'Brien J. Use of adjuvants for enhancement of rectal absorption of cefoxitin in humans. Antimicrob Agents Chemother 1985; 28(2): 211-5.

[247] Hauck ML, LaRue SM, Petros WP, Poulson JM, Yu D, Spasojevic I, *et al*. Phase I trial of doxorubicin-containing low temperature sensitive liposomes in spontaneous canine tumors. Clin Cancer Res 2006; 12(13): 4004-10.

[248] Poon RT, Borys N. Lyso-thermosensitive liposomal doxorubicin: A novel approach to enhance efficacy of thermal ablation of liver cancer. Expert Opin Pharmacother 2009; 10(2): 333-43.

[249] Dromi S, Frenkel V, Luk A, Traughber B, Angstadt M, Bur M, *et al*. Pulsed-high intensity focused ultrasound and low temperature-sensitive liposomes for enhanced targeted drug delivery and antitumor effect. Clin Cancer Res 2007; 13(9): 2722-7.

[250] Yarmolenko PS, Zhao Y, Landon C, Spasojevic I, Yuan F, Needham D, *et al*. Comparative effects of thermosensitive doxorubicin-containing liposomes and hyperthermia in human and murine tumours. Int J Hyperthermia 2010; 26(5): 485-98.

[251] Banno B, Ickenstein LM, Chiu GN, Bally MB, Thewalt J, Brief E, *et al*. The functional roles of poly(ethylene glycol)-lipid and lysolipid in the drug retention and release from lysolipid-containing thermosensitive liposomes *in vitro* and *in vivo*. J Pharm Sci 2010; 99(5): 2295-308.

[252] Mills JK, Needham D. Lysolipid incorporation in dipalmitoylphosphatidylcholine bilayer membranes enhances the ion permeability and drug release rates at the membrane phase transition. Biochim Biophys Acta 2005; 15(2): 77-96.

[253] de Smet M, Langereis S, van den Bosch S, Grull H. Temperature-sensitive liposomes for doxorubicin delivery under MRI guidance. J Control Release 2010; 143(1): 120-7.

[254] Woo J, Chiu GN, Karlsson G, Wasan E, Ickenstein L, Edwards K, *et al*. Use of a passive equilibration methodology to encapsulate cisplatin into preformed thermosensitive liposomes. Int J Pharm 2008; 349(1-2): 38-46.

[255] Allen TM, Sapra P, Moase E. Use of the post-insertion method for the formation of ligand-coupled liposomes. Cell Mol Biol Lett 2002; 7(3): 889-94.

[256] Brown SL, Li XL, Pai HH, Worthington AE, Hill RP, Hunt JW. Observations of thermal gradients in perfused tissues during water bath heating. Int J Hyperthermia 1992; 8(2): 275-87.

[257] Wust P, Nadobny J, Szimtenings M, Stetter E, Gellermann J. Implications of clinical RF hyperthermia on protection limits in the RF range. Health Phys 2007; 92(6): 565-73.

[258] Chopra R, Curiel L, Staruch R, Morrison L, Hynynen K. An MRI-compatible system for focused ultrasound experiments in small animal models. Med Phys 2009; 36(5): 1867-74.

[259] Ruoslahti E. RGD and other recognition sequences for integrins. Annu Rev Cell Dev Biol 1996; 12: 697-715.

[260] Dong X, Mattingly CA, Tseng M, Cho M, Adams VR, Mumper RJ. Development of new lipid-based paclitaxel nanoparticles using sequential simplex optimization. Eur J Pharm Biopharm 2009; 72(1): 9-17.

[261] Ponce AM, Viglianti BL, Yu D, Yarmolenko PS, Michelich CR, Woo J, *et al*. Magnetic resonance imaging of temperature-sensitive liposome release: Drug dose painting and antitumor effects. J Natl Cancer Inst 2007; 99(1): 53-63.

[262] Peller M, Schwerdt A, Hossann M, Reinl HM, Wang T, Sourbron S, *et al*. MR characterization of mild hyperthermia-induced gadodiamide release from thermosensitive liposomes in solid tumors. Invest Radiol 2008; 43(12): 877-92.

[263] Abraham SA, Edwards K, Karlsson G, MacIntosh S, Mayer LD, McKenzie C, *et al*. Formation of transition metal-doxorubicin complexes inside liposomes. Biochim Biophys Acta 2002; 1565(1): 41-54.

[264] Haran G, Cohen R, Bar LK, Barenholz Y. Transmembrane ammonium sulfate gradients in liposomes produce efficient and stable entrapment of amphipathic weak bases. Biochim Biophys Acta 1993; 19(2): 201-15.

[265] Maurer-Spurej E, Wong KF, Maurer N, Fenske DB, Cullis PR. Factors influencing uptake and retention of amino-containing drugs in large unilamellar vesicles exhibiting transmembrane pH gradients. Biochim Biophys Acta 1999; 12: 1-2.

[266] Hong, K., Drummond, D.C., Kirpotin, D. Liposomes useful for drug delivery. US8329213 (**2012**) & AU13207554A1 (**2013**).

[267] Mamot C, Drummond DC, Greiser U, Hong K, Kirpotin DB, Marks JD, *et al*. Epidermal growth factor receptor (EGFR)-targeted immunoliposomes mediate specific and efficient drug delivery to EGFR- and EGFRvIII-overexpressing tumor cells. Cancer Res 2003; 63(12): 3154-61.

[268] Poul MA, Becerril B, Nielsen UB, Morisson P, Marks JD. Selection of tumor-specific internalizing human antibodies from phage libraries. J Mol Biol 2000; 301(5): 1149-61.

[269] Neve RM, Nielsen UB, Kirpotin DB, Poul MA, Marks JD, Benz CC. Biological effects of anti-ErbB2 single chain antibodies selected for internalizing function. Biochem Biophys Res Commun 2001; 280(1): 274-9.

[270] Ali HM, Urbinati G, Raouane M, Massaad-Massade L. Significance and applications of nanoparticles in siRNA delivery for cancer therapy. Expert Rev Clin Pharmacol 2012; 5(4): 403-12.

[271] Barros SA, Gollob JA. Safety profile of RNAi nanomedicines. Adv Drug Deliv Rev 2012; 64(15): 1730-7.

[272] Bora RS, Gupta D, Mukkur TK, Saini KS. RNA interference therapeutics for cancer: Challenges and opportunities (review). Mol Med Rep 2012; 6(1): 9-15.

[273] Ghafouri-Fard S. siRNA and cancer immunotherapy. Immunotherapy 2012; 4(9): 907-17.

[274] Yan L, Chen WL, Zeng SG, Wang YJ, Bai ZB. Inhibition of VEGF expression in tongue squamous cancer cells via RNA interference silencing of iNOS gene. Int J Oral Maxillofac Surg 2009; 38(4): 369-73.

[275] Yang WQ, Zhang Y. RNAi-mediated gene silencing in cancer therapy. Expert Opin Biol Ther 2012; 12(11): 1495-504.

[276] Bumcrot D, Manoharan M, Koteliansky V, Sah DW. RNAi therapeutics: A potential new class of pharmaceutical drugs. Nat Chem Biol 2006; 2(12): 711-9.

[277] de Fougerolles A, Vornlocher HP, Maraganore J, Lieberman J. Interfering with disease: A progress report on siRNA-based therapeutics. Nat Rev Drug Discov 2007; 6(6): 443-53.

[278] Fabian MR, Sonenberg N, Filipowicz W. Regulation of mRNA translation and stability by microRNAs. Annu Rev Biochem 2010; 79: 351-79.

[279] Liu Z, Sall A, Yang D. MicroRNA: An emerging therapeutic target and intervention tool. Int J Mol Sci 2008; 9(6): 978-99.

[280] Sledz CA, Williams BR. RNA interference in biology and disease. Blood 2005; 106(3): 787-94.

[281] Cervantes A, Alsina M, Tabernero J, Infante JR, LoRusso P, Shapiro G, *et al*. Phase I dose-escalation study of ALN-VSP02, a novel RNAi therapeutic for solid tumors with liver involvement. J Clin Oncol (Meeting Abstracts) 2011; 29(supplement 15): 3025.

[282] Gollob JA, Bumcrot D, Toudjarska I, Bastian BC, Sah DW, Vaishnav AK. Progress in the delivery of siRNA therapeutics: Potential in uveal melanoma. Drugs Future 2011; 36(3): 229-39.

[283] Koldehoff M, Steckel NK, Beelen DW, Elmaagacli AH. Therapeutic application of small interfering RNA directed against bcr-abl transcripts to a patient with imatinib-resistant chronic myeloid leukaemia. Clin Exp Med 2007; 7(2): 47-55.

[284] Strumberg D, Schultheis B, Traugott U, Vank C, Santel A, Keil O, *et al*. Phase I clinical development of Atu027, a siRNA formulation targeting PKN3 in patients with advanced solid tumors. Int J Clin Pharmacol Ther 2012; 50(1): 76-8.

[285] Gilmore IR, Fox SP, Hollins AJ, Akhtar S. Delivery strategies for siRNA-mediated gene silencing. Curr Drug Deliv 2006; 3(2): 147-5.

[286] Ozpolat B, Sood AK, Lopez-Berestein G. Nanomedicine based approaches for the delivery of siRNA in cancer. J Intern Med 2010; 267(1): 44-53.

[287] Dey S, Maiti TK. Superparamagnetic nanoparticles and RNAi-mediated gene silencing: Evolving class of cancer diagnostics and therapeutics. J Nanomater 2012; 2012: doi:10.1155/2012/129107.

[288] Huang PI, Lo WL, Cherng JY, Chien Y, Chiou GY, Chiou SH. Non-viral delivery of RNA interference targeting cancer cells in cancer gene therapy. Curr Gene Ther 2012; 12(4): 275-84.

[289] Kesharwani P, Gajbhiye V, Jain NK. A review of nanocarriers for the delivery of small interfering RNA. Biomaterials 2012; 33(29): 7138-50.

[290] Leng Q, Woodle MC, Lu PY, Mixson AJ. Advances in systemic siRNA delivery. Drugs Future 2009; 34(9): 721.

[291] Wu SY, McMillan NA. Lipidic systems for *in vivo* siRNA delivery. AAPS J 2009; 11(4): 639-52.

[292] Ishida, T., Huang, C.L., Wada, H. Liposome containing shRNA molecule targeting a thymidylate synthase and use thereof. US20130058996 (**2013**).

[293] Huang, C.L., Wada, H. RNAi molecule for thymidylate synthase and use thereof. EP2415870 (**2012**).

[294] Galvani E, Peters GJ, Giovannetti E. Thymidylate synthase inhibitors for non-small cell lung cancer. Expert Opin Investig Drugs 2011; 20(10): 1343-56.

[295] Johnston PG, Lenz HJ, Leichman CG, Danenberg KD, Allegra CJ, Danenberg PV, *et al*. Thymidylate synthase gene and protein expression correlate and are associated with response to 5-fluorouracil in human colorectal and gastric tumors. Cancer Res 1995; 55(7): 1407-12.

[296] Yeh KH, Shun CT, Chen CL, Lin JT, Lee WJ, Lee PH, *et al*. High expression of thymidylate synthase is associated with the drug resistance of gastric carcinoma to high dose 5-fluorouracil-based systemic chemotherapy. Cancer 1998; 82(9): 1626-31.

[297] Doi Y, Okada T, Matsumoto H, Ichihara M, Ishida T, Kiwada H. Combination therapy of metronomic S-1 dosing with oxaliplatin-containing polyethylene glycol-coated liposome improves antitumor activity in a murine colorectal tumor model. Cancer Sci 2010; 101(11): 2470-5.

[298] Nagano S, Perentes JY, Jain RK, Boucher Y. Cancer cell death enhances the penetration and efficacy of oncolytic herpes simplex virus in tumors. Cancer Res 2008; 68(10): 3795-802.

[299] Iyer AK, Khaled G, Fang J, Maeda H. Exploiting the enhanced permeability and retention effect for tumor targeting. Drug Discov Today 2006; 11(17-18): 812-8.

[300] Kano MR, Bae Y, Iwata C, Morishita Y, Yashiro M, Oka M, *et al*. Improvement of cancer-targeting therapy, using nanocarriers for intractable solid tumors by inhibition of TGF-β signaling. Proc Natl Acad Sci USA 2007; 104(9): 3460-5.

[301] Seynhaeve AL, Hoving S, Schipper D, Vermeulen CE, de Wiel-Ambagtsheer G, van Tiel ST, *et al*. Tumor necrosis factor alpha mediates homogeneous distribution of liposomes in murine melanoma that contributes to a better tumor response. Cancer Res 2007; 67(19): 9455-62.

[302] Bergers G, Song S, Meyer-Morse N, Bergsland E, Hanahan D. Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors. J Clin Invest 2003; 111(9): 1287-95.

[303] Hoffman JA, Giraudo E, Singh M, Zhang L, Inoue M, Porkka K, *et al*. Progressive vascular changes in a transgenic mouse model of squamous cell carcinoma. Cancer Cell 2003; 4(5): 383-91.

[304] Ruoslahti E. Specialization of tumour vasculature. Nat Rev Cancer 2002; 2(2): 83-90.

[305] Eliceiri BP, Cheresh DA. The role of alphav integrins during angiogenesis: Insights into potential mechanisms of action and clinical development. J Clin Invest 1999; 103(9): 1227-30.

[306] Pasqualini R, Koivunen E, Ruoslahti E. Alpha v integrins as receptors for tumor targeting by circulating ligands. Nat Biotechnol 1997; 15(6): 542-6.

[307] Arap W, Pasqualini R, Ruoslahti E. Cancer treatment by targeted drug delivery to tumor vasculature in a mouse model. Science 1998; 279(5349): 377-80.

[308] Boehm T, Folkman J, Browder T, O'Reilly MS. Antiangiogenic therapy of experimental cancer does not induce acquired drug resistance. Nature 1997; 390(6658): 404-7.

[309] Wu, H.-C., Lin, C.-T., Lee, T.-Y., Kuo, S.-Y. Peptide-conjugates that bind to VEGF-stimulated or tumor vasculature and methods of treatment. US8088887 (**2012**).

[310] Zalipsky S, Mullah N, Harding JA, Gittelman J, Guo L, DeFrees SA. Poly(ethylene glycol)-grafted liposomes with oligopeptide or oligosaccharide ligands appended to the termini of the polymer chains. Bioconjug Chem 1997; 8(2): 111-8.

[311] Huynh NT, Passirani C, Saulnier P, Benoit JP. Lipid nanocapsules: A new platform for nanomedicine. Int J Pharm 2009; 379(2): 201-9.

[312] Beija M, Salvayre R, Lauth-de Viguerie N, Marty JD. Colloidal systems for drug delivery: From design to therapy. Trends Biotechnol 2012; 30(9): 485-96.

[313] Song H, Nie S, Yang X, Li N, Xu H, Zheng L, *et al*. Characterization and *in vivo* evaluation of novel lipid-chlorambucil nanospheres prepared using a mixture of emulsifiers for parenteral administration. Int J Nanomedicine 2010; 5: 933-42.

[314] Lasa-Saracibar B, Estella-Hermoso de Mendoza A, Guada M, Dios-Vieitez C, Blanco-Prieto MJ. Lipid nanoparticles for cancer therapy: State of the art and future prospects. Expert Opin Drug Deliv 2012; 9(10): 1245-61.

[315] Lucks, J.S., Müller, R.H. Medication vehicles made of solid lipid particles (solid lipid nanospheres - SLN). EP0605497 (**1996**).

[316] Battaglia L, Gallarate M. Lipid nanoparticles: State of the art, new preparation methods and challenges in drug delivery. Expert Opin Drug Deliv 2012; 9(5): 497-508.

[317] Wissing SA, Kayser O, Muller RH. Solid lipid nanoparticles for parenteral drug delivery. Adv Drug Deliv Rev 2004; 56(9): 1257-72.

[318] Pardeike J, Hommoss A, Muller RH. Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. Int J Pharm 2009; 366(1-2): 170-84.

[319] Muller RH, Radtke M, Wissing SA. Nanostructured lipid matrices for improved microencapsulation of drugs. Int J Pharm 2002; 242(1-2): 121-8.

[320] Ruggiero A, Riccardi R. Platinum Drugs in Children with Cancer. In: Bonetti A, Leone R, Muggia FM, Howell SB, Eds. Platinum and other Heavy Metal Compounds in Cancer Chemotherapy: Molecular Mechanisms and Clinical Applications. 1st ed. New York, Humana Press; 2009, pp. 365-71.

[321] Graham MA, Lockwood GF, Greenslade D, Brienza S, Bayssas M, Gamelin E. Clinical pharmacokinetics of oxaliplatin: A critical review. Clin Cancer Res 2000; 6(4): 1205-18.

[322] Gasco, M.R., Gasco, P., Bernareggi, A. Nanoparticle formulations of platinum compounds. US7611733 (**2009**).

[323] Hamelers IH, van Loenen E, Staffhorst RW, de Kruijff B, de Kroon AI. Carboplatin nanocapsules: A highly cytotoxic, phospholipid-based formulation of carboplatin. Mol Cancer Ther 2006; 5(8): 2007-12.

[324] Farrel, N.P., Menta, E., Di Domenico, R., Spinelli, S. Bis-platinum complexes with polyamine ligands as antitumor agents. US6022892 (**2000**).

[325] Gasco, M.R. Method for producing solid lipid microspheres having a narrow size distribution. US5250236 (**1993**).

[326] Boulikas, T. Therapy for human cancers using cisplatin and other durgs or genes encapsulated into liposomes. US20090280164 (**2009**).

[327] Goldstein JL, Brown MS. Regulation of the mevalonate pathway. Nature 1990; 343(6257): 425-30.

[328] Graaf MR, Richel DJ, van Noorden CJ, Guchelaar HJ. Effects of statins and farnesyltransferase inhibitors on the development and progression of cancer. Cancer Treat Rev 2004; 30(7): 609-41.

[329] Relja B, Meder F, Wang M, Blaheta R, Henrich D, Marzi I, *et al*. Simvastatin modulates the adhesion and growth of hepatocellular carcinoma cells via decrease of integrin expression and ROCK. Int J Oncol 2011; 38(3): 879-85.

[330] Yanae M, Tsubaki M, Satou T, Itoh T, Imano M, Yamazoe Y, *et al*. Statin-induced apoptosis via the suppression of ERK1/2 and Akt activation by inhibition of the geranylgeranyl-pyrophosphate biosynthesis in glioblastoma. J Exp Clin Cancer Res 2011; 30: 74.

[331] Mo H, Elson CE. Studies of the isoprenoid-mediated inhibition of mevalonate synthesis applied to cancer chemotherapy and chemoprevention. Exp Biol Med (Maywood) 2004; 229(7): 567-85.

[332] Sylvester PW. Synergistic anticancer effects of combined gamma-tocotrienol with statin or receptor tyrosine kinase inhibitor treatment. Genes Nutr 2012; 7(1): 63-74.

[333] Lonardo A, Loria P. Potential for statins in the chemoprevention and management of hepatocellular carcinoma. J Gastroenterol Hepatol 2012; 27(11): 1654-64.

[334] Broughton T, Sington J, Beales IL. Statin use is associated with a reduced incidence of colorectal adenomatous polyps. Int J Colorectal Dis 2012; 28(4):469-76.

[335] Spampanato C, De Maria S, Sarnataro M, Giordano E, Zanfardino M, Baiano S, *et al*. Simvastatin inhibits cancer cell growth by inducing apoptosis correlated to activation of Bax and down-regulation of BCL-2 gene expression. Int J Oncol 2012; 40(4): 935-41.

[336] Campbell MJ, Esserman LJ, Zhou Y, Shoemaker M, Lobo M, Borman E, *et al*. Breast cancer growth prevention by statins. Cancer Res 2006; 66(17): 8707-14.

[337] Maltese WA, Defendini R, Green RA, Sheridan KM, Donley DK. Suppression of murine neuroblastoma growth *in vivo* by mevinolin, a competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. J Clin Invest 1985; 76(5): 1748-54.

[338] Seeger H, Wallwiener D, Mueck AO. Statins can inhibit proliferation of human breast cancer cells *in vitro*. Exp Clin Endocrinol Diabetes 2003; 111(1): 47-8.

[339] Shibata MA, Kavanaugh C, Shibata E, Abe H, Nguyen P, Otsuki Y, *et al*. Comparative effects of lovastatin on mammary and prostate oncogenesis in transgenic mouse models. Carcinogenesis 2003; 24(3): 453-9.

[340] Shibata MA, Ito Y, Morimoto J, Otsuki Y. Lovastatin inhibits tumor growth and lung metastasis in mouse mammary carcinoma model: A p53-independent mitochondrial-mediated apoptotic mechanism. Carcinogenesis 2004; 25(10): 1887-98.

[341] Rogers AE, Lee SY. Chemically-induced mammary gland tumors in rats: Modulation by dietary fat. Prog Clin Biol Res 1986; 222: 255-82.

[342] Sylvester PW, Russell M, Ip MM, Ip C. Comparative effects of different animal and vegetable fats fed before and during carcinogen administration on mammary tumorigenesis, sexual maturation, and endocrine function in rats. Cancer Res 1986; 46(2): 757-62.

[343] Sundram K, Khor HT, Ong AS. Effect of dietary palm oil and its fractions on rat plasma and high density lipoprotein lipids. Lipids 1990; 25(4): 187-93.

[344] Goh SH, Hew NF, Norhanom AW, Yadav M. Inhibition of tumour promotion by various palm-oil tocotrienols. Int J Cancer 1994; 57(4): 529-31.

[345] Parker RA, Pearce BC, Clark RW, Gordon DA, Wright JJ. Tocotrienols regulate cholesterol production in mammalian cells by post-transcriptional suppression of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. J Biol Chem 1993; 268(15): 11230-8.

[346] Nazzal, S.M., Sylvester, P.W. Tocotrienol compositions. US20110052704 (**2011**) & EP2473044A4 (**2013**).

[347] Chaturvedi SP, Kumar V. Production techniques of lipid nanoparticles: A review. RJPBCS 2012; 3(3): 525-41.

[348] Dash, A.K., Trickler, W.J. Mucoadhesive nanoparticles for cancer treatment. US8242165 (**2012**).

[349] Sternberg LR, Byrd JC, Hansson GC, Liu KF, Bresalier RS. Alternative splicing of the human MUC2 gene. Arch Biochem Biophys 2004; 421(1): 21-33.

[350] Chhieng DC, Benson E, Eltoum I, Eloubeidi MA, Jhala N, Jhala D, *et al*. MUC1 and MUC2 expression in pancreatic ductal carcinoma obtained by fine-needle aspiration. Cancer 2003; 99(6): 365-71.

[351] Howard KA, Rahbek UL, Liu X, Damgaard CK, Glud SZ, Andersen MO, *et al*. RNA interference *in vitro* and *in vivo* using a novel chitosan/siRNA nanoparticle system. Mol Ther 2006; 14(4): 476-84.

[352] Shikata F, Tokumitsu H, Ichikawa H, Fukumori Y. *In vitro* cellular accumulation of gadolinium incorporated into chitosan nanoparticles designed for neutron-capture therapy of cancer. Eur J Pharm Biopharm 2002; 53(1): 57-63.

[353] Jeong YI, Kim SH, Jung TY, Kim IY, Kang SS, Jin YH, *et al*. Polyion complex micelles composed of all-trans retinoic acid and poly (ethylene glycol)-grafted-chitosan. J Pharm Sci 2006; 95(11): 2348-60.

[354] Koudelka S, Turanek J. Liposomal paclitaxel formulations. J Control Release 2012; 163(3): 322-34.

[355] Ye L, He J, Hu Z, Dong Q, Wang H, Fu F, *et al*. Antitumor effect and toxicity of Lipusu in rat ovarian cancer xenografts. Food Chem Toxicol 2013; 52: 200-6.

[356] Jinturkar KA, Anish C, Kumar MK, Bagchi T, Panda AK, Misra AR. Liposomal formulations of Etoposide and Docetaxel for p53 mediated enhanced cytotoxicity in lung cancer cell lines. Biomaterials 2012; 33(8): 2492-507.

[357] Desai, N.P., Soon-Shiong, P., Sandford, P.A., Grinstaff, M.W., Suslick, K.S. Methods for *in vitro* delivery of substantially water insoluble pharmacologically active agents and compositions useful therefor. US5439686 (**1995**).

[358] Taylor P. Ostwald ripening in emulsions. Adv Colloid Interface Sci 1998; 75(2): 107-63.

[359] Singh, C.U. Solid nanopaticle formulation of water insoluble pharmaceutical substances with reduced Ostwald ripening. US20090238878 (**2009**) & EP2054036A4 (**2013**).

[360] US National Cancer Institute website. Available at: http://nano.cancer.gov/learn/understanding/faq.asp. (Accessed on: March 11, 2013).