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NOVEL DELIVERY APPROACHES FOR CANCER THERAPEUTICS

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Abstract

Currently, a majority of cancer treatment strategies are based on the removal of tumor mass mainly by surgery. Chemical and physical treatments such as chemo- and radiotherapies have also made a major contribution in inhibiting rapid growth of malignant cells. Furthermore, these approaches are often combined to enhance therapeutic indices. It is widely known that surgery, chemo- and radiotherapy also inhibit normal cells growth. In addition, these treatment modalities are associated with severe side effects and high toxicity which in turn lead to low quality of life. This review encompasses novel strategies for more effective chemotherapeutic delivery aiming to generate better prognosis. Currently, cancer treatment is a highly dynamic field and significant advances are being made in the development of novel cancer treatment strategies. In contrast to conventional cancer therapeutics, novel approaches such as ligand or receptor based targeting, triggered release, intracellular drug targeting, gene delivery, cancer stem cell therapy, magnetic drug targeting and ultrasound-mediated drug delivery, have added new modalities for cancer treatment. These approaches have led to selective detection of malignant cells leading to their eradication with minimal side effects. Lowering, multi-drug resistance and involving influx transportation in targeted drug delivery to cancer cells can also contribute significantly in the therapeutic interventions in cancer.

Graphical Abstract

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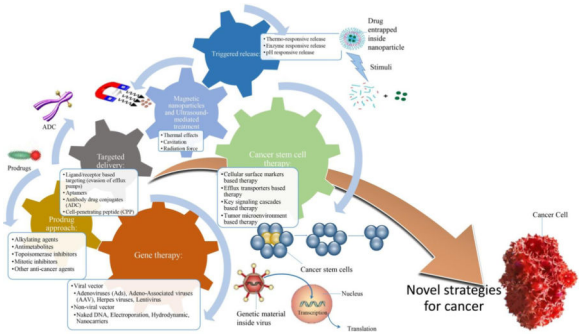
CONFLICT OF INTEREST

Authors do not have any conflict of interest.



Keywords

Chemotherapeutics; Tumor; Cancer; Targeted Drug Delivery; Stem cells; Cell Penetrating Peptides; Efflux Pumps; Gene therapy; Immunotherapy

1. INTRODUCTION

Cancer is a group of diseases involving uncontrolled growth and spread of abnormal cells. Such cells undergo transformations to obtain inexhaustible replication and thus traverse to other organs leading to malignancy. Failure to regulate or prevent such a spread of cancerous cells often leads to death of the patient. Cancer represents the most common cause of death in the US, and accounts for nearly 1 of every 4 deaths [3]. Approximately, 14.5 million Americans with a history of cancer were surviving on January 1, 2014. Survival statistics vary with cancer type and diagnosis stage. In U.S., 1,658,370 new cancer cases are expected to be diagnosed in 2015 and about 589,430 Americans are expected to die of cancer. Within the years 2004–2010, the relative survival rate for all cancers diagnosed was 68%, compared to 49% in 1975–1977 [3]. Several internal and external factors are responsible for this deadly disease [4][5]. External factors include infectious organisms, unhealthy diet, pesticides, environmental toxins and tobacco while internal factors includes inherited genetic mutations, immune conditions and hormones. These factors may act together or in series to develop cancer. There are several stages in cancer progression which is generally established with tumor size, extent of primary tumor and spreading capability to nearby lymph nodes or other organs. Diagnosis and staging are essential elements to initiate therapy. The conventional cancer treatment methods include surgery, radiation and chemotherapy.

Several differences in normal and cancer biology render cancer therapy as a multidisciplinary task. Targeted therapy based on distinct tumor type aiming to maximize efficacy and minimize toxicity has remained extremely challenging. Targeted therapy has not been particularly effective in treating certain tumors [4][6].The cancer genome atlas (TCGA) was founded to explore opportunities that may provide a holistic approach for classification. TCGA researchers hypothesized a multiplatform analysis of 12 cancer types, unravelling similarity of tumors based on genetics and molecular biology. This analysis generated a reclassification of approximately 10% of tumors based on their cell origin instead of tissue site. Interestingly, TCGA's integrative analysis unified classification of 12

cancer types into 11 major subtypes. Eleven major cancer types include lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), breast adenocarcinoma (BRCA), uterine corpus endometrial carcinoma (UCEC), glioblastoma multiforme (GBM), head and neck squamous cell carcinoma (HNSC), colon and rectal carcinoma (COAD, READ), bladder urothelial carcinoma (BLCA), kidney renal clear cell carcinoma (KIRC), ovarian serous carcinoma (OV) and acute myeloid leukemia (AML) which were standardized according to their mutational data from 3281 tumors. Analysis revealed that some tumors are molecularly heterogeneous while others are homogenous [6]. As mentioned above, histological classification includes 100 different types of cancer that are classified into 6 major categories: i.e. carcinoma, sarcoma, myeloma, leukemia, lymphoma and mixed types (Table 1).

2. Novel strategies for cancer targeted delivery

For effective cancer therapy, it is necessary to improve and develop novel strategies for effective delivery of chemotherapeutics to cancer cells. Conventional chemotherapeutic agents accumulate both in normal and tumor cells due to non-specificity. The ultimate goal of cancer therapy is to reduce the systemic toxicity and improve the quality of life. The landscape of cancer treatment has improved significantly over the past four decades.

Direct drug administration may be associated with embolism, non-specificity and drug induced toxicity. Additionally, orally administered drug regimen is required to overcome biological barriers, protein binding and first pass metabolism to reach therapeutic concentrations in cancer cells. Direct drug administration into the tumor environment may be effective when the cancer is benign. However, the prognosis is completely different when tumors metastasize and invade surrounding normal tissues. Under such conditions (metastasis) tumor cells invade other organs by altering phenotype. Moreover, such cells over express efflux pumps (P-glycoprotein, MRP and BCRP) and metabolizing enzymes relative to normal cells [8]. Such overexpression aids tumor cell survival and imparts resistance to xenobiotics (anticancer agents). For example, glioblastoma multiforme (GBM) is one of the metastatic diseases which is very challenging to treat till now.

Delivery of anticancer agents to metastatic cancer cell at therapeutic levels is extremely challenging. Targeted drug delivery may counteract metastatic tumor and minimize off target toxicity. Targeted drug delivery may be achieved by exploiting overexpression of transporters and receptors on cancer cell plasma membrane. Also ion channels such as potassium (K+), sodium (Na+), calcium (Ca+2), chloride (Cl-) and AQP4 channels may be targeted to regulate tumor metastases. Cancer cells, particularly GBM utilize these channels for migration [8].

2.1. Ligand/receptor based targeting

Ligand/receptor targeting has proven to be an effective method for drug delivery [9]. In order to improve efficacy chemotherapeutic agents need to be delivered into tumor cell cytoplasm or sub-cellular organelles such as nucleus and mitochondria. Such targeting may be achieved by proper selection, tailoring and designing ligands. Specificity of antibody targeting an antigen overexpressed in cancer cells needs to be well delineated. In addition, if

a linker is involved such as cell penetrating peptide (CPP) drug conjugates, a stable covalent bond formation is essential between the ligand/receptor and the drug. However, mechanism of drug release at tumor site is crucial. Premature drug release may result in systemic toxicity.

Tumor targeting ligands such as antibody, aptamer, siRNA, and peptides are being explored to target metastatic cells and block migration and invasion. Targeted drug delivery may be broadly classified as active and passive. Active targeting includes ligand mediated drug delivery. These ligands may be covalently conjugated to active agent or on to the surface of a carrier system such as nanoparticles, liposomes or nanomicelles [16]. Passive targeting exploits systemic and lymphatic systems in tumor architecture. Several targeting strategies have been developed for anticancer drug delivery, of which a few are discussed in this review article.

2.1.1. Antibody-drug conjugates (ADC)—Development of monoclonal antibodies targeting various antigens highly expressed on malignant cells is a novel approach. ADC represents a broad class of effective biopharmaceutical agents designed for targeted therapy including cancer. Antibodies may target malignant cells that overexpress a specific antigen. A few antibody drug conjugates have been marketed for oncology therapeutics such as brentuximab vedotin (Adcetris®) and trastuzumab emtansine (Kadcyla®). Currently, a majority of antibody drug conjugates are in different stages of clinical trials and some biologics are still in early stages of development. Robertson et al. studied the application of a recombinant human interleukin 18 (rhIL-18), a cytokine with antitumor activity conjugated to Rituximab. This CD20 monoclonal antibody against B cell lymphoma is highly effective for the treatment of Non-Hodgkin Lymphoma. Phase I studies (19 patients) with intravenous rituximab (375 mg/m2/wk) and rhIL-18(2 hours IV infusion/wk) demonstrated elevated plasma levels for pro-inflammatory cytokines, transient lymphopenia, and baseline lymphocyte count [8]. In another study, DM1, a cytotoxic agent that binds and destabilizes microtubule activity was studied by Lopus et al. The researchers established the application of antibody-DM1 conjugates in cancer therapy. Such antibody-drug conjugates were formulated as Trastuzumab emtansine (T-DM1) consisting of monoclonal antibody trastuzumab (Herceptin) conjugated to DM1 [10, 11]. Trastuzumab emtansine has been recently approved by FDA for breast cancer treatment. T-DM1 targets epidermal growth factor receptor 2 (HER2) overexpressed in aggressive breast cancer. Trastuzumab emtansine destroys cancer cells due to interaction of microtubules and DM1 [12]. Brentuximab Vedotin is another example for antibody-drug conjugate approved for treating Hodgkin lymphoma (HL) and systemic anaplastic large cell lymphoma (sALCL). Brentuximab Vedotin targets cell membrane protein (CD30) and an antimitotic agent, monomethyl auristatin E (MMAE) [13]. A phase II study on relapsed/ refractory sALCL indicated that 86% patients reached the objective response (57% complete remission and 17 % partial remission) [14]. However, phase II study on relapsed/ refractory diffuse large B cell lymphoma (DLBCL) demonstrated 44 % objective rate achievement [15]. Based on the presence of CD33 on acute myeloid leukemia (AML) blasts and some leukemic stem cells, gemtuzumab ozogamicin (GO), an anti-CD33 antibody carrying the cytotoxic calicheamicin has been developed to treat AML [231]. Several other markers including CD44 [232],

IL-3RA or CD123 [233], the immunoglobulin mucin TIM-3 [234] and folate receptor beta (FRβ) [235], have been extensively explored to specifically target leukemic stem cells in human AML.

2.1.2. Aptamers—These macromolecules are single stranded DNA or RNA that binds to proteins and peptides with higher specificity and affinity. Aptamers recognize various targets ranging from small molecules to macromolecules [16, 17]. Therefore, aptamers are utilized in therapeutic and diagnostic applications [18]. Several studies demonstrated that aptamer drug conjugates minimized systemic toxicity and enhanced drug delivery at the tumor site [19, 20]. For example, modified TLS11a-GC aptamer conjugated to doxorubicin (DOX) demonstrated high binding affinity (7.16 ±0.59 nM) towards hepatocellular carcinoma cells (LH86). However, negligible binding was observed towards normal human liver cell line (Hu1082) [21, 22]. Similarly, Cao et al. studied cy-apt 20 aptamer as a biomarker and selected it as a targeting ligand for gastric cancer [23]. Results indicate a significant rise in binding of cy-apt 20 to gastric carcinoma cells (AGS) with prolonged incubation time. In addition, concentration dependent increase in binding affinity was demonstrated. Cell uptake studies with FITC labelled cy-apt 20 AGS on Hepatoma cells (HepG2) and colon carcinoma cells (SW620) confirmed significant staining of AGS cells. Negligible staining was observed in HepG2 and SW620 [23]. Similar results were obtained with cy-apt 20, a diagnostic agent for gastric cancer. This study found cy-apt 20 aptamer to possess high and specific binding for AGS cells. Similar results were obtained with aptamer in human prostate cancer. Elevation in prostatic acid phosphatase (PAP) is associated with prostate cancer [24]. Kong and Byun reported that 6N 2’-FY RNA aptamer with 50 nucleotide sequence contains two hairpins that facilitate binding with PAP. Moreover, 6N 2’-FY RNA aptamer demonstrated higher binding (118 nM) for positive PAP mammalian cells (LNCaP and PC-3) relative to normal human lung fibroblast (IMR-90) [24]. These results indicate potential application of 6N 2’-FY RNA aptamer as a targeting ligand.

2.1.3. siRNA—Surface of calcium carbonate nanoparticles decorated with siRNA demonstrated active targeting to vascular endothelial growth factor C (VEGF-C) in gastric tumors. Similarly, surface modified nanoparticles generated higher transfection efficiency in human gastric cell line (SGC-7901) relative to unconjugated blank nanoparticles. *In vivo* studies suggest that siRNA decorated calcium carbonate nanoparticles can inhibit angiogenesis and growth of cancer cells [25]. Wang et al. demonstrated targeted delivery of anti CD47 siRNA conjugated to liposomal protamine hyaluronic nanoparticles (LPH-NPs) for lung cancer cells. The study demonstrated significant cancer metastasis inhibition (~27%) suggesting that active targeted siRNA delivery is highly effective [26]. Targeted drug delivery can prolong residence time at tumor site. *In vivo* studies demonstrated that GBM therapy with DOX PLGA nanoparticles surface decorated with siRNA and angiopep-2 (ANG) caused significantly higher DOX accumulation in glioma region [27]. Main advantages of such targeting include low dose requirement, reduced frequency of drug administration and minimal toxicity. None or minimal hematopoietic toxicity was observed with anti-CD47 siRNA encapsulated in a lipostamine-hyaluronic acid (LPH) formulation targeting CD47 receptors. This study indicated that LPH formulation was safe and well-tolerated [26].

2.1.4. Peptides—Chlorotoxin, a 36 amino acid peptide derived from scorpion venom [28] and AaCtx (70% homology with chlorotoxin peptide) are examples of such peptides which potentiate the anti-angiogenic effect of bevacizumab and may be targeted as anti-angiogenic agents for tumors [29]. Phase display peptide library series were screened at large to identify peptides with high affinity towards cancer cells. Recently, McGuire, et al. isolated 11 peptides with high affinity towards non-small lung cancer cells and low affinity for normal cells [30]. Ligands are designed to generate high binding affinity to plasma membrane of cancer cells [31–33]. For example, conjugation of paclitaxel to a peptide ligand demonstrated significant tumor size reduction relative to paclitaxel alone. Pallechia et al. conjugated paclitaxel (PTX) to dYNH peptide [ySAYPDSVP(L-norleucine)(L-homoserine)S] and YNH peptide [YSAYPDSVP(L-norleucine)(L-homoserine)S] to target EphA2 receptor overexpressed on various neoplastic cells including prostate cancer cells. dYNH-PTX- conjugate appeared to be more stable relative to YNH-PTX conjugate. Additionally, tumor reduction was more noticed with dYNH-PTX relative to mice treated with YNH-PTX [34]. A comparison of two studies indicate that dYNH peptide displays higher affinity and more anti-tumor activity than YNH peptide. Enhanced permeation and retention (EPR) effect due to hyper vascularization, poorly differentiated vasculature and ineffective lymphatic drainage are mostly responsible for development of weak, fragile and leaky vasculature [33, 35]. Such passive targeting exploits systemic and lymphatic systems in tumor architecture. Certain aggressive tumors may develop a 100 to 800 nm pore due to neovascularization [36]. Drug carriers with nanometer size range may take advantage of such pores and accumulate in the tumor site due to EPR effect. There are reports that small particle size (20 nm-100 nm) with surface pegylation may prolong circulation. Such carrier properties may aid in higher particulate accumulation at tumor site and enhance diffusion within tumor tissues [37, 38].

2.1.5. Cell-penetrating peptide (CPP)—CPP can serve as an effective ligand for targeting conventional as well as oligonucleotide based cancer therapeutics. Cell-penetrating peptides are generally composed of 5–30 amino acids, basic or amphiphilic [39]. CPP efficiently translocates plasma membrane and may aid in drug translocation across cell membrane. Hence, CPPs may be a potential targeting ligands for cancer chemotherapeutics or delivery systems. Charge specific CPP conjugated to DOX was studied by Lelle et al. [40]. Positively charged octa-arginine and negatively charged proline rich aliphatic CPP conjugated to DOX were studied. *In vitro* cytotoxicity of DOX conjugated to octa-arginine peptide on MCF-7 and HT-29 demonstrated an IC_{50} of 11.4μM and 19.0 μM, respectively. However, DOX conjugated to proline rich peptide displayed a higher IC_{50} of 27.0 μM on MCF-7 and 24.7 μM on HT-29 relative to DOX alone on MCF-7 (0.4 μM) and on HT-29 (1.2 μM) [40]. Confocal laser scanning microscopy revealed that oligo-arginine-DOX had significantly higher cellular uptake relative to proline peptide DOX indicating octa-arginine peptide as an efficient targeting ligand. Furthermore, a 16 base pair CPP (KKLFKKILKKL— NH_2) (B16) was conjugated to chlorambucil (CLB) to form CLB-BP16 demonstrated an IC_{50} value of 8.7 μM. On the other hand, an arginine analogue with 308 base pair (BP308) conjugated to CLB (CLB-BP308) had an IC_{50} of 25.5μM against CAPAN-1, MCF-7, PC-3, 1BR3G and SKMEL-28 cell lines compared to CLB alone which had an IC_{50} of 73.7μM. A significant improvement in activity of CLB was evidenced when

conjugated to either at the N or C-terminal of B16 [41]. A number of small molecular weight peptides have also been applied to deliver targeted MNP (see section: **Magnetic nanoparticles)**

3. Intracellular targeted drug delivery

The most effective techniques to target tumor cells is to direct DNA inhibiting drug molecules to nuclei of cancer cells. Nuclear targeting not only causes primarily tumor cell death but simultaneously minimizes damage to surrounding normal cells. The major problem of such targeted drug delivery is to avoid translocation of active agents into endosomal or lysosomal vesicles. Drug delivery mechanism requires active molecules to escape from subcellular cytoplasmic vesicles and translocate into nuclei [42]. The cancer cells develop intracellular resistance mechanisms such as overexpression of drug efflux pumps, metabolism and sequestration into acidic compartments and deactivation [43]. Sui et al. described two nuclear drug delivery strategies. One strategy involves indirect nuclear targeting in which drug molecules are carried into cytosol in large quantities subsequently allowing complete sequestration of nuclear DNA. The other strategy provides direct nuclear targeting in which nanocarriers carry molecules to cancer cells across the cell membrane into cytosol and finally localize in the nuclei where the active molecules may be released [44].

3.1. Barriers for nuclear drug delivery

(a) Plasma membrane: The foremost barrier to nuclear drug delivery to mammalian cells is the plasma membrane that restrict the passage of charged and large hydrophilic molecules [45]. Large nanocarriers are taken by different endocytotic mechanisms into the cell [46]. Physical properties of nanocarriers such as geometry may have an effect on phagosome formation. Pinocytosis may be subdivided into clathrin mediated endocytosis (CME), caveolin-mediated endocytosis, clathrin and caveolin- independent endocytosis, and macropinocytosis. Trafficking of nanocarriers through different types of pinocytotic mechanisms is an important factor to develop targeted therapies. For instance, polarized MDCK epithelial cells that lack caveolae on apical surface cationic as well as anionic nanoparticles are trafficked by clathrin mediated endocytosis (CME) [47]. However, anionic particles are trafficked through CME and caveolae mediated endocytosis whereas cationic particles are restricted to CME and micropinocytosis in non-polarized HeLa cells [48]. In MDCK cells anionic particles access lysosomal compartments whereas cationic particles are directed to transcytosis [49]. These studies highlight the significance of shape and charge of the particulate delivery system which must be considered to overcome the foremost barrier of nuclear drug delivery.

(b) Nuclear membrane and nuclear transport: Multicellular nature of eukaryotes allows for compartmentalized architecture to regulate cell differentiation. The nuclear envelope surrounds the nucleus separating the nucleoplasm and genetic material from cytoplasm. The nuclear envelope comprises nuclear pore complex (NPC) through which exchange of molecules occurs [50]. NPC consists of a central channel, a nuclear and cytoplasmic ring which are made up of 50 different proteins called nucleoporins [51]. Each individual NPC translocates approximately 1000 proteins per second in a bidirectional way [52]. Molecules

≥45KDa or 9nm in diameter are transported though targeting signals to enter or exit the nucleus. On the other hand, small molecules pass through the NPC by passive diffusion [53]. Interestingly, a few studies reported the entry of particles over 100 nm [54]. Therefore, there is no consensus about exact mechanisms of transport across the NPC [55].

3.2. Strategies for targeted nuclear delivery

Four major nuclear targeted delivery systems have been studied extensively, i.e., 1) NLS mediated delivery system 2) TAT conjugated nuclear targeting 3) cationic polymer based delivery system and 4) pH triggered charge reversal approach. *In vitro/In vivo* studies demonstrated effective nuclear localization of NLS functionalized nanocarriers which are presented in Table 2. Positive charge residues in NLS and CPP sequences render them non-specific for *in vivo* applications. Even though brain penetration of CPP was documented, CPP and NLS exhibit rapid clearance mechanisms [56, 57]. Block copolymer micelles (BCM) surface-grafted with Trastuzumab-Fab-NLS generated 5-fold higher tumor uptake in HER2-overexpressing mice model. Moreover, the clearance of NLS conjugated Tm-Fab-NLS conjugates is attributed to mononuclear phagocyte system (MPS) without lowering tumor accumulation [58]. TAT functionalized liposomes conjugated to DOX increased cancer cell apoptosis by 37.1%. PEG modified liposomes are activated extracellularly by cysteine to achieve higher *in vivo* tumor uptake [59]. Similarly, DOX conjugated dextran and phenylboronic acid cholesterol (chol-PBA) nanomicelles demonstrated significant lysosomal-acidity dependent nuclear uptake of DOX nanomicelles. DOX-loaded Dex/Chol-PBA nano assembly demonstrated 100% survival rate and reduced tumor volume in mice model [60]. Cytotoxicity of DOX is largely dependent on its intercalation between two base pairs of DNA forming DNA adduct thereby inducing separation of DNA strands and DNA helicase [61]. Increased DNA cleavage activity and nuclear accumulation are dual functions that may enhance the efficacy of an anticancer drug. On the other hand, Wang et al. developed Graphene Quantum Dots conjugated DOX (DOX/GQD) and reported significant nuclear DOX accumulation and enhanced DNA cleavage. Further, at cellular level DOX/GQD conjugates evaded efflux transporters overexpressed in MCF-7/ADR cell lines [62].

3.3. Gene Therapy

Apparently, more than 2100 gene therapy clinical trials have been conducted as well as approved early till to date. Several gene therapy approaches have been introduced which include suicide gene therapy, immunomodulatory gene therapy, genetic modulation, pro-apoptotic and corrective gene therapy, antiangiogenic gene therapy, and siRNA therapy [68, 69]. In comparison to conventional chemotherapy which can cause high toxicity due to the lack of specificity, gene therapy provides a unique and powerful approach to combat cancer. Gene therapy works at molecular level, in which genetic materials or functioning genes are inserted into patients' cells to either repair or replace the defective genes. The cancer cells carry mutated genes such as p53, bax and other oncogenes. Therefore, gene therapy can play crucial role in treating cancer.

3.3.1. Mechanism of gene therapy in cancer treatment—Gene therapy involves the delivery of genetic materials into cancer cells. A vector is employed as a carrier to ensure

the delivery of genetic materials. After therapeutic genes are transported into cells, these sequences exert their action through various mechanisms such as silencing, up or down-regulation, repair or modification of the particular target genes. Suicide genes may cause cell death and/or tumor necrosis. Gene silencing inhibits cell growth and tumor regression. While gene modification may lead to higher response from other combination therapies (e.g. chemotherapy, immunotherapy, or radiation).

3.3.2. Gene delivery—Several delivery methods have been developed for gene therapy, which generally can be divided into two categories, viral and non-viral delivery system.

3.3.2.1. Viral vector: Viruses are microscopic infectious agents that can replicate in living cells [70]. Researchers have used viruses to deliver therapeutic genes into cell nuclei because of high transfection efficiency, ability to penetrate, express and replicate in host cells [71]. In order to utilize virus as vector, it is necessary to remove the pathogenic part of viral genes and replace with the therapeutic genes [71]. The remaining non-pathogenic segments in virus carries therapeutic gene constituting the viral vector.

(a) Adenovirus (Ads) are non-enveloped viruses that possess linear ds (double stranded) DNA [72]. Ads can cause transduction safely along with high transgene expression, which makes it a very powerful vector to treat glioblastoma multiforme (GBM) [72]. However, the application of Ads in gene therapy is limited by the low therapeutic efficacy after systemic administration and severe toxicity [73]. Many attempts have been made to minimize toxicity and improve therapeutic efficacy. Yao et al. demonstrated higher tumor-selective transgene expression with PEGylated adenovirus vector relative to Ads [74, 75]. The breakthrough in Ads gene therapy was the approval of Gendicine (Ad-p53) in 2003 in US and Oncorine/ ONYX-015 (E1B-defective Ad) in 2005 in China. Both drugs are approved for treating head and neck squamous cell carcinoma [76].

(b) Adeno-Associated viruses (AAV) are small single-stranded (ss) DNA viruses which offer several advantages such as wide host range, low immune response (due to lack of pathogenicity) and long-term expression [77]. Recently several AAV-mediated genes have been developed to treat cancers such as prostate cancer, glioblastoma, cervical and breast cancer, nasopharyngeal carcinoma and lung carcinoma [78–81]. The safety of cytotoxic T lymphocytes (CTLs) with recombinant AAV has been evaluated in cancer patients [82]. AAV-mediated bevacizumab has been reported to inhibit ovarian tumor growth [83]. In another study, down-regulations of p16 (INK4), p27 (KIP1), p21 (WAF1), and p53 tumor suppressors using AAV type 2 was noted [84]. Another breakthrough of AAV associated cancer therapy was the approval of Glybera (alipogene tiparvovec) by European Union in 2013. It is an adeno-associated viral vector to treat lipoprotein lipase deficiency [85].

(c) Herpes viruses are large enveloped dsDNA viruses that can carry large transgene. A combination of oncolytic herpes simplex virus and vinblastine to deliver interleukin (IL)-12 to enhance antitumor effect in prostate cancer model has been evaluated [86]. Zeng et al. demonstrated synergistic effects of a combination therapy with paclitaxel and oncolytic herpes simplex virus G47Δ [87] in breast cancer. Similarly, Goshima et al. also showed

promising effect of a combination of HF-10, herpes simplex virus (HSV) and granulocyte-macrophage colony-stimulating factor (GM-CSF) in murine ovarian cancer [88].

(d) Lentiviruses are ssRNA genome retroviruses. They have emerged as a promising vector in cancer gene therapy. Lentiviruses possesses many advantages over other types of viral systems i.e. in low immunogenicity and the capability of transducing a variety of cells [89]. Various attempts have been made with lentivirus as vector in cancer gene therapy. Lentivirus mediated RNA interference was utilized to target protein phosphatase magnesium/manganese-dependent 1D (PPM1D) [90] or high mobility group box 1(HMGB1) [91]. These constructs suppress growth of bladder cancer. Similarly, other research groups have applied lentivirus-mediated short hairpin RNA to knockdown PPM1D in lung [92] and colorectal carcinoma [93].

3.3.2.2. Non-viral: Many non-viral systems have been investigated for gene delivery, including the injection of naked DNA or physical methods such as electroporation, gene gun, hydrodynamic distribution, sonoporation, and nanocarriers (nanoparticles and neutral or cationic liposomes) [94]. Non-viral methods are superior for large-scale production, low immunogenicity and they provide high level of transfection efficiencies compared to viral methods [95].

(a) Naked DNA: This is the simplest way to deliver therapeutic genes with direct injection of free DNA into particular tissues resulting in gene expression. In cancer gene therapy, DNA can be directly injected either inside the tumor or into tumor-surrounding tissues to express tumor antigens that might work as cancer vaccine. This method is less immunogenic and comparatively economical in terms of production cost. However, the low overall expression level restricts its use. Despite limitations, some success has been reported regarding clinical trials of naked DNA plasmid delivery [96], [97], [98].

(b) Electroporation (or electro-permeabilization) is a technique in which electrical field is applied to enhance the penetrability of DNA into cells [99] [100]. Electroporation offers several advantages such as accurate delivery of therapeutic genes, localized gene expression and less adverse effects. Studies have been conducted to ensure the safety and tolerability of this method in cancer and infectious diseases. The first clinical trial started in 2004 [101], [102]. Daud et al. have demonstrated the effectiveness of interlieukin-12 plasmid electroporation in metastatic melanoma patients in a phase I trial [103], [104]. Another study reported an intravaginal therapeutic DNA vaccine by electroporation. This involves coding calreticulin linked to E7 (CRT/E7) against cervicovaginal cancer [105].

(c) Hydrodynamic is another simple but effective non-viral gene delivery method. It works by employing a physical force that increases intravascular pressure [106]. This process is widely applied for gene delivery in animals. Hydrodynamic gene delivery is capable of delivering transgenes into mammalian cells in efficient and safe manner [107]. Yazawa et al. have examined hydrodynamic injection of plasmid encoding a soluble form of fetal liver kinase-1 (Flk-1) gene for angiogenesis inhibition in mouse model [108].

(d) Nanocarriers is another widely studied area in cancer gene therapy involving nanoparticle-based delivery of genetic material. These artificially synthesized non-bioactive nonviral vectors provide an efficient way to deliver genetic material to cells. Low immunogenic, less toxic and flexible for chemical modifications are unique advantages associated with this approach. However relatively low transfection efficiency is the main drawback with this method [109]. The nano-vectors i.e. nanoparticles or nanocapsules are usually prepared with biodegradable materials. These 10 to 100 nm particles form a nano complex by encapsulation or adsorption of genetic materials. The flexibility to chemical modification of nanomaterials provides excellent capability to adsorb, concentrate and protect genetic materials. Such nano-vectors can be divided into two categories: polymeric nano-vectors made of dendrimers, lipids, PLGA, and chitosan; and inorganic nano-vectors made of silica, iron oxide, and gold nanoparticles [110] [111] [112, 113]. Endocytosis is considered as the main delivery mechanism. In addition, coupling of specific molecules (antibodies/monoclonal antibodies, and peptides) to the surface of nanoparticles may be utilized to target tumor complex through binding to specific receptors on cell surface. The targeted genes can thus be safely and effectively transfected. The nanocarriers used for gene therapy in cancer are summarized in Table 3.

3.4. Cancer stem cell therapy

Self-renewal and proliferation of cancer stem cell (CSC) cause tumor initiation, development, metastasis and recurrence. So far, CSC has been discovered in a wide variety of solid tumors, including lung, colon, prostate, ovarian, and brain cancer, as well as melanoma. The primary reasons for treatment failure in multiple malignancies include resistance and lack of selectivity for chemotherapeutics. Furthermore, CSC populations are more resistant to convention chemotherapy relative to non-CSC population. Therefore, elimination of CSC is crucial in cancer treatment. Recently, multiple novel therapeutic systems have been explored for elimination of CSC and alteration of microenvironment which supports the growth of such cells. Chen et al. has reported various targets of current cancer treatment strategies. (Fig. 1). Surface markers and signaling pathways are the two potential targets. Multiple potential CSC therapeutic targets, including the ABC superfamily, anti-apoptotic factors, detoxifying enzymes, DNA repair enzymes and distinct oncogenic cascades have been identified. Currently, a few therapeutic strategies have been developed that can destroy CSC successfully while others are still under preclinical and clinical stages [127].

3.4.1. Key signaling cascades based therapy—Understanding the anti-apoptotic pathway and inactive pro apoptotic pathway/ mechanisms are emerging areas for the development of cancer chemotherapeutics. Notch1 signaling plays a major role in the development of tumors. Xanthohumol (XN) that inhibits Notch1 signaling pathway leading to accelerated apoptosis of tumor cells [128]. Merkelbach and co-workers described apoptosis modulation of KIT. Several mRNAs can induce apoptosis such as the PI3K/AKT signaling pathways in gastrointestinal stromal tumors [129]. Several other signaling cascades including c-Met [130], MAPK [131], TGF-β, Wnt [132], NF-κB [133], Hedgehog [134], APC/βcatenin, Ras/Raf/Mek/Erk and others play an important role in the recurrence and maintenance of CSC.

3.4.2. Tumor microenvironment based therapy—Tumor microenvironment also plays a vital role in creating a niche for nursing and protecting CSC from drug induced apoptosis. Caro et al. reported an important role of tertiary lymphoid tissue in T cell recruitment and local activation of immune cells in the tumor microenvironment. Several approaches involving targeting myeloid cells [135], bone microenvironment [136], Versican, (a 'bridge' connecting inflammation with tumor progression) [137], spliceosome [138] and NADPH oxidase-derived reactive oxygen species [139] have been explored.

4. Cancer Immunotherapy

Cancer immunotherapy is an alternative treatment intended to activate the immune system to induce disease stabilization [140]. In contrast to the other delivery approaches, immunotherapy primarily aims to prevent the metastatic spread of the disease. Cancer immunotherapy can utilize specificity of the immune system for the treatment of melanoma. Although the immune system is capable of recognizing and eliminating cancer cells but the tumor interferes with immune responses [141]. During tumor growth, cancer cells gain the ability to conquer the surrounding healthy tissue, thereby spreading the disease. Owing to genetic instability, cancer cells usually express abnormal proteins. Tumor-associated antigens (TAAs), produce none or limited expression on non-cancer cells. Such TAAs expose new, potentially immunogenic epitopes, which can be recognized by the host immune system. Therefore, tumor cells must be determined at early stages by immune system causing destruction of these abnormal cells [142]. Currently, immune check point inhibitors have been utilized to treat cancer. The immune system check points include PD-1 (programmed cell death protein 1) and CLTA-4 (Cytotoxic T-lymphocyte-associated antigen 4) which can protect normal cells. Targeting of these check points may be a new tool for the treatment of cancer [143]. Approaches to cancer immunotherapy include, active and passive immunotherapy. Cytokines regulate both the cells of the innate immune system and the adaptive immune system. Cytokines exert the effects upon binding to their respective receptors on the target cells. US Food and Drug Administration (FDA) approved IL-2 and IFN-α2b for treatment of a variety of cancers. IL-2 is indicated in the treatment of renal cell carcinoma, leukemia and lymphoma. IFN-α2b has been approved for the treatment of Kaposi’s sarcoma and various types of leukemia. Active immunotherapy includes cancer vaccines in which tumor antigen(s) are co-administered with an adjuvant to raise a specific T cell or B cell response. Passive immunotherapy includes monoclonal antibodies that block immune checkpoints such as CTLA-4 and PD-1 [142], [140]

5. Strategies for controlled drug delivery

5.1. Triggered Release

Noninvasive stimuli-responsive controlled drug delivery is attractive since such systems allow remote, repeatable, and reliable switching on or off drug release based on need. A complete noninvasive remote-controlled drug delivery system comprises of drug, an external/internal stimulus, stimulus-sensitive materials, and stimulus-responsive carriers. The external stimulus can be a magnetic field, light, ultrasound or radio-frequency. The internal stimulus can be pH, temperature or cellular enzymes. With the help of these response-triggered delivery systems, not only one can achieve targeted delivery of

therapeutic agents, but can control the duration and extent of drug release also into tumor cell. A schematic has been providing to explain the different types of triggered release systems (Fig. 2).

5.2. Thermo-responsive release

Temperature-responsive or thermoresponsive polymers exhibit a sharp and discontinuous change of their physical properties with temperature [144]. These polymers can be functionalized with groups that bind to specific biomolecules. The polymer-biomolecules-conjugate can be precipitated from solution by a small change in temperature. Several examples of thermoresponsive delivery systems have been discussed. Gnaim et al. synthesized and characterized a novel γ-cyclodextrin (γ-CD) based carrier for molecular encapsulation of cancer chemotherapeutic agent DOX. The γ-CD derivative, with a β-naphthyl alanine residue attached in its primary surface, exhibited potent binding with DOX. The encapsulation efficiency was assessed under various temperatures and pH. The carrier-DOX inclusion complex is highly stable under a wide range of acidic conditions (pH 1.0–7.0). However, the encapsulated drug is slowly released under hyperthermic conditions (up to 50°C). Cell culture studies showed that the complexation of DOX with the carrier inhibited cellular uptake thereby significantly lowering toxicity. Thermo-triggered DOX release was validated and rise in cellular uptake was observed [145]. Magnetic-based core-shell particles (MBCSPs) were developed to target skin cancer cells while delivering chemotherapeutic drugs in a controlled fashion. MBCSPs consist of a thermoresponsive shell of poly (N-isopropylacrylamide–acrylamide–allylamine) and a core of poly (lactic-co-glycolic acid) (PLGA) embedded in magnetite nanoparticles. To target melanoma cancer cells, MBCSPs were conjugated with Gly–Arg–Gly–Asp–Ser (GRGDS) peptides that specifically bind to the a5b3 receptors of melanoma cells. MBCSPs consist of unique multifunctional controlled drug delivery properties. Specially, these particles can provide dual drug release mechanisms (a sustained release through degradation of PLGA core and a controlled release in response to changes in temperature via thermo-responsive polymer shell), as well as dual targeting mechanisms (magnetic localization and receptor-mediated targeting). Results from *in vitro* studies demonstrated that GRGDS-conjugated MBCSPs were less than 300nm in diameter. No cytotoxicity was observed in human dermal fibroblasts. These particles sustained the release of curcumin. A temperature-dependent release of doxorubicin from the shell of MBCSPs was observed. The particles also produced a dark contrast signal in magnetic resonance imaging. Finally, the particles accumulated at the tumor site in a B16F10 melanoma orthotopic mouse model, especially in the presence of a magnet [146]. To improve the efficacy of gemcitabine (GEM) in the treatment of advanced pancreatic cancer a multi-functional nanoplatform permitting both *in vivo* heating and drug delivery was developed by Kim et al. [147]. The researchers utilized a chemo-hypothermia approach to achieve high intra-tumoral GEM concentrations simultaneously inducing hyperthermia for enhanced tumor cell death as well as growth inhibition. MRI visible hydroxypropyl cellulose (HPC) grafted porous magnetic drug carrier may permit *in vivo* visualization of bio distribution. The magnetic drug carriers produced strong T2 weighted image contrast and permitted efficient heating with low magnetic field intensities. The thermo-mechanical response of HPC triggered GEM release was confirmed by *in vitro* drug release studies. *In vitro* studies confirmed that, pancreatic cancer cell growth was

significantly inhibited (~82% reduction) with chemohyperthermia compared to chemotherapy or hyperthermia alone. Chemohyperthermia with intra-tumoral injections of GEM-magnetic carriers (followed by heating) resulted in significant rise in apoptotic cells compared to tumors treated with GEM-magnetic carrier injections. Chemohyperthermia with GEM-magnetic carrier offers the potential to significantly improve the therapeutic efficacy of gemcitabine in the treatment of pancreatic cancer [147].

5.3. Enzyme responsive release

Enzymes are key components of the bio-nanotechnology toolbox that provides exceptional bio-recognition capabilities and outstanding catalytic properties. Combined with unique physical properties of nanomaterials, the resulting enzyme-responsive nanoparticles can be designed to perform efficiently with specificity for the triggering stimulus (Fig.3). This powerful concept has been successfully applied in the fabrication of drug delivery systems where the tissue of interest is targeted via release of cargo triggered by the biocatalytic action of an enzyme [2] [148]. When the enzymatic activity associated to a particular tissue is expressed at higher concentrations at the target site, the nanomaterial can be programmed to deliver drugs via enzymatic conversion of the carrier [149]. Excitingly, the detection of enzyme activity can be an extremely useful tool in diagnostics, up and down regulation of enzyme expressions that may be associated with many disorders. Also, the exceptional efficiency of enzymes in catalyzing a chemical reaction can be harnessed to amplify the signal generated by the recognition of a certain analyte, i.e. immunoreaction in enzyme-linked immunoassays. In some cases, the nanoparticles are prepared with a material that is sensitive to enzymatic transformation stemming from recognition of chemical structure by a biocatalyst and/or transformation of an enzymatic reaction by the product. Polymeric nanoparticles incorporating biological motifs are cleaved via such enzymatic digestion. Under these conditions, it is possible to program the nanomaterials to release cargo (e.g. anticancer agent) by triggering the degradation of the polymeric shell [150]. Nazli et al. developed magnetic iron oxide nanoparticles (MIONPs) coated with matrix-metalloproteinase (MMP)-sensitive PEG-hydrogel. High concentrations of proteolytic enzymes such as MMP are secreted by tumor tissues. These enzymes can degrade the basement membrane and natural extracellular matrix opening up more space for tumor growth. MMP-2 and MMP-9 are highly expressed in some malignant tissues such as breast, colon and brain tumors [151]. Elevated concentrations of MMP in tumor tissues can lead to proteolytic degradation of peptide linkages conjugated to drugs and/or delivery systems. Multifunctional MIONP was designed to serve as contrast agent for MRI as well as carry chemotherapeutic doxorubicin to target overexpressed receptors on cancer cell membrane. Nanoparticles consist of proteolytically degradable PEG hydrogel coatings with integrin-binding RGDS domains. In presence of MMP, such nanoparticles may degrade due to cleavage of the MMP sensitive domains causing anticancer drug release. Studies demonstrated that such nanoparticles may penetrate cancer cells eleven times more efficiently than blank nanoparticles. Confocal laser scanning microscopy further showed that the targeted nanoparticles released doxorubicin into the nuclei of HeLa cells within 2 hrs. This strategy may become a promising and highly efficient alternative to existing methods of cancer treatment [151].

5.4. pH responsive release

Among the different types of stimuli, pH sensitive system has been the most widely employed nano-systems in cancer therapy [1]. It is well known that pH varies significantly in different tissues or organs, such as stomach and liver, and in disease states, such as ischemia, infection, inflammation, and tumorigenesis. Due to high rate of glycolysis in cancer cells, aerobic and anaerobic conditions and lower pH in tumors can be exploited to target chemotherapeutics to these cells. Tumors have been demonstrated to exhibit acidic pH values ranging from 5.7 to 7.8, while the pH of normal tissue is 7.4 [152][153]. Several approaches have been developed to design pH-responsive drug release. One of the most commonly used approach is to introduce an “ionizable” chemical group such as amines, phosphoric and carboxylic acids among others, into polymeric structures. These groups, with different chemical structures and pKa values, can accept or donate protons and undergo pH-dependent changes in physical or chemical properties such as swelling ratio and/or solubility, resulting in drug release [154]. Another approach is to introduce acid-labile chemical bonds either by covalently conjugating drug molecules directly to the surface of existing nanocarrier or to construct a new nanocarrier. These acid-labile linkages are stable at neutral pH but easily degraded or hydrolyzed under acidic environment. The acid-labile linkers most commonly employed are acetal, orthoester, hydrazone, imine, and cis-aconyl bonds. A novel approach to prepare pH-responsive delivery systems is to incorporate carbon dioxide-generating precursors that generate CO_2 in an acidic environment, leading to disintegration of the carrier and release of active molecules [155]. For effective delivery of anticancer drugs, pH-sensitive nanosystems may encapsulate and stabilize the anticancer agent at physiological pH but rapidly release the cargo at tumor pH.

6. Prodrug approach: analog / chemical conjugation for cancer chemotherapeutics

Prodrug approach in the field of anticancer drug delivery has gained tremendous interest. This strategy can improve the efficacy and reduce systemic or unwanted tissue/organ toxicity of the parent drugs. The structures of parent anticancer drugs and their prodrugs developed in last two years have been summarized in the following sections:

6.1. Prodrugs of alkylating agents

Selective DNA cross linking triggered by oxidation, reduction, fluoride induction and photo-irradiation have been developed. Han et al. recently described a series of binitroimidazole prodrugs that generated DNA inter-strand cross–links (ICL) and direct strand breaks (DSB) upon UV irradiation. Such DNA cross linking may lead to highly selective inhibition of DNA replication and gene expression, thereby leading to cell death [156]. Paci and co-workers designed preactivated ifosfamide (IFO), an oxazaphosphorine (alkylating agent). This prodrug requires bioactivation by cytochrome P450 enzymes to release the active entity. This approach led to the inhibition of toxic metabolites which is the major drawback of IFO delivery [157]. A hypoxia-selective anti-tumor agent, demonstrated preparation of mono-N-oxide analogue by removal of 4-oxide from tirapazamine (parent di-N-dioxide). It bears a nitrogen mustard unit, which exhibits significant alkylating properties [158]. Peng and group developed H_2O_2 activated aromatic nitrogen mustard based prodrugs where the

DNA alkylating agent is connected to a H_2O_2 responsive trigger by an electron withdrawing group. Such an approach of ROS-activation provided better specificity and lower toxicity [120, 159–161]. A melphalan derived prodrug, melflufen (J1) which releases the parent drug by hydrolytic cleavage of the peptide bond by aminopeptidase N (APN) thereby eliciting anti-anigiogenic effects [162]. Wietrzyk and co-workers developed ester prodrugs of isophosphoramide mustard (iPAM), an active metabolite of IFO which can be easily hydrolyzed by esterases to release the parent drug [163]. Boger et al. reported reductively cleaved novel phenolic prodrugs of duocarmycin that alkylates DNA in a selective sequential manner [164]. Oxidative quenching of quinone methide precursors prevented quinone methide regeneration leading to formation of transient derivatives that can cause reversible alkylation in DNA duplex [165].

Platinum drugs (cisplatin, carboplatin, and oxaliplatin) are not grouped under alkylating agents. However, these compounds cause tumor cell death following same mechanism as alkylating agents. Recently, Lippard and co-workers developed a series of Pt (IV) prodrugs of cisplatin and carboplatin bearing axial halides by oxidative halogenation [166, 167]. Similarly derivatives of oxaliplatin with axial valproato ligands were developed [168]. Cisplatin analogs conjugated with cyclooxygenase (COX) inhibitors were also designed to overcome cisplatin resistance [169, 170]. Cisplatin-based aliphatic bis(carboxylato) Pt(IV) prodrugs with varying carbon chain length are reported by Osella and group [171].

Recently, several nanoformulations of anticancer drugs have gained attention. Bilgicer and co-workers developed photosensitive Pt (IV)-azide prodrug-loaded nanoparticles that selectively induce active Pt (II) at the tumor site by UVA irradiation [172]. The platin-A prodrug is developed to overcome the nephrotoxicity and ototoxicity of cisplatin [173]. Xing and group developed near-infrared (NIR) light-activated nanoplatform for a platinum (IV) prodrug [174]. Many other prodrugs with nano formulations of anti-tumor agents are currently under development [175–182]

6.2. Prodrugs of antimetabolites

Sakamoto and co-workers developed prodrug which releases 5-fluorouracil (5-FU) anticancer drug from oligonucleotide strands following photo irradiation [183]. Prodrugs of gemcitabine containing bioorthogonal Pd(0)-cleavable groups that generate biologically inert precursors of gemcitabine [184]. Cui et al. discussed a tripeptide prodrug, 13F-1 of 5-FU that provides activity against tumor cells by targeting an enzyme aminopeptidase N [185]. N-Acyl-phosphoramidates derivatives of gemcitabine can overcome acquired resistance to gemcitabine caused by deoxycytidine kinase deficiency [186]. Similarly, Mcguigan et al. have disclosed NUC-1031, a gemcitabine phosphoramidate prodrug that has shown significant reduction in viability of tumor cells [187]. Zhao et al. demonstrated the efficacy of cell penetrable dendrimer as a potential antitumor drug carrier by 5-FU and lysine dendrimer conjugates [188]. Several nanocarriers like PEGylated lipids for squalenoyl amphiphilic prodrugs of gemcitabine and dideoxycytidine [189] and polymeric micelles for stearoyl-gemcitabine [190] are being developed to improve the efficacy and sustainability of the anticancer properties. A glycol chitosan (GC) grafted with 2,3-dimethylmaleic acid (DMA) and fullerene (C60) nanogels display both photo and low pH responsive properties

for cancer therapy [191]. A few other recent advancements in the field of prodrugs of cancer therapy have been listed; prodrugs activated by histone deacetylases and a tumour-associated protease [192]; squalene-based prodrug [193]; fluorouracil/zidovudine glyceryl prodrug [194]; 1,2- and 1,3-diacylglycerophosphates of clofarabine [195].

6.3. Prodrugs of anthracyclines/ anti-tumor antibiotics

A non-hormonal therapeutic approach is a growing area that provides an alternative to various treatment modalities for hormone refractory cancers. Antibody-directed enzyme prodrug therapy (ADEPT) and gene-directed enzyme prodrug therapy (GDEPT) are being explored recently to overcome nondiscriminatory drug exposure to normal tissues by chemotherapeutics. Kim and co-workers recently developed an induced phenotype targeted therapy (IPTT) where DEVD-S-DOX prodrug containing DOX linked to a peptide moiety (DEVD) is cleaved by an enzyme caspase-3. Caspase three expression is elevated in tumor region by radiation induced apoptosis [196]. Several enzyme cleavable peptide prodrug conjugates and formulations of DOX have been recently developed to increase the specificity and tolerability of chemotherapeutic agents [197–205]. Selective DOX prodrug activation using photoirradiation [206], pH active linkages [176, 207–209], photothermal linkages [210], and reduction [211] have paved the way for the development of new selective chemotherapeutic agents.

6.4. Prodrugs of topoisomerase inhibitors

Kim and co-workers developed a prodrug of SN-38, having a biotin subunit for localization and a piperazine-rhodol moiety for fluorescent based monitoring. These two segments are connected by a self-immolative linker through disulfide bonds. Such a strategy can offer better targeting and drug release. It can also aid in cancer diagnosis and treatment [212]. Ashley and group developed a macromolecular prodrug of SN-38 by a linker between the macromolecular carrier and the drug molecule. The linker undergoes self-cleavage by a non-enzymatic B-elimination which is highly predictable. This process in turn prolongs the circulation time and lower glucoronidation of the parent drug [213]. Several hyaluronan [214], methylenthiol group [215], folate targeted [216] based prodrugs of campothecin and malic acid based ester prodrugs of etoposide [208] have been reported. Wang et al. developed a prodrug of SN-38, which releases the parent drug upon hydrolysis in the presence of glutathione. It leads to reactive oxygen species which can elicit high anticancer therapeutic activity [217]. Nano-formulations such as glutathione responsive nanoparticles [218], phosphorylcholine micelles responsive to reducing agent [219] and PEG based nanomicelle [220] have been prepared recently. Chen and co-workers came up with a very innovative approach of co-delivering camptothecin and small interfering RNA in the form of a prodrug (CPTssR5H5) to overcome multi-drug resistance [221].

6.5. Prodrugs of mitotic inhibitors

Recently the development of mitotic chemotherapeutic agents has made rapid progress. The representative drugs for this class include PTX. Poor aqueous solubility and systemic toxicity led to derivatization of PTX prodrugs. Conjugation of PTX with a hydrophilic macromolecule through a bio-cleavable linker is one of the strategies widely studied [222–226]. Silicate ester derivatives of PTX and docetaxel (Dtx) also demonstrated improved

physiochemical properties. Targeted therapy based on distinct tumor types faces a major challenge. As classification has been primarily based on histology of tumor, targeted therapy has not been effective in treating tumors with similar histology [6, 227]. Similarly, several Dtx derivatives with better targetibility and stability were developed such as CD44 targeted-hyaluronic acid based derivatives [228]. Sun and co-workers reported a prodrug with fluorinated Dtx conjugated to rhodamine B (imaging reporter/targeting domain) through a biodegradable ester linkage. This prodrug demonstrated high plasma/blood stability and specificity to mitochondria. [229]. Other derivatives of colchicine [230], estradiol [231], combretastatin [232, 233] and phenstatin [234] have been synthesized and have shown better efficacy relative to parent drug.

6.6. Steroid prodrugs as anticancer agents

Corticosteroids are also known to exhibit anticancer activity. However, several serious side effects are associated with their use. Gilmer and co-workers prepared nitrophenyl based prodrugs of prednisolone, budesonide and celecoxib where the parent drugs generated by the nitro reductase action of the colonic microflora thus improving the targetibilty and lowering the toxicity of parent drugs [235].

7. Magnetic nanoparticles (MNP)

Widder and colleagues were the first to utilize magnetic microsphere to direct anticancer agents to tumor tissue with the aid of external magnetic field [236]. Magnetism-assisted therapy has undergone a significant evolution to become a sophisticated theranostic (combination of therapeutics and diagnostics) approach. Magnetic nanoparticles (MNP) are flexible systems offering a variety of modification for therapeutic and diagnostic applications. Owing to super paramagnetic property, MNP have proven to be excellent magnetic resonance imaging (MRI) contrast agents. These agents are biocompatible and well tolerated. As a result, a number of MNP's are in clinical trials or approved as contrast agent for MRI to detect a variety of cancers. Iron oxide NP may produce reactive oxygen species in vivo (Fenton reaction) leading to DNA damage and toxicity [237]. However, this can be easily avoided by coating MNP's with polymers, surfactant, inorganic metals or oxides. Some of the commonly used materials for preparation of MNP's include magnetite (Fe_3O_4), maghemite (γ-Fe_2O_3), iron-based metal oxides ($CoFe_2O_4$, $NiFe_2O_4$, $MnFe_2O_4$), iron alloys (FePt and FeAu), rare earth metal alloys and transition metals. Cobalt, nickel and chromium are less preferred as biomedical agents, because these metals are highly toxic and require impervious coating. Iron oxide and iron alloy based materials are generally safer to use.

7.1. Therapeutic applications of MNP

MNP's are typically 10 to 100 nm in size with very narrow size distribution, which allow them to exploit enhanced permeation and retention effect (passive targeting). This particle size range aids in evasion of both renal clearance and phagocytosis of circulating particles. Chemotherapeutic agents can be incorporated in the polymeric film of MNP or chemically conjugated to polymer chains via suitable linker. MNP can also be directed to the tumor site by using an external magnetic field to provide a localized delivery of anticancer agents

thereby limiting off-target toxicity. In a clinical trial, MNP's were employed to deliver DOX hydrochloride to hepatocellular carcinoma assisted by external magnetic field. Particle localization was monitored by MRI. In a similar study Wilson et al. treated inoperable hepatocellular carcinoma with MNP targeted DOX hydrochloride. Using an external magnet DOX hydrochloride coated MNP's were directed to tumor. The fraction of tumor volume (ranged from 0.64 to 0.91) was treated with minimal off target effect [238].

Surface coating also provides numerous advantages such as prevention of agglomeration of sub-nano particles. It also limits nonspecific interactions, improves pharmacokinetics and allows ligand mediated active targeting. Coating also enables development of multifunctional nanocarriers facilitating delivery of a multiple class of therapeutic agents simultaneously. A schematic representation of multifunctional nanoparticles is shown in Fig. 4. MNP can be actively targeted to cancer cells via receptor-ligand mediated specific interactions. A number of cancer cell specific surface biomarkers have been targeted for this purpose. Ligand-targeted MNP have been utilized for diagnosis as well as theranostics application for MR imaging. Location of tumor lesions as small as ~2–3 mm in diameter can be identified following cellular uptake [239]. Chlorotoxin-conjugated MNP was utilized to target glioma cells. Active targeting resulted in higher accumulation in cancer cells with significantly improved contrast MR imaging [240]. Targeting ligands conjugated to MNP was exploited for theranostics purposes ranging from small molecule nutrient transporters such folate [241–243] to macromolecules like Trastuzumab [244–246], single-chain anti-epidermal growth factor antibody [247], anti-epidermal growth factor receptor antibody [248], anti-vascular endothelial growth factor antibody (VEGF) [249, 250] and aptamers [251], [252]. A number of small molecular weight peptide have also been investigated including arginylglycylaspartic acid (RGD) [243, 253], bombesin [254, 255], and luteinizing hormone releasing hormone [256, 257]; highly cationic peptides like CPP [258], myristoylated poly-arginine peptide (MPAP) [259, 260] and HIV-TAT [261]. Multifunctional MNP have also been developed where nanocomposites are surface modified with fluorescent probes enabling both fluorescence and MR imaging for enhanced *in vivo* tracking. Lin et al. developed methotrexate loaded folate receptor targeted dual probe MNP where cyanin dye (Cy5.5) was used as fluorescent probe [262]. Such theranostic nanocomposite can be delivered to treat tumors and monitored in real time via MRI or fluorescence imaging.

Hyperthermia is another application of superparamagnetic iron oxide nanoparticles (SPIONs) [263]. MNP can raise local temperature to ~ 41–46 °C under alternating magnetic field which has been shown to destroy cancer cells [264]. Exposure to moderate temperature 41–46 °C leads to activation of myriad of intra and extracellular degradation mechanisms such as protein denaturation, protein folding, aggregation and DNA cross linking. Temperatures above 46 °C (up to 56 °C) causes direct tissue necrosis, coagulation or carbonization leading to cell death. MNP in the 14–16 nm range have been reportedly most effective in producing hyperthermia [265]. However, this technique requires precise special controls to avoid damage to normal cells. Hyperthermia has also been employed as a mechanism to trigger chemotherapeutic release within tumor microenvironment, potentially minimizing off-target effect due to premature drug release. Hyperthermia-mediated drug

release is achieved via either bond cleavage, where drug is chemically conjugated to MNP via thermolabile bond or enhanced permeability where chemotherapeutics are encapsulated in polymer film coated MNP. Thermoresponsive poly (N-isopropylacrylamide) (PNiPAM) microgel coated MNP containing anticancer agents were encapsulated inside microgel. Release is achieved via enhanced permeation mechanism [266]. Similarly, PNiPAM based hydrogel was encapsulated with SPIONs. Model agents (vitamin B12 and methylene blue) showed drug release in the presence of oscillating magnetic field via enhanced permeation mechanism [267]. Derfus et al. have reported multifunctional MNP for remote controlled delivery of fluorescein-labeled DNA via oscillating magnetic field [268].

8. Current non-invasive strategies for cancer treatment

8.1. Ultrasound-mediated treatment

Ultrasound as a traditional diagnostic tool has been indicated in non-invasive therapy and anticancer drug delivery. The potential mechanisms of ultrasound for drug delivery involves three strategies including thermal effects, cavitation and radiation forces. Ultrasound has been utilized to facilitate intracellular delivery of a specific drug as well as to enhance the overall efficiency of the cytotoxic effects from carriers such as microbubbles and nanobubbles [269–274]. Ultrasound as a component of drug delivery system has the potential to be coupled with various drug carriers for the treatment of cancer [275–277].

8.1.1. Thermal effects—Localized tissue heating depends on the absorption of energy, intensity and frequency of the ultrasound, and the rates of thermal diffusion and conversion. Even a moderate temperature increase may significantly increase permeability of blood capillaries and/or cause cell membrane fluidization [278, 279]. Thermal effect of ultrasound has been utilized with temperature sensitive liposomes which is the most commonly investigated ultrasound-responsive drug delivery vehicle. In combination with localized hyperthermia under ultrasound, thermosensitive liposomes improved the delivery of various anticancer drugs to tumors [280–282]. Liposomes undergo a gel-to-fluid phase transition in the phospholipid membrane and become more permeable. This process allows rapid drug release in the target region within non-destructive hyperthermia range (39–41 °C) [279, 280, 283, 284]. An increase in local drug delivery causes significant inhibition of tumor growth [285].

8.1.2. Cavitation—Acoustic cavitation causes oscillation, and collapse of small stabilized gas bubbles under an ultrasonic field in a fluid medium. It is considered to be the most important of all non-thermal ultrasound mechanisms. This mechanism has the potential to produce cavitation in biological tissues, especially for enhancing drug delivery. Cavitation can be considerably improved by combining gas-filled microbubbles [271]. There are two distinct types of acoustic cavitation activity such as non-inertial (or stable) cavitation and inertial (or transient) cavitation [286]. The non-inertial cavitation bubbles may stably oscillate and persist for a many acoustic cycles. Non-inertial cavitation of systemically injected microbubbles induces alternating invagination and distention of vascular walls. In turn it causes damage to the endothelial lining and temporarily raises blood vessel permeability for enhanced extravasation and can improve delivery to whole tissue [287–

289]. In ultrasound, inertial (transient) cavitation can also alter the permeability of individual cells for improved delivery of genes and drugs. Inertial cavitation bubbles grow and expand two to three times of their resonant size, and finally collapse. Inertial cavitation is generally considered as the primary mechanism for structurally altering intact cells including irreversible damage and non-destructive membrane permeability [290, 291]. Inertial cavitation of microbubbles causes microjets and shock waves which create holes in blood vessels and cell membranes causing higher permeability of drugs and the carriers. The process of ultrasound-induced creation of pores in cell membranes is known as sonoporation [292–295]. Acoustic cavitation approach involves the path way of ultrasound for deploying drugs from the carrier. However, the exact mechanism by which the interactions between the bubbles and the carriers create these effects is still unclear. Ultrasound-induced cavitation has been incorporated for opening liposomal membranes and ultrasound-responsive stable liposomes demonstrated prolonged circulation time and effective tumor targeting [296–299]. Drug-loaded microbubbles are attractive and ultrasound-responsive drug carriers may be very beneficial for drug targeting to intravascular targets [300–306]. Targeted and ultrasound-triggered liposomes co-modified with single stranded DNA aptamers can target platelet-derived growth factor receptors (PDGFRs) expressed in breast cancer cells. Poly (NIPMAM-co-NIPAM) as the thermosensitive polymer can sensitize these liposomes at high temperature [307]. Ideal ultrasound-mediated tumor-targeted drug carrier requires good drug stability in circulation, long drug retention until activated, small size allowing extravasation through defective tumor vasculature and high ultrasound responsiveness [308]. However, currently used contrast agents as tumor-targeted drug carriers have many inherent difficulties. The commercially available microbubbles failed to effectively extravagate into tumor tissue for effective drug targeting due to their very short circulation time and relatively large size (2–10 microns). After release from microbubbles into circulation, a majority of drug fraction may circulate within the systemic circulation and eventually reach off-target sites. As a result only a small fraction reaches the tumor site.

8.1.3. Radiation force—Radiation has been defined as a unidirectional force that is generated with a transfer of momentum from the ultrasound wave to the medium under relatively high amplitudes of ultrasound exposures. Radiation forces are proportional to the rate of energy being applied and the absorption coefficient of the medium while it is inversely proportional to the speed of the ultrasound wave in the medium. [309]. Acoustic streaming can reduce heating through the process of increasing the mass transport of nanoparticles for improved transdermal delivery [310, 311]. Acoustic streaming and radiation force can also facilitate nanoparticle transfer across blood capillaries, consequently enhancing extravasation of drug carriers and/or macromolecular drugs [312–317]. Additionally, ultrasound radiation force may assist in modulating ligand exposure onto the surface of nanoparticles. The ligand in the droplet of primary nanoparticle can be exposed to the cell receptor with ultrasound [318]. Ultrasound effect on drug carriers and biological tissues is to enhance perfusion, extravasation of drugs and/or carriers, and drug diffusion through tumor tissue. Overall effect is drug penetration through various biological barriers. Enhanced intracellular uptake of nanoparticles, genes, and drugs extensively improve therapeutic efficacy of these agents [312, 319–325]. Furthermore, ultrasound treatment has also been associated with an induced immune response to tumors [326–328].

9. Chemotherapy and Drug Resistance

Chemotherapy is one of the foremost therapeutic interventions in cancer. Despite advances in drug discovery and treatment protocols, patients acquire multidrug resistance (MDR). Consequently response to chemotherapy remains far below expectations. MDR is a phenomenon where tumor cells develop resistance to functionally and structurally unrelated anticancer drugs [329]. While cancer cells initially respond to chemotherapy but relapse is common. Clinically drug resistance ensues prior to or in response to chemotherapy. It is either acquired or inherent and can be caused via multiple pathways. The mechanisms of drug resistance adapted by cancer cells include modifications in drug metabolism and transport, gene mutation, amplification of drug targets and genetic rewiring leading to gene repair and impaired apoptosis. Tumor heterogeneity (heterogeneous population of cells with distinct genetic fingerprints) is another aspect of drug resistance where a small subpopulation remain dormant and unresponsive to chemotherapy. But these cells later emerge as virulent type which become difficult to treat [330]. Onset of drug resistance is a complex process. It involves pharmacokinetic and pharmacodynamic mechanisms. Extensive reviews are available on the mechanisms of drug resistance [331–334]. Pharmacokinetic mechanisms involving sub-therapeutic concentration, elevated efflux, due to overexpression of MDR genes and enhanced biotransformation by cytochrome P450 (CYP) metabolizing enzymes are implicated. All these mechanisms indicate up-regulation of efflux transporters and metabolizing enzymes that constitute a major resistance phenotype. A concerted effects of efflux transporters and metabolizing enzymes leading to MDR may possibly occur due to overlapping substrate specificity and coordinated regulation of their expression. The expressions of MDR genes and CYP are mainly governed by nuclear receptors, particularly pregnane X receptor (PXR). These mechanisms may function independently or synergistically, leading to treatment failure. Therefore, ways to overcome such integrated role of efflux transporters [P-glycoprotein (P-gp), multidrug resistant proteins (MRP) and breast cancer resistance protein (BCRP)] along with CYP activity have been discussed in the following sections:

9.1. Role of Transporters in Drug Delivery

Transporters are integral part of cell membrane proteins. These proteins are intricately associated with selective absorption of endogenous substances (substances such as anions and cations, vitamins, sugars, nucleosides, amino acids, and peptides) and exclusion of toxic elements. Indeed, influx transporters allow absorption of essential nutrients and ions whereas efflux transporter eliminate cellular metabolites and xenobiotics. These proteins play a vital role in drug absorption, distribution, elimination, as well as drug-drug interactions. Although the occurrence of multidrug resistance in bacteria was identified more than fifty years ago, role of P-gp as a major factor for efflux of xenobiotics has been established recently. It is clear now that, these efflux proteins are highly expressed on different tumor cells which leads to drug resistance in chemotherapy.

In fact, these transporters play an important role in the process of drug absorption, distribution, metabolism and elimination (ADME). Almost 2000 genes have been identified for expression of transporters or transporter-related proteins [335]. Approximately 400

membrane transporters, in two super families, have been identified and characterized for specific tissue localization and a number of these transporters have been cloned [335]. From pharmacological perspective two major super families, ABC (ATP binding cassette) and SLC (solute carrier) transporters, are most important. Importance of drug transporters in the process of ADME and drug-drug interactions is now well documented. Recently US Food and Drug Administration (FDA) and International Transporter Consortium (ITC) together have considered a few transporters i.e., organic anion transporter (OAT), organic anion transporting polypeptide (OATP), organic cation transporter (OCT), peptide transporter (PEPT), P-gp, multidrug resistance protein (MRP) and breast cancer resistance protein (BCRP) which are crucial for xenobiotics absorption, and drug-drug interactions. From pharmacokinetic view point, localization and function of these transporters in the intestine, liver and kidney are receiving foremost consideration.

(a) ABC Transporters: The ABC transporter genes stand for the largest family of transmembrane proteins. So far 49 human genes have been recognized. Based on sequence homology and domain structure, these proteins are categorized into seven different classes (ABCA-ABCG). So far, thirteen different transporters have been identified from classes A, B, C and G and these transporters play a significant role in the development of multidrug resistance (MDR) [334, 336]. Biedler and Riehm [337] reported that Chinese hamster ovary (CHO) cells identified for resistance to actinomycin D, also impart cross-resistance to many other drugs (duramycin, democoline, mithramycin, mytomycin C, puromycin, vinblastin and vincristine). These efflux pumps also impart resistance to HIV-protease inhibitors. Mammalian cells can acquire cross resistance to a variety of therapeutic agents with diverse chemical structures and functions. This phenomenon is referred to as multidrug resistance. Juliano and Ling revealed that drug resistance in CHO cells is due to the presence of P-glycoprotein. These ABC transporters function in a unidirectional manner as opposed to several others transporters which are bidirectional [338]. All ABC proteins show in general a minimal requirement of two transmembrane domains (TMDs) and two nucleotide binding domains (NBDs) except BCRP. The hydrolysis of ATP is required as a standard power for translocation by these transporters [339]. The structural requirements of the ABC transporter for drug binding have been discussed previously [340, 341].

(b) Efflux transporters: Three important ABC transporters: P-gp, MRP and BCRP are expressed at physiological barriers (e.g. intestinal absorption barrier, BBB, placental barrier, corneal and retinal barriers), in the liver, cancer cells, and other tissues and are responsible for clearance and excretion of xenobiotics. These transporters significantly influence ADME of a number of drugs and their metabolites and cause drug resistance for many therapeutic agents. Such proteins along with primary metabolizing enzyme cytochrome P450 (CYP) together constitute a highly efficient barrier for oral drug absorption. P-gp is the most extensively studied efflux transporter which functions as a biological barrier by extruding toxins and xenobiotics into extracellular fluid. BCRP and MRP also belong to the same ABC super family. Substrate specificity and tissue localization of P-gp and BCRP differ from MRP. As a consequence of efflux, drug absorption is reduced and bioavailability of xenobiotics diminishes at the target tumor tissue. In recent reviews we have elaborately

discussed the role of these efflux transporters in drug resistance [342]. A list of anticancer agents that are substrate for various efflux transporters is presented in Table 4.

9.2. Strategies to overcome efflux

A major impediment in the effective delivery of anticancer agents is efflux. Hence developing strategies to circumvent efflux proteins are indispensable.

9.2.1. Pharmacological inhibition of efflux proteins—Co-administration of appropriate chemical agents that can reverse the action of efflux proteins either by competitive or non-competitive binding may be a smart strategy. The efflux modulators can efficiently prevent the activity of efflux proteins and consequently augment the permeability of desired drug molecules in the targeted tumor. A schematic of this mechanism is depicted in Fig. 5.

(a) First generation MDR modulators: Clinically approved, Ca^{2+} channel blocker verapamil can enhance cellular accumulation of vincristine in P-gp overexpressing cell lines [343, 344]. Though these first generation inhibitors are potent for MDR reversal *in vitro*, these compounds require very high doses to inhibit drug efflux pumps in human causing severe toxicity [345].

(b) Second generation MDR modulators: In order to circumvent the complications and toxicities associated with the first generation modulators, MDR modulators with lower toxicities were developed. Structural analogues of verapamil and cyclosporine A, such as dexverapamil and PSC-833 (valspodar) respectively are generated as second-generation of MDR modulators [346, 347]. These compounds alter the pharmacokinetic properties of co-administered anticancer drugs, such as paclitaxel, and DOX [348, 349] along with nonspecific interactions with CYP enzymes and other resistance proteins such as P-gp [350]. Often a consequence of these changes is associated with dose adjustment of co-administered anticancer drugs, which may otherwise adversely affect the outcome of therapy.

(c) Third generation MDR modulators: These agents are designed to overcome the limitations of the earlier modulators. Zosuquidar [351], tariquidar [352] and elacridar [353] are the MDR inhibitors that have shown potential in pre-clinical mouse models and in the clinic. These agents do not cause pharmacokinetic interactions with other therapeutic agents and drug metabolizing (CYP-450) enzymes. Preclinical studies indicate MDR-bearing human tumour reduction in mice and prolonged survival rate [354]. No alterations in pharmacokinetic parameters of anticancer drugs such as DOX, etoposide, daunorubicin, vincristine and paclitaxel, have been reported upon co-administration. Several Phase I and phase II studies were conducted with co-administration of zosuquidar with docetaxel and duanorubicin and DOX for the treatment of breast cancer, leukaemia and non-Hodgkin's lymphoma [355–358]. The results indicate clinical improvements. Several tyrosine kinase inhibitors (TKI) and other kinase inhibitors are being developed as MDR modulators. Quantitative structure-activity relationship to overcome the problems associated with first and second generation modulators are ongoing. TKI modulators (imatinib, nilotinib, ponatinib, icotinib, laptinib, erlotinib, canertinib, telatib, sunitinib, and vandetanib) are very effective at nanomolar concentrations and clinically relevant for cancer therapy [359].

9.3. Metabolizing enzymes

Apart from MDR, metabolizing enzymes such as cytochrome P450 (CYP450) are also induced by anticancer drugs causing rapid biotransformation resulting in treatment failure. Several chemotherapeutic agents require enzymatic activation to exert cytotoxic effects. For example, a nucleoside drug cytarabine (indicated for leukemia) requires phosphorylation by deoxycytidine kinase to active cytarabine triphosphate. To reduce the drug effect, cancer cells acquire resistance by lowering drug activation. Such resistance develops via down regulation/mutation of involved metabolic enzyme [360, 361].

Two main approaches have been explored so far to overcome efflux pumps and metabolizing enzymes. These include either co-administering an anticancer agent with an efflux modulator or employing anticancer agents which are not substrates of these efflux transporters. Unfortunately there are no modulators, so far identified, which can act at molecular level to prevent activation of MDR gene and metabolizing enzymes simultaneously. A novel common modulator which can inhibit the resistance mechanisms (activation of efflux transporters and metabolizing enzymes) needs to be explored for successful chemotherapy. Table 5 depicts representative chemotherapeutic agents which are metabolized by CYP450.

9.4. Ritonavir and reversal of chemo-resistance

Our laboratory has examined the strategy of combining ritonavir-a retroviral protease inhibitors with anticancer drugs to control drug efflux, and metabolism thereby allowing sufficient drug entry into tumor cells [342]. Vinblastine mediated induction of efflux transporters (MDR1, MRP2), metabolizing enzyme (CYP3A4) and nuclear receptor (PXR) is reversed in the presence of ritonavir in human colon adenocarcinoma cells (LS-180). Vinblastine induced overexpression of these genes completely deactivated co-treated cells with ritonavir. Uptake of [^{3}H] Lopinavir and VIVID™ assay further confirmed the functional activity of transcribed genes upon co-treatment. When any one of the anticancer agents (DOX, paclitaxel, tamoxifen and vinblstine) was combined with ritonavir, a significantly diminished cell proliferation, cell migration and augmented caspase activity are observed. This process leads to enhanced apoptosis of human breast adenocarcinoma cells (T47D) and prostate cancer cells (PC-3) (Tables 6 and 7). These results indicate enhanced activity of chemotherapeutics in the presence of ritonavir. In summary, combination therapy of anticancer drug with ritonavir may overcome drug resistance by deactivating the overexpression of efflux transporters and metabolizing enzymes and reprograming cell death. Therefore, drug regimens containing ritonavir may enhance therapeutic exposure of cancer cells to anticancer agents, potentially improving chemotherapeutic efficacy with lower resistance development.

10. CONCLUSION

Cancer has emerged as a leading cause of death worldwide. Although conventional chemotherapy has been the keystone to combat cancer, it is associated with normal cell toxicity. Due to lack of specificity, conventional cancer treatments often cause severe side effects and toxicities. Severity of cancer makes it imperative to develop novel approaches to

treat such diseases. Current challenges of anticancer drug development include the site specific delivery with low systemic toxicity. An ebbing tumor represents a dynamic environment with changes in its angiogenic status, cell mass, and extracellular matrix composition, among other factors. With recent advancements and progress in drug delivery approaches, preventive interventions are being identified. New chemopreventive agents may be delivered through novel cell targeting approaches. Focus of such novel approaches is based on cancer treatment with innovative methods. Consequently, these promising technologies offer new opportunities for cancer prevention and treatment with minimal/lower toxicity to normal cells which can be realized in the clinic in the very near future. In this review, we discussed various novel approaches such as ligand and receptor based targeting, triggered release methods, gene delivery, prodrug approach and analogue/chemical conjugation to treat cancer cells. This comprehensive review paper has discussed many recent approaches such as intracellular drug targeting, cell penetrating peptides, aptamer based targeting, cancer stem cells therapy, cancer immunotherapy, magnetic drug targeting and ultrasound-mediated drug therapy to deliver cancer therapeutics. Such approaches have been investigated to overcome the limitations of conventional therapy. This article covers various aspects of multidrug resistance (MDR), metabolizing enzymes and consequently response to chemotherapy.

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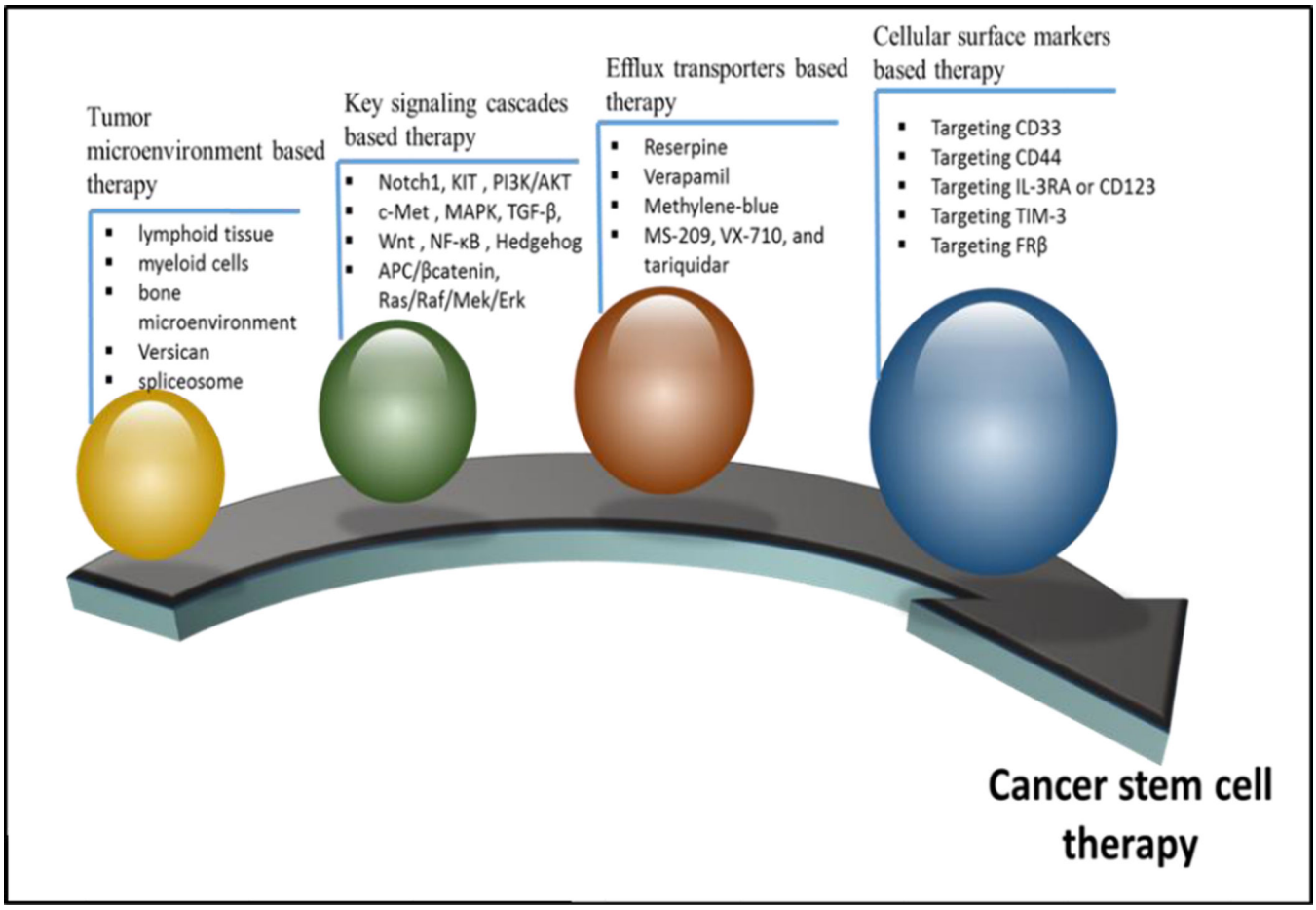

Fig 1.
Cancer Stem Cell Therapy

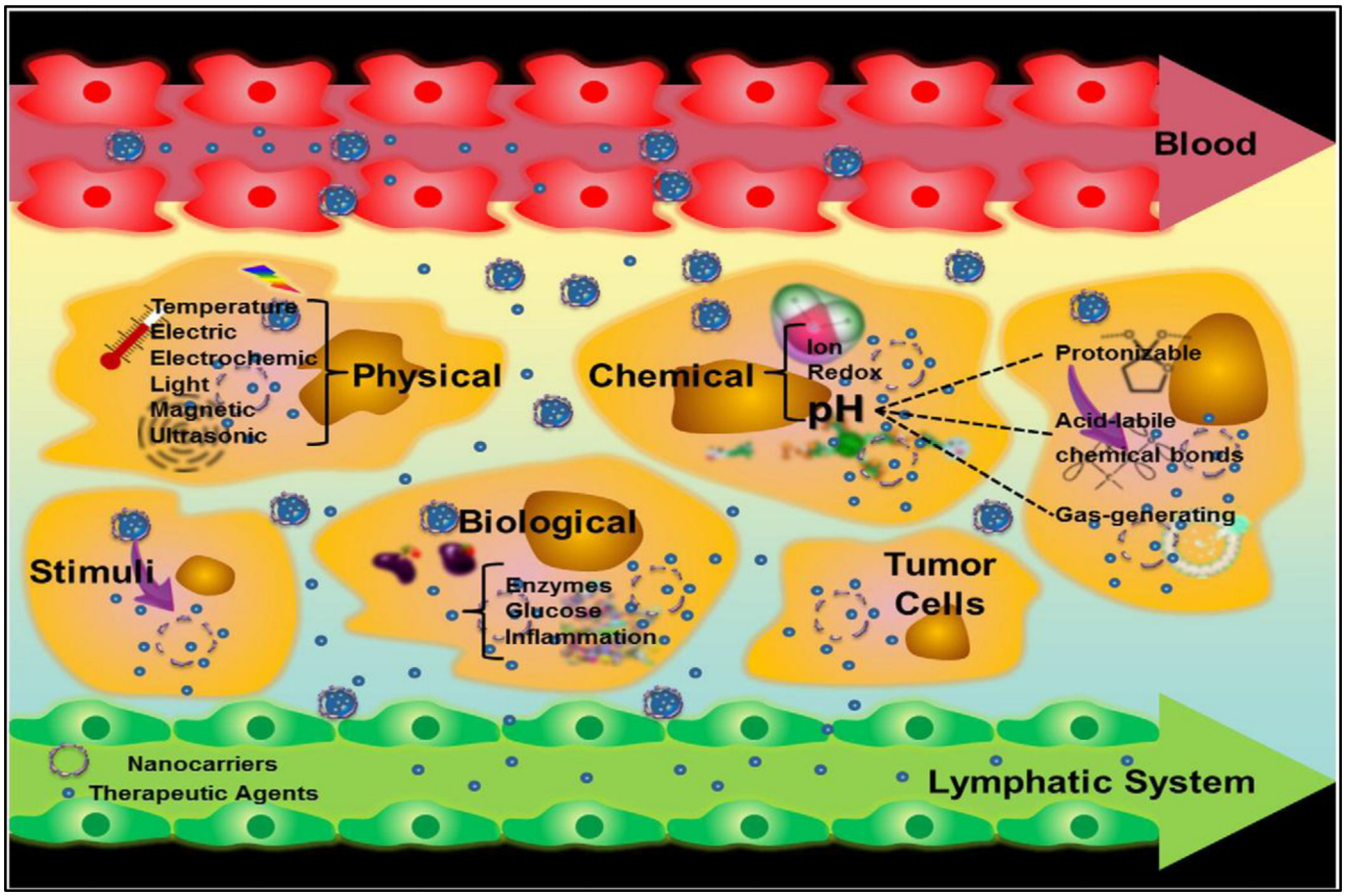

Fig. 2.

Nanocarriers enhance the permeability and retention of drugs in tumors. The figure depicts various stimuli that can be used to trigger drug-release from appropriately responsive materials in tumor cells. Reproduced with the permission from Liang et al. [1]

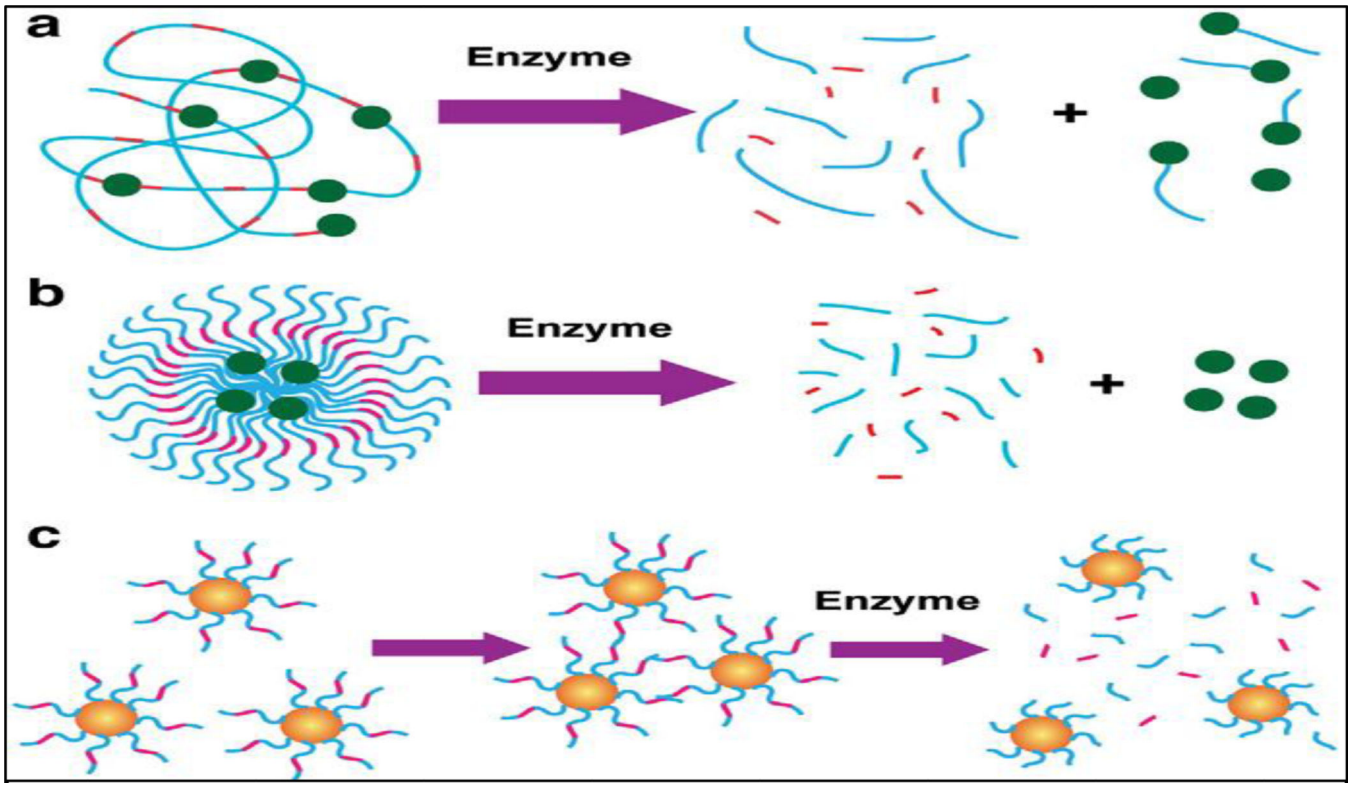

Fig. 3.

Enzyme-responsive nanomaterials for drug delivery and diagnostics. a) polymer based nanoparticles covalently modified with drugs through an enzyme cleavable linker with enzyme triggered drug delivery in malignant tissue; b) polymer stabilized liposomes loaded with drugs, where programmed degradation triggered by an enzyme; c) Inorganic nanoparticles used for diagnostics where the activity of the target hydrolase the assembly or disassembly of the nanoparticles. Reproduced with the permission from Stevens et al. [2]

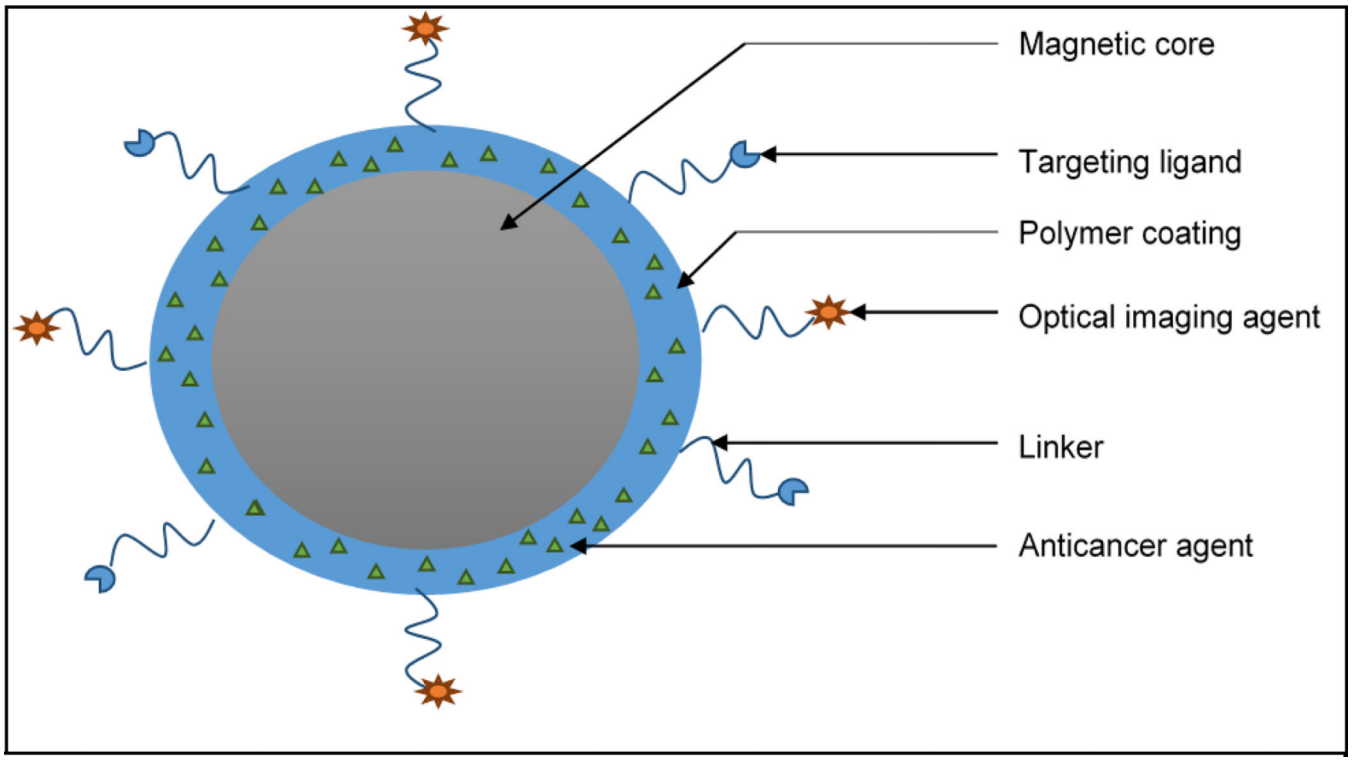

Fig.4.

Schematic representation of core shell type magnetic nanoparticles. The external polymer coating entrapping anticancer agent can be decorated with imaging agent for enhanced imaging and/or targeting ligand for active targeting via a suitable linker.

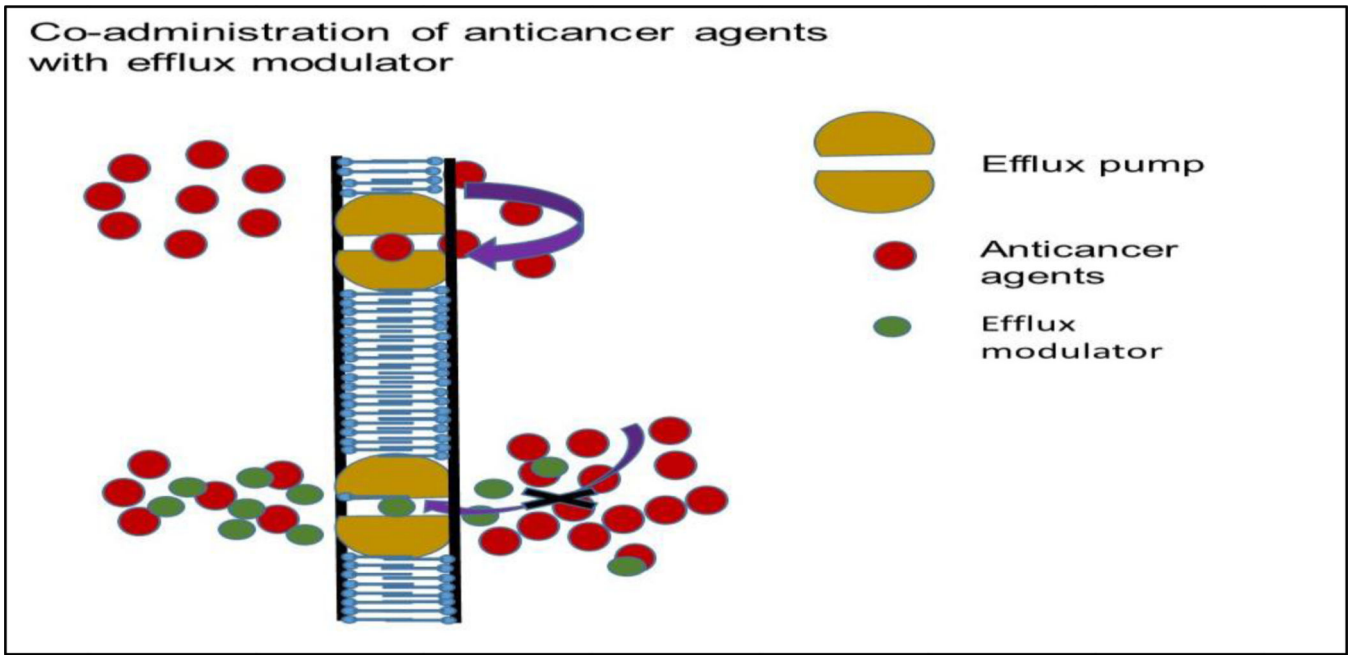

Fig. 5.

Mechanism of action of combination therapy: efflux pump modulator upon co-administration with anticancer agents- substrates of MDR efflux transporters

Table 1

Summary of six major categories of cancer [7]

Category	Types	Examples
Carcinoma	Adenocarcinoma Squamous Cell Carcinoma	Breast, Lung, Colon, Prostate, Bladder
Sarcoma	Osteosarcoma	Bone
	Chrondosarcoma	Cartilage
	Leiomyosarcoma	Smooth muscle
	Rhabdomyosarcoma	Skeletal muscle
	Mesothelial sarcoma	Membranous linings
	Fibrosarcoma	Fibrous tissue
	Angiosarcoma	Blood vessels
	Liposarcoma	Adipose tissue
	Glioma or astrocytoma	Neurogenic tissue in brain
	Myxosarcoma	Embryonic connective tissue
	Mesenchymous	Mixed connective tissue
Myeloma	--	Plasma cells of bone marrow
Leukemia	Myelogenous or granulocytic	Myeloid and granulocytes
	Lymphatic, Lymphoblastic	Lymphoid and lymphocytic
	Polycythemia vera or erythemia	Red cells and blood cells
Lymphoma	Hodgkin Lymphoma, Non Hodgkin Lymphoma	Stomach, breast or brain
Mixed types	Adenosquamous carcinoma Mixed mesodermal tumor Carcinosarcoma	Breast, Lung, Colon, Prostate etc.

Table 2

Drug delivery vehicles and their effect on nuclear translocation

Drug	Formulation	*In vitro*	*In vivo*	Reference
Doxorubicin	Dox-NLS loaded PLGA NP	six times higher uptake than native DOX in MCF-7 cell line	N/A	[63]
Carboplatin	PEGylated NLS-carboplatin conjugate	Rapid internalization and accumulation in nucleus in M109FR cell lines that have overexpressed folic acid receptors	N/A	[64]
Doxorubicin	TAT modified micelles consisting 2 block co polymers		Suppressed tumor growth in Xenograft mice models	[65]
Camptotheticin	PLL & PAMAM dendrimer	Facilitates nuclear accumulation by endosomal and lysosomal uptake in SKOV3 ovarian cancer cells	N/A	[66]
Doxorubicin	Targeted charge reversal nanoparticles [TCRN]	Increased antitumor activity in SKOV3 ovarian cancer cells	N/A	[67]

Table 3

Nanocarriers for gene therapy in cancer

Nanoparticle types	Gene/Drugs	Target Cancer	Reference
Cationic Liposomes	ZEB1 siRNA and doxorubicin	lung cancer	[114]
	Anti- Prostate-specific membrane antigen (PSMA) liposome complex	prostate cancer	[115]
Neutral/zwitterionic Liposomes	Chlorotoxin-coupled nanoparticles for antisense oligonucleotides or siRNAs	glioblastoma	[116, 117]
Magnetic nanoparticles	Vascular endothelial growth factor (VEGF)- siRNA	ovarian cancer	[118]
	siRNA with multimodal mesoporous silica-based nanocarrier.	lung cancer	[119]
	Notch-1 shRNA	breast cancer	[120]
Gold nanoparticles	MicroRNA	prostate and breast cancer	[121]
	siRNA delivery with polyion complexes and gold nanoparticles.	cervical cancer	[122]
	siRNA by novel epithelial cell adhesion molecule antibody conjugated polyethyleneimine-capped gold NPs	retinoblastoma	[123]
Chitosan-based	systemic delivery of survivin (SVN) siRNA	prostate cancer	[124]
	siRNA	ovarian cancer	[125]
Hyaluronic acid based	siRNA	lung cancer	[126]

Table 4

A list of chemotherapeutic agents which are substrates for various efflux transporters

Efflux transporters	**Chemotherapeutic substrates**
MDR1	Bisantrene, daunorubicin, docetaxel, DOX, epirubicin, etoposide, idarubicin, methotrexate, mitoxantrone, paclitaxel, teniposide, vinblastine, vincristine
MRP1	Daunorubicin, DOX, epirubicin, etoposide, imatinib, melphalan, methotrexate, mitoxantrone, paclitaxel, vinblastine, vincristine
MRP2	Cisplatin, DOX, etoposide, irinotecan, methotrexate, SN-38, vinblastine, vincristine
MRP3	Carboplatin, cisplatin, etoposide, methotrexate, teniposide
MRP4	Methotrexate, topotecan
MRP5	5-Fluorouracil, methotrexate
MRP6	Cisplatin, etoposide, teniposide
MRP7	Docetaxel, paclitaxel, vinblastine, vincristine
MRP8	5-Fluorouracil
BCRP	Bisantrene, daunorubicin, DOX, epirubicin, etoposide, gefitinib, imatinib, irinotecan, methotrexate, mitoxantrone, SN-38, teniposide, topotecan

Table 5

Chemotherapeutic agents metabolized by different isoforms of CYP450

CYP450 isoforms	Chemotherapeutic substrates
1A1	Dtx, erlotinib, tamoxifen
1A2	Erlotinib, etoposide, flutamide, imatinib, tamoxifen
2B6	Cyclophosphamide, ifosfamide, tamoxifen
2C9	Cyclophosphamide, ifosfamide, imatinib, tamoxifen
2C19	Cyclophosphamide, ifosfamide, imatinib, tamoxifen, teniposide
2D6	Imatinib, tamoxifen, vinorelbine
2E1	Cisplatin, etoposide, tamoxifen, vinorelbine
3A4/5	Cisplatin, cyclophosphamide, cytarabine, dexamethasone, Dtx, DOX, erlotinib, etoposide, exemestane, flutamide, fulvestrant, gefitinib, ifosfamide, imatinib, irinotecan, letrozole, medroxyprogresterone acetate, mitoxantrone, paclitaxel, tamoxifen, targretin, teniposide, topotecan, vinblastine, vincristine

Table 6

Summary of activity changes (folds) of chemotherapeutics in presence of ritonavir in T47D cells

Drug	Cell Proliferation (IC_{50}) ⇩	Migration ⇩	Apoptosis ⇧
Doxorubicin	2.86	2.08	1.49
Paclitaxel	3.66	2.08	1.53
Tamoxifen	3.18	1.41	1.41
Vinblastin	7.71	1.52	1.78

Table 7

Summary of activity changes (folds) of chemotherapeutics in presence of ritonavir in PC-3 cells

Drug	Cell Proliferation (IC_{50}) ⇩	Migration ⇩	Apoptosis ⇧
Doxorubicin	3.91	2.96	2.79
Paclitaxel	4.32	3.63	2.01
Tamoxifen	2.98	2.18	2.47
Vinblastin	3.17	2.51	3.17